INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

University of Alberta

Expression and Synthesis of Lewis Antigens in *Helicobacter pylori*

by

David Allan Rasko 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Medical Microbiology and Immunology

Edmonton, Alberta

Fall 2000

University of Alberta

Library Release Form

Name of Author: David Allan Rasko

Title of Thesis: Expression and Synthesis of Lewis Antigens in *Helicobacter pylori*

Degree: Doctor of Philosophy

Year this Degree Granted: 2000

Box 12, Castor, Alberta, TOC OXO

Date: Aug 15, 00

Abstract

Helicobacter pylori is one of the world's most ubiquitous pathogens with, by some estimates, greater than 50% of the population being infected. Clinical symptoms of *H. pylori* infection are not exhibited by all people, but can include gastritis, formation of gastric or duodenal ulcers, as well as, a mucosa-associated lymphoid tissue or gastric cancer. *H. pylori* has been designated a class 1 carcinogen by the World Health organization and is the only bacterium to have such a designation. The demonstration that *H. pylori* contained human histo-blood group antigens (Lewis antigens) as part of the lipopolysaccharide (LPS) was thought to be key in the infectious processes of this organism. My research has focused on the distribution of these antigens among the *H. pylori* isolated from individuals with, and without, symptoms, as well as functionally characterizing the *H. pylori* fucosyltransferase enzymes that are involved in the synthesis of Lewis antigens.

Investigations of the distributions of the Lewis antigens among the *H. pylori* isolated from the aforementioned groups revealed that the *H. pylori* LPS isolated from the asymptomatic individuals demonstrated an absence of type I antigens, Lewis A and Lewis B, a decreased prevalence of the Lewis X antigen, as well as a significant increase in the number of isolates expressing LPS that did not contain any of the Lewis antigens. The reasons for the alteration of the *H. pylori* LPS phenotypes among the isolates from this group of individuals are unclear, but they do demonstrate that the Lewis antigens, as well as the LPS, possibly plays a role in the pathogenesis of this organism. Also, an increase in the variability of these antigens among genetically identical *H. pylori* isolates was observed.

While investigating the *H. pylori* Lewis antigen expression, some of the fucosyltransferase enzymes, which are responsible for the final steps of Lewis antigen synthesis, were cloned and functionally characterized. The *H. pylori* fucosyltransferase enzymes exhibit limited nucleotide homology with human homologues, and were

demonstrated to be functionally similar but not identical. I identified novel *H. pylori* fucosyltransferase enzymes that are responsible for the synthesis of both type I and type II antigens. The biosynthetic pathway of the Lewis B antigen was also investigated and characterization of the enzymes involved demonstrated that the Lewis B antigen is not synthesized in a similar manner to the human Lewis B antigen, but rather *H. pylori* utilizes a unique biosynthetic pathway. Overall, my results indicate the LPS phenotype of *H. pylori* isolated from asymptomatic individuals is markedly different than the LPS of *H. pylori* isolated from symptomatic individuals, and the enzymes utilized in the synthesis of these antigens are unique, both genetically and functionally, from their human homologues.

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Expression and Synthesis of Lewis Antigens in *Helicobacter pylori* submitted by David Allan Rasko in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. D. E. Taylor (Supervisor)

Dr. G. D. Armstrong (Supervisor)

Dr. M. S. Peppler

Dr. J. A. Robertson

Dr. M. M. Palcic

Dr. C. Whitfield

Date: Aug 16/00

For H.J.K.

Acknowledgments

I would firstly like to thank my supervisors Dr. D.E. Taylor and Dr. G.D. Armstrong for their support, both financial and otherwise. The members of both laboratories who have crossed my path during my stay in the lab I thank you all but some people need to mentioned specifically. I would like to thank Michelle Rooker, Qin Jiang and George Mulvey for providing enough technical assistance to help me but still making me do things on my own. Zhongming Ge for teaching me how to do science, your skills are unsurpassed, thank you for letting me learn a fraction of what you know. I have to thank San Ving for his with my figures.

This work was funded by a Doctoral Research Award from the Medical Research Council of Canada (MRC), Studentship and Incentive Award from the Alberta Heritage Foundation for Medical Research (AHFMR) and The Canadian Bacterial Diseases Network (CBDN) for funding provided to the laboratories of Dr. Taylor and Dr. Armstrong. I would also like to thank the CBDN and the University of Alberta for providing travel funding over the years.

I would also like to thank my family for their support over the years. I would like to especially thank my wife, Yvonne, for providing emotional support in the good times and the bad, for the helpful pushes as well as helping me to see when to step back and realize it was time for a day off. I could not have done it without you.

Table of Contents **PAGE**

PAGE

List of Figures

List of Tables

List of Abbreviations

3'SL	3' sialyllactose
α(1,3/4) FucT	fucosyltransferase with both α(1,3) and α(1,4) FucT activity
α(1,3(4)) FucT	fucosyltransferase enzymes that may contain α(1,3) and α(1,4) FucT activity
BCA	bicinchoninic acid
BHI-YE agar	brain heart infusion yeast extract agar
bp	base pair
Cag	cytotoxin associated gene
CAT	chloramphenicol acetyltransferase
DNA	deoxyribonucleic acid
ECL	enhanced chemiluminesence
ELISA	enzyme linked immunosorbant assay
FAB-MS	fast atom bombardment-mass spectrometry
Fuc	fucose
fucT	fucosyltransferase gene
FucT	fucosyltransferase enzyme
FUT	human fucosyltransferase gene
G+C	guanosine plus cytosine
Gal	galactose
Glc	glucose
GlcNAc	*N*-acetylglucosamine
H^+K^+ ATPase	proton, potassium ATP transport pump
H Type I	complex carbohydrate shown in Figure 1.3
H type II	complex carbohydrate shown in Figure 1.3
Hep	heptose

HRP	horse radish peroxidase
IgG	immunoglobulin G
IgM	immunoglobulin M
IL-8	interleukin 8
kb	kilobase
kDa	kilodalton
LacNAc	*N*-acetylactosamine
LB	lauria broth
Le^a	Lewis A
Le^b	Lewis B
Le^x	Lewis X
Le^y	Lewis Y
LPS	lipopolysaccharide
MAb	monoclonal antibody
MALT	mucosa associated lymphoid tissue
ml	millilitres
mM	millimolar
munits	milliunits
nCi	nanocuries
nm	nanometers
NTHp	nontypable *H. pylori*
ORF	open reading frame
ODU	optical density units
PAI	pathogenicity island
PCR	polymerase chain reaction
PE	phosphatidylethanolamine
PFGE	pulsed field gel electrophoresis

poly A	poly adenosine
poly C	poly cytosine
RAPD-PCR	random amplified polymorphic DNA -polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate - polyacrylamide gel electrophoresis
SLe^x	Sialyl-Lewis X
TFC	translational frameshift cassette
Tir	translocated intimin receptor
TNFα	tissue necrosis factor alpha
Tris	tris (hydroxymethyl) aminomethane
Tween	polyethylenesorbitan monolaurate
µl	microlitres
µm	microns
und-PP	undecaprenol pyrophosphate
UV	ultraviolet
vol/vol	volume to volume
wt/vol	weight to volume

Chapter 1

INTRODUCTION

1.1. General Introduction

Helicobacter pylori is a Gram-negative bacterium that colonizes the gastric epithelium of humans. In 1983, the first description of the isolation of a *Campylobacter*-like organism from gastric samples was reported (184). This was not the first description of the organism. In the early part of the last century, pathologists had noted that a curved bacterium could be stained and observed on the gastric tissue of many individuals (15). However, it was assumed by many that the harsh gastric conditions prevented colonization by any organism and the lack of bacterial culture by any microbiologists reinforced this idea. The isolation of *H. pylori*, at that time referred to as a *Campylobacter*-like organism, was fortuitous. The primary culture plates that were being used at the time were left in the incubator over the Easter break and when the technician returned from vacation small colonies were identified (B. Marshall, personal communication). Since then, continued isolations with prolonged incubation periods, as well as low oxygen tension has become standard for the culture and isolation of this organism (159).

In the 17 years since the discovery of *H. pylori*, there have been many significant advances in the understanding of the biology and pathogenesis of this microorganism. For example, it was discovered that the *H. pylori* infection could be linked to ulcer formation in human patients (18, 61) and further work established that *H. pylori* infection can be a causative agent in gastric cancers (137). Antibiotic treatment of the *H. pylori* infection cured many of the ulcer-related symptoms. However, antibiotic resistance is beginning to be an important point to consider in the treatment of this bacterium (68). There has been a great deal of investigation into the pathogenic processes of *H. pylori* including adhesion, toxins, carbohydrate antigens as well as other antigens that have been demonstrated to be essential in the infectious process. It was recently noted that between 1991 and 1997, ~7.5% of all scientific papers published pertained to the genus *Helicobacter* and the number of papers specifically concerning *H. pylori* has tripled over that same period of time (58). Probably the most significant advances in the field were the publication of the

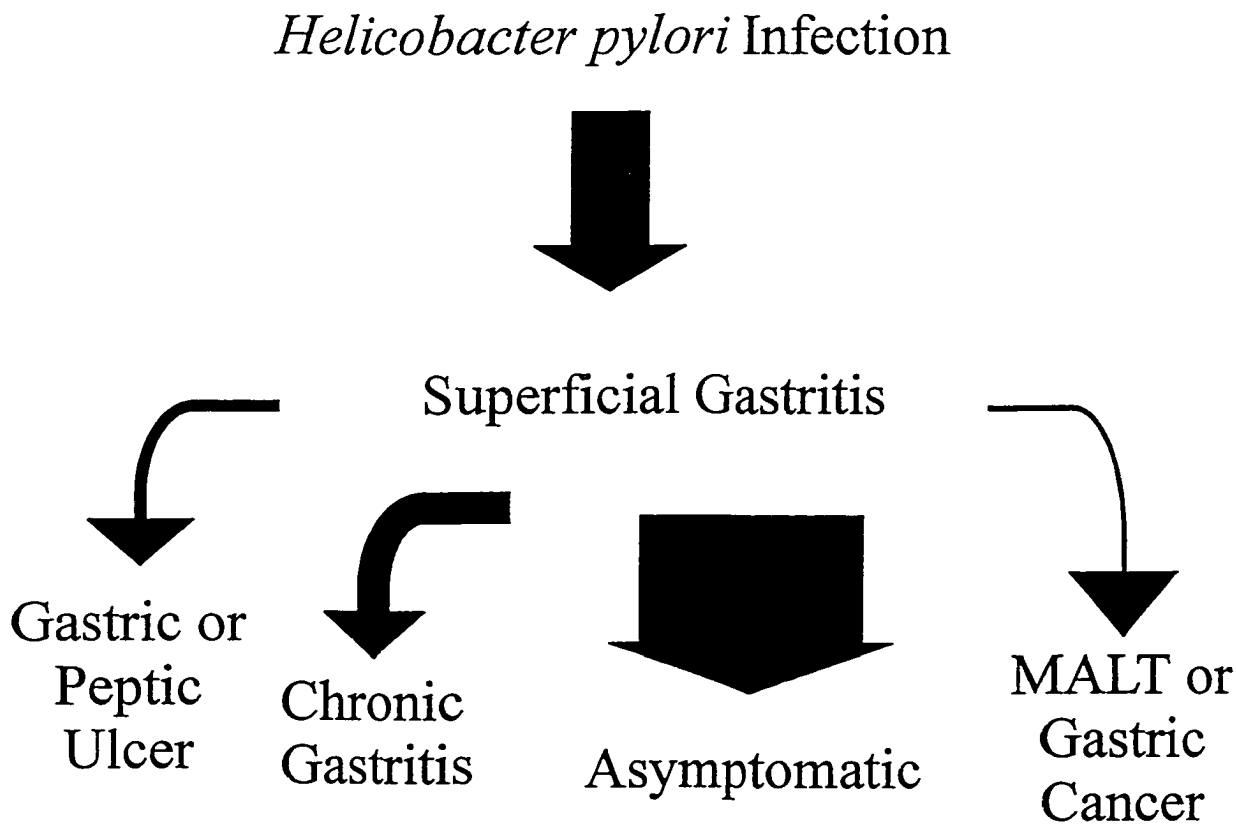
genome sequences of strains 26695 (163) and J99 (3). Although we have made significant strides in the understanding of the pathogenic processes utilized by *H. pylori* in the infection of humans, there are many important questions that remain unanswered.

1.2. *Helicobacter pylori* Pathologies

A great deal of research has gone into the understanding of *H. pylori* pathogenesis and infection but there has been very little headway gained in the investigation of the routes of transmission of this organism (41). It has been suggested that a route of transmission may be fecal-oral as *H. pylori* has been isolated from the feces of infected children (162). *H. pylori* isolation from fecal matter appears to be rare as no other group has confirmed the initial report. It has been suggested that this is the result of intermittent shedding of the organism. One other possible route of transmission is oral-oral, which may be common in areas of the world where mothers premasticate the food for infants and thus pass on the *H. pylori* (107). One uncommon route of infection is via contaminated medical equipment used during gastric biopsy collection (41). The investigation of the route of transmission continues and as methods become more sensitive it may be possible to determine and even interfere with transmission pathways.

With more than 50% of the world's population being infected with *H. pylori*, one would assume that the need for treatment would be astronomical, but it has been reported that the majority of people (>80%) who are infected with *H. pylori* do not report any negative effects of the infection and do not seek medical attention (19, 41). These people are considered to have an asymptomatic infection and this appears to be the norm for *H. pylori* infection. It has been suggested that all people who are infected with *H. pylori*, asymptomatic or not, develop superficial gastritis to some degree (Figure 1.1)(18). The gastritis can be superficial and thus not recognized or may progress to be more invasive, in which case the individual may seek medical intervention. This condition is known as chronic gastritis (61) and is characterized by localized increases in pro-inflammatory cytokines (IL-8 and TNF-α), increased numbers of infiltrating neutrophils and T cells,

Figure 1.1. The progression of *H. pylori* pathologies. The width of the arrow is indicative of the percentage of patients that progress to that disease. All people infected with *H. pylori* experience a superficial gastritis. Most people (greater than 80% according to some studies) do not report any further clinical symptoms. Some individuals approximately 15-20% progress to clinical symptoms, most report a gastritis, whereas a few ,~1% of the symptomatic individuals, progress to gastric or peptic ulcer and a very small proportion develop a MALT lymphoma or gastric cancers.



as well as a variable level of epithelial degradation (61). Atrophy of the gastric tissue is observed in some cases but not all.

H. pylori has also been shown to be a causative factor in the progression to both gastric and duodenal ulceration. It is suggested that approximately 15-20% of infected symptomatic individuals develop some type of ulcer (51, 153). Ulcers are characterized by an erosion of the gastric epithelium to produce an area in which the underlying tissue is exposed to the low pH of the gastric lumenal contents (~ pH 3-5)(61). The area of the ulceration is also often identified by a lack of specialized cells, i.e. secretory or mucous cells, as well as an increase in inflammatory cells (61). The local environment has an increased amount of pro-inflammatory cytokines that further potentiate the immune response. Duodenal ulcers may also be caused by *H. pylori*, but they usually occur in older patients (> 60 years old) suggesting that the length of time of infection may be important in the outcome of the disease (20, 61).

The association of *H. pylori* with gastric cancer in a number of well-designed studies have lead to the assignment of class I carcinogen status for this organism (75, 136, 137). This is the only microorganism to have such a designation. The risk factors involved in the progression from the asymptomatic or ulcerogenic status of infection to the cancer-causing state are not presently understood. The observation of mucosal-associated lymphoid tissue (MALT) in some cases can be "cured" by the eradication of the *H. pylori* (61). It has been demonstrated that *H. pylori* infection increases the risk of gastric cancer (135). One hypothesis in the development of gastric cancer is that the presence of *H. pylori* infection causes an increase in the number of cell generations, thus increasing the chances an oncogene will be activated, resulting in a malignancy (20). One other hypothesis that needs to be further explored is the possibility that *H. pylori* can transfer portions of bacterial DNA into host epithelial cell which may integrate into the host chromosomes and activate oncogenes. The machinery to accomplish this has recently been demonstrated, in that a Type IV secretion pathway has been shown to insert the Cag A

protein into the eukaryotic cell (24, 146) (see section 1.5.2). A similar pathway is utilized in bacteria to move DNA from one cell to another via conjugation (32). It has been established that *H. pylori* is the cause of ulcers and some malignancies and it has been hypothesized that the increased eradication of *H. pylori* has lead to a decrease in the number of deaths from gastric cancers (19). It has also been demonstrated that fewer people are being infected by *H. pylori* (B. Marshall, personal communication).

The reasons for the different outcomes of *H. pylori* infection may be a result of three factors. The first factor is that each *H. pylori* strain that infects an individual has a different pathogenic potential. It is known that each *H. pylori* isolate can express different amounts of the various virulence factors (see section 1.5) (58). Additionally, it has been noted that the colonization density and the primary interaction of the *H. pylori* with the host epithelial cells may affect the outcome of infection (62, 104). The second factor for a difference in the outcome of *H. pylori* infection is the host response. Each person has the potential to react differently to the same antigens, and thus the outcome of the infection can be individualized depending on how and which antigens cause a reaction (20). The final factor responsible for differences may likely be environmental cofactors. This is one aspect that cannot be easily investigated because *H. pylori* may infect an individual for a lifetime that is filled with exposures to other potential carcinogens. Diet and environmental factors have been implicated in the development of some gastric cancers and *H. pylori* may provide an additional cofactor (20).

Further work is needed to identify the factors, both bacterial and host, that are involved in the development of all the aforementioned pathological states and long-term studies with appropriate animal models will be needed to explain the progression to the cancer-causing states.

1.3. Genomic Comparison

There are now more than 40 complete prokaryotic genomes available to the public (www.tigr.org/mdb) and it should be noted that *H. pylori* is the first organism to have two

genome sequences completed. The advances that have arisen from the genome projects have been many-fold, and their impact is still not fully appreciated. Previously, the genomes of *H. pylori* and other organisms were physically mapped using pulsed-field gel electrophoresis (PFGE). From this work it was determined that the *H. pylori* genome was variable in gene order (79), although there did appear to be a conservation of the order of certain genes (58). The sequencing of two non-related *H. pylori* isolates allowed the comparison of the genomes to determine if there was as much variability as the physical mapping had indicated. It has been determined by genome comparison that the two isolates that were sequenced were much closer to one another than the isolates examined by PFGE (58). Some research groups have also questioned the variability ascribed to the *H. pylori* genomes by investigation of porin proteins (64). Their hypothesis is that the porins were almost identical between the two strains' genomes and were similar to other porins that had been sequenced by individual research groups (64). This analysis is based on a small and conserved group of proteins that play a role in the transport of essential molecules into the bacterial cell and must contain certain conserved elements to maintain function.

The two *H. pylori* genomes share the identical G+C content of 39% and are within the established range of 1.6-1.73 megabases (79), as 26695 is 1,667,867 bp and J99 is 1,643,831 bp (58). As would be imagined from the diversity proposed for this species each of the isolates contains some strain-specific genes, 26695 containing 117 (~8%) and J99 having only 89 (~6%), but 1406 genes are common to the two isolates indicating a significant level of similarity. Each strain has a significant number of open reading frames (ORF) that have no ascribed function (~20% in each J99,275; 26695,290) which is a common proportion encountered by genome sequencing projects. The identification of most of the ORFs is based exclusively upon sequence data and functional work must be completed to definitively identify the functions of most ORFs. One likely example of erroneous ORF designation due to genetic homology is the *rfaG* (*waaG*) gene of which

J99 has four homologues and 26695 has three. In *E.coli* this gene product is responsible for the addition of glucose to the inner core of the lipopolysaccharide (LPS) in an 1->2 linkage (83). This linkage is not observed in *H. pylori* LPS (116) and thus it is assumed that these homologues have other glycosyltransferase function(s). It is likely that many of these genes encode sugar transferases, as many transferases that are responsible for the production of LPS are not identified in either genome sequence (16).

By comparison of the two *H. pylori* genomes, the most common mutation observed was at the codon third position and thus not affecting the amino acid sequence of the proteins but may possibly have an effect on restriction profiles that are relied upon to create the physical maps by PFGE (3, 181). Analysis of the two genomes by mapping the neighbouring genes provides a more complete picture. Approximately 85% of the genes identified have the same two neighbouring genes whereas 1.8% contain only a single common neighbour (3) indicating that the majority of the genes are in the same relative area of the genome. A number of conserved blocks or units of genes have been identified such as the 40kb of the Cag pathogenicity island in which the gene order is conserved between these isolates and others (3, 163). There are also ten inversions identified between the two *H. pylori* genomes which are though to have been mediated by insertion sequences, such as IS605, that are numerous in some strains (3). Overall, the genomic organization of the *H. pylori* may be less divergent than was once thought, but still must be considered extremely variable.

1.4. Antigenic Variability

A degree of variability has been identified in a number of *H. pylori* antigens, including the Lewis antigens of the LPS (5, 8), VacA (13, 14, 155, 171), CagA (108, 196) and the adhesin BabA (74). The regulation of variable antigens by *H. pylori* is a poorly understood process. There are a number of genetic mechanisms in place which could lead to increased variability of antigens including the existence of homopolymeric nucleotide tracts within genes, natural transformation, a number of restriction modification

systems and the absence of many DNA repair mechanisms (103, 157, 181). The use of one or more of these mechanisms by *H. pylori* may be responsible for the observed antigenic variability.

Homopolymeric repeat regions have been identified in over 40 genes in the *H. pylori* genomes. Many of the identified genes encode surface exposed antigens or are involved in the expression of surface exposed antigens (3, 143, 163). Several of these polymeric repeats occur in the sequences encoding the amino terminus of the protein or in the promoter region, indicating that there may be an element of phase variation in the control of these gene products. Polynucleotide repeats can be the repeat of a single base (homopolymeric) or dinucleotides (two bases) ranging in length from as few as 5 dinucleotides (10 bases) to as many as 11 dinucleotides (22 bases). Also, single-base repeats ranging from 9 to 16 nucleotides have been identified (103). It has been demonstrated that DNA containing the polymeric repeat units is subject to a higher mutation frequency (181) by a slipped strand mispairing mechanism (5). Under normal conditions the misincorporated nucleotides will be excised, but the lack of DNA repair mechanisms in *H. pylori* may allow the mutations to persist (181). The ability of a bacterial population to rapidly alter its surface characteristics may allow avoidance of the host immune response. A basal level of mutation will allow "natural" variants to occur that will survive after an immune response may have eliminated much of the population.

There are multiple examples of mosaicism among the surface expressed antigens of *H. pylori* (103). The BabA protein, the adhesin of Le^b, is an example of a such a gene (74). Three gene homologues of *babA* are present in some isolates (NCTC11637) and two in others (26695), but it appears as though only one produces a functional protein (74). The other copy or copies of the gene are thought to be present to allow for the excision of the existing ORF or portion thereof and the insertion of a similar portion from the other non-coding gene. This type of change which may effect protein expression can

be carried out by the natural DNA-processing elements that are present within the *H. pylori* cell (74, 181).

H. pylori is a naturally transformable organism (69, 183) enabling it to acquire DNA from the environment and incorporate it into the chromosome. It is thought that natural transformation allows for increased variability in the genetic material that can be expressed by any given organism (69). The mechanism of natural transformation in *H. pylori* is not understood, but the *comB* locus has been implicated (69). In some organisms, certain sequences are known to mediate the DNA uptake, but no such sequence has been identified in *H. pylori* (69). There are also a number of identified restriction modification systems identified in *H. pylori* that are thought to process environmental DNA prior to incorporation or destruction (3, 163, 183). A conjugation-like process in *H. pylori* has also recently been described (90). This process would allow for the exchange of genetic material without the need for the DNA to pass unprotected through the environment. Homologues of a type IV secretory pathway were found in the Cag pathogenicity island (24, 32), which may mediate DNA transfer.

A number of antigens such as flagellin subunits, LPS O side chain and the sialic acid specific adhesin appear to be expressed in variable amounts and composition depending on the *in vitro* growth conditions (5, 39, 70, 82, 110). It is not clear exactly how the expression of these *H. pylori* genes are controlled as very few regulatory proteins were identified in the genome sequences (40). The combination of all of the genetic tools employed by *H. pylori* allow it to be a genotypically and phenotypically variable organism.

1.5. Virulence factors

There has been a significant amount of research into the virulence factors that allow *H. pylori* to cause disease in humans. Much of this work has focused on the classical bacterial virulence factors such as toxins (33), excreted proteins (32) and adhesins (39). Furthermore, virulence factors that are unique to *H. pylori* and allow colonization of the gastric epithelium such as motility factors (43) and urease (114) have been identified

(Table 1.1). Although a number of factors which aid the *H. pylori* infection of the gastric tissue have been identified, there are undoubtedly more virulence factors that have not been discovered. The following section briefly discusses the best characterized *H. pylori* virulence factors (Table 1.1).

1.5.1. Vacuolating toxin

The vacuolating cytotoxin (VacA) is considered to be one of the major virulence factors of *H. pylori*. The cytotoxic activity of the VacA protein causes vacuolation of eukaryotic cells (34). The vacuoles can be visualized via addition of a neutral red dye that is taken up into the vacuoles because they become acidified (34). The process by which the VacA protein works has not been clearly elucidated, but the presence of rab7 and other membrane marker molecules indicates that the vacuoles are formed from endocytic vesicles (115, 133). Recently, there have been reports that VacA is also able to form anion-selective channels on the eukaryotic cell membrane or activate existing channels which may also be responsible for vacuolation within the cell (158, 164). The two proposed activities of the toxin are not mutually exclusive as suggested by Tombola *et al.* (164) who indicated that both phenotypes could be attributed to VacA in different locations within the host cell.

The *vacA* gene encodes a 140 kDa protein, showing no homology with any other known toxin (33). The full length protein is processed twice to produce the active, mature form of the cytotoxin (33, 161). A 47 kDa carboxyl-terminal portion is responsible for the *H. pylori* membrane association after toxin autosecretion and is cleaved to produce the secreted product (33). The VacA protein is secreted as a 90 kDa protein which is further processed to 34 and 58 kDa protein subunits that must remain associated for biological activity (161, 197). It is thought that these subunits represent two distinct toxin subunits. The 58 kDa protein subunit has been implicated in the adhesion of the cytotoxin to the eukaryotic cell membrane (55) and so it was assumed that the 34 kDa fragment was the active subunit (33). However, the 34 kDa subunit alone does not contain any

Table 1.1. Major Virulence Factors of *H. pylori*

Virulence Factor	Essential	Role
VacA	?	Vacuolization
Pathogenicity Island	?	Delivery of *H. pylori* CagA protein
Flagella	yes	Motility
Urease	yes	Acid Resisitance
Adhesins	yes	Adherence/colonization
Lipopolysaccharide	?	Immune evasion? Low reactivity, Persistence?

cytotoxic activity (197). It has been clearly demonstrated that both subunits of the toxin are required for the vacuolization activity, and the minimal portions of each subunit have been identified by subcloning (197). The VacA proteins oligomerize into a flower-like structure (101) but it is unclear whether monomers also contain biological activity.

H. pylori vacuolating cytotoxin activity is present in only 50% of the clinical isolates but the *vacA* gene is present in all strains investigated. (13). It is thought that the mosaic structure of the VacA protein leads to some of the observed differences in toxin activities (13, 33). Atherton *et al.* (13) demonstrated that there are two regions of the *vacA* gene which are significantly variable and may lead to variations in protein expression or vacuolating activity. The first region is an amino-terminal signal sequence, termed the s region, of which four types have been identified, s1a, s1b, s1c and s2 (13, 171). It is thought that this region is responsible for the secretion of VacA as isolates with mutations in this region produce but do not secrete the VacA protein (13). The s region is also implicated in the lack of vacuolating activity from isolates containing the s2 sequence as the vacuolating activity from these isolates is usually nonexistent (13). The second *vacA* region containing marked differences is the middle region, m region, containing the putative active components of the VacA toxin (13). It has been demonstrated that this region is responsible for some cell-specific vacuolating activity (132) and mutations in this region may be responsible for the lack of vacuolating toxin activity observed in some isolates (13). There have been 4-6 m regions identified among the *vacA* alleles investigated, depending on the definition of a new subunit type (13, 102, 155, 171). The association of s and m alleles has been noted in isolates obtained from patients with increased symptoms (13, 36). *H. pylori* isolates with s1/m1 are associated with ulcer formation, whereas isolates with the s2 allele is not usually isolated from patients with ulcer disease (13, 36). However, some studies have indicated that the m region is not correlated with disease outcome (155). Further work will determine which portions of the VacA protein are essential for disease progression.

The mosaicism that is observed within this protein has also been demonstrated to have a geographic distribution with the s1a/m1 *vacA* gene being prevalent among *H. pylori* from patients of Japanese origin (77), the m1a allele among *H. pylori* isolates from German patients (155) and the s1c from the *H. pylori* isolated from East Asian patients (171). The reason for this mosaicism and geographic distribution is not understood at this time but may be related to the genetic disposition of individuals in these areas of the world. It may be that the people of certain ethnic groups express receptors which allow for the increased binding and internalization of VacA containing a certain allele and thus the *H. pylori* have adapted to that. It was demonstrated that differences in the genetic makeup of the protein could be responsible for the differences observed in toxicity (132). Further work is needed to identify how exactly this toxin exerts its effects and the molecular mechanisms behind that activity.

1.5.2. Cag (cytotoxin associated gene) Pathogenicity Island

The Cag pathogenicity island (PAI) is an approximately 40 kb fragment of DNA that encodes 31 ORFs which have been associated with the development of gastric ulcers (24). The Cag PAI exhibits characteristic features of pathogenicity islands such as inverted repeats and a G+C content that is not consistent with the rest of the genome (63). The Cag PAI is flanked by 31 base direct repeats (1) and is inserted into the glutamate racemase gene (24). The G+C content of the PAI is 35%, whereas the remainder of the *H. pylori* genomes have G+C contents of 39% (103). There has been the assignment of two classes of *H. pylori* types based upon the PAI architecture (24). Type I *H. pylori* have the entire PAI in one contiguous unit with enhanced virulence whereas, the type II strains do not contain the PAI and thus are less likely to be associated with disease (24, 58).

There are a number of genes contained within the Cag PAI that would appear to suggest the presence of a Type IV secretion system (24). This system has recently been demonstrated to be responsible for the insertion of the CagA protein into eukaryotic cells,

where CagA is phosphorylated (11, 32, 146, 154). The dominant ORF, *cagA*, for which the PAI is named, encodes a 120-130 kDa protein that is found in *H. pylori* culture supernatants. The *cagA* gene product is immunogenic (31). Antibodies against this protein can be identified in the sera from *H. pylori* infected patients (31, 35). CagA can also be identified in the culture supernatants of *H. pylori* that exhibit cytotoxin activity (24, 35), but the deletion of Cag PAI does not affect production of the cytotoxin (1). The importance of the insertion of the CagA protein into the eukaryotic cell has not been elucidated, but its insertion and subsequent phosphorylation may play a role in the formation of adhesion pedestals (11, 32, 146, 154). This appears to be similar to the insertion of the Tir protein in Enterpathogenic *E. coli* (32, 86). In the *E. coli* system, the Tir protein is inserted by bacterial proteins, phosphorylated by the host, and then acts as an adhesin for one of the major bacterial adhesins, intimin (86). It has not been demonstrated if the CagA protein also has this function or fulfills another function in the gastric epithelial cell. It was noted by Segal *et al.* (146) that two human epithelial cell phenotypes were observed after *H. pylori* contact; one that was Cag-dependent and one that was Cag-independent. Further work will be needed to investigate if other bacterial proteins are secreted into the host cell and define what role these proteins play in pathogenesis.

After many years of being designated as an accessory protein it appears as though the CagA protein may indeed play a role in *H. pylori* pathogenesis. Multiple forms of the CagA protein have been identified (108, 196). The basis of the variation is found in repeated regions in the 3' end of the *cagA* gene. What purpose these protein repeats may play in the pathogenicity of this organism remains to be identified. If the function of CagA is similar to the Tir protein system acting, as the bacterial receptor (86), then the CagA variation should be mirrored by a variation in the protein that attaches to the CagA protein. Alternatively, if the variable regions are responsible for interactions with host signaling pathways, the variable portions of the proteins may result in different degrees of actin polymerization and pedestal formation during infection (146).

1.5.3. Motility

Eaton *et al.* demonstrated that in the gnotobiotic piglet model that the flagella are needed to colonize and cause infection (43). The flagella are not the only factors involved in the motility of *H. pylori* as both the shape of the organisms and the ability to direct movement via chemotaxis have also been shown to be important (38).

H. pylori cells resemble a corkscrew and it has been shown that this form is an advantage to the organisms in the penetration of the viscous gastric mucous (19, 41). The factors determining the shape of the organisms have not been identified from the genome sequence. The *H. pylori* cell morphology has been demonstrated to be an indicator of culture viability as the other morphological form of the bacterial cell is a coccoid shape, and likely indicates that the cells are no longer viable (92).

In the genomes sequenced to date there are 38 genes identified that are involved with the assembly, processing or activation of the *H. pylori* flagella (38). *H. pylori* produce between one and five polar flagella that are each covered by a sheath (38). The importance of the flagella as a virulence factor has been established in many gram-negative organisms (52) including *H. pylori*. Because of the interest in the flagella of other organisms there are a large number of gene homologues available for comparison and many of the flagellar-associated proteins have been identified within the *H. pylori* genomes (3, 163). The *H. pylori* flagellum is composed of two subunits, FlaA and FlaB (81, 95). The ratio at which these two subunits are incorporated into the flagella is variable (82). The reason for the alteration of the flagellar subunit ratios are unknown, but a change in environment may require *H. pylori* to alter the flagella for increased or decreased motility under particular conditions.

It had been reported previously that the genes encoding these products were a source of antigenic variation, but comparison of the two genome sequences indicates that the FlaA proteins are identical and the FlaB differ by only a single amino acid (3). One difference between the *H. pylori* flagella and the flagella of other gram-negative organisms

is the sheath that encapsulates the entire flagellum and is capped with a terminal bulb (138). The composition of the sheath has not been conclusively determined as of yet (38), and the role is also unknown. However, two theories are predominant. The first theory is that the sheath protects the flagella from the acidic conditions that are encountered in the gastric lumen (pH 3-5) (38). It has been suggested that at the low gastric pH the flagella would depolymerize creating an organism that is non-motile and consequently not infectious (38). Alternatively, the sheath may bleb off and act as an immune decoy allowing *H. pylori* cells to survive without the added pressure of the immune response.

One other area of *H. pylori* motility being investigated is the chemotactic ability of *H. pylori* (112, 201). A system that detects nutrients or areas of increased pH and initiates movement towards them is essential for survival in the harsh gastric environment (201). It has been demonstrated that *H. pylori* has increased motility towards many amino acids (194), urea and bicarbonate (112, 123) indicating that detection of these nutrients and movement towards them is possible by *H. pylori*. The chemotaxis systems studied to date have been similar to systems described in other gram negative organisms (102).

1.5.4. Urease. The colonization of the gastric epithelium is one of the features of *H. pylori* which makes this pathogen unique (113). To survive in this niche *H. pylori* produces a highly effective urease enzyme (114). It has been demonstrated that isolates that do not contain a functional urease enzyme do not colonize in animal models (42, 166). Urease hydrolyses urea to produce carbon dioxide, bicarbonate and ammonia. The carbon dioxide and bicarbonate create a localized region of increased pH surrounding the bacterial cell, while the ammonia is utilized in the synthesis of amino acids (56).

Seven genes are required for the production of a functional urease enzyme (114). Five are accessory proteins (*ureE, ureF, ureG, ureH* and *ureI*) involved in the incorporation of nickel into the active enzyme, which consists of the oligomerized subunits of the gene products of *ureA* and *ureB* (71, 114). The two structural subunits of the urease enzyme form a multimeric complex of 550 kDa (71). There appears to be a large

degree of genetic variability in the urease structural genes. This variability has been exploited to develop a typing system based upon the restriction of *ure* gene specific PCR products (150). Active urease enzyme has been localized to cytoplasmic as well as the membrane fractions of *H. pylori*. The process by which urease arrives at these locations is unclear (114), but both are essential for the survival of *H. pylori* in acidic conditions (89). It has been suggested that the surface localization of the urease enzyme is achieved by altruistic autolysis, in which one *H. pylori* cell sacrifices itself for the good of the population, providing urease for the surface of the other *H. pylori* (89). Alternatively, Vanet *et al.* believe that a specific secretion process is required for the surface localization of the urease enzyme (174). Further work is required to determine which hypothesis is correct.

Since nickel is required for urease enzyme function, the level of this compound within the *H. pylori* cell is tightly regulated (102). Multiple gene products are involved with nickel ion regulation including the nickel binding proteins *nixA*, *hspA* and *hspB*, as well as an ABC transport operon (*abcA-D*), and a P Type ATPase which are responsible for nickel transport (58, 114). The inactivation of any one of these systems causes a decrease in urease activity, but not a total loss of enzyme activity, indicating that these systems are redundant for the importation of nickel for the urease enzyme (114). Although these gene products are not directly involved in the production of the urease enzyme, their functions are essential to *H. pylori* survival in the acidic gastric contents (114). The redundancy that is demonstrated in the *H. pylori* nickel regulation is an indicator of the importance of the function of this enzyme to bacterial survival (114).

1.5.5. Adhesion

There has been significant research into the identification of the adhesions responsible for attachment of *H. pylori* to the gastric epithelium (26, 39, 177) Table 1.2). A number of adhesins and corresponding receptors have been identified, but no one has

Table 1.2. Proposed Adhesins and Receptors of *H. pylori*

Proposed Receptor	Protein Size	Protein Name
N -acetylneuraminyl-α(2-3)-lactose *(HpaA= protein)*	26-30 kDa	Hemagglutinins Sialic Acid Dependent Sialic Acid Independent
Sialic Acid	??	??
Integrin	??	??
Extracellular Matrix	LPS -25 kDa	??
Lipids (phosphatidylethanolamine)	63 kDa	??
Fucose containing Carbohydrates (i.e. Lewis B, H Type I)	75-61 kDa	BabA
Mucin- glycoproteins Sulfated Lewis Ags	16 kDa	NAP

been demonstrated to be dominant *in vivo*. Identifying the dominant adhesin and finding an inhibitor for that adhesin could lead to an anti-adhesive therapy for *H. pylori* rather than, or in combination with, the antibiotic regimes that are available to increase the effectiveness of eradication therapy.

1.5.5.1. Hemagglutinins

One of the classical ways of determining the specificity of an adhesin of an organism is to perform a hemagglutination experiment (70). When these tests were first performed on *H. pylori* with a panel of red blood cells from different animal species the hemagglutination profile was inconsistent. Over time it became clear that there was more than one group of *H. pylori* based upon hemagglutination ability (39, 94). Hemagglutination was shown to be sensitive to heat, proteinase and alkylating agents suggesting that it was proteinaceous in nature (124). There also appeared to be differences with respect to the inhibitors that would affect the hemagglutination, with some sialic acid-containing sugars inhibiting the hemagglutination of some isolates but not others (94). *H. pylori* hemagglutination has been grouped into sialic acid-dependent or sialic acid-independent binding based on the adhesion capabilities of the isolate. The protein responsible for hemagglutination in either group has not been not identified. It has also been discovered that the hemagglutinating ability of the *H. pylori* isolate is a poor predictor of infectivity, colonization or disease causing capabilities (131).

1.5.5.2. Sialic Acid

The concept of hemagglutination is based upon binding to receptors on red blood cells. This is an extremely artificial system as *H. pylori* rarely, if ever, comes into contact with red blood cells. The binding of *H. pylori* to cultured cells *in vitro* was investigated and it was demonstrated that some isolates bound in a sialic acid-dependent manner (45, 46). Studies have demonstrated that *H. pylori* binding was inhibited by pretreatment with sialidases to remove sialic acid or by addition of fetuin, a highly branched carbohydrate molecule, whereas other studies indicated that there was no effect by these pretreatments

(47, 50). The use of different cell lines by investigators make interpretation of these binding assays difficult. A study by Simon *et al.* (151) demonstrated binding to sialic acid-containing synthetic carbohydrates as well as a decrease in the adhesion of the *H. pylori* to epithelial cells that were pretreated with neuraminidase to remove the sialic acid-containing residues on the cell surface. Simon *et al.* (151) further utilized the 3'sialyllactose (3'SL) structure of the gastric epithelia to create synthetic analogues. These analogues decreased the adherence of *H. pylori* to cultured cells (151).

The protein hypothesized to be the sialic acid adhesin is HpaA (47, 80, 151). HpaA is a 20 kDa protein which was first isolated by Evans *et al.* (47) as a sialic acid-dependent hemagglutinin. The protein is lysine-rich and has similarity to the sialic acid binding motif of the SfaS adhesin of S-fimbriated *E.coli* (134) and colonization factor antigen I (CFA/I) of enterotoxigenic *E.coli* (84). Hopkins *et al.* (70) first noted that there was a decrease in the sialic acid binding with the longer an isolate was passaged. A similar phenotype is also described by Miller-Podraza *et al.* (109, 110), who found that agar-grown *H. pylori* are less adherent to sialic acid-containing carbohydrates than broth-grown bacteria of the same isolate. Studies continue to discover new receptors involving sialic acid constituents.

1.5.5.3. Integrins

The ability of *H. pylori* to adhere to the integrin family of molecules provides yet another example of this organism's ability to bind molecules that are expressed on many different cell types (156). The integrins are a large family of molecules that are utilized in cell-to-cell adherence or in adhesion to substratum material (73). Of interest, in cell culture conditions *H. pylori* will adhere primarily around the edges of the cells rather than to the body of the cell (65). This phenotype may be explained by the availability of the integrins at this location (73). Van Putten *et al.* (172) demonstrate that the OpaA protein of *Neisseria gonorrhoeae* can associate with heparan sulfate (another *H. pylori* receptor about which very little is known (168)) containing glycosaminoglycans and the amino terminus

of fibronectin. Fibronectin, in turn, associates with the αβ1 integrins which leads to the uptake of the bacteria. It still remains to be demonstrated that *H. pylori* binds directly to the integrin molecules. The direct adhesion of *H. pylori* to an integrin molecule may provide an opportunity for the bacterium to access the signaling pathways of the host epithelial cells (156). It has been demonstrated that *H. pylori* interaction with the epithelial cell surface can induce changes at a molecular level including phosphorylation of host proteins and polymerization of actin (146, 147), but this phenomenon may also be attributed to other proteins such as CagA (see section 1.5.2).

1.5.5.4. Extracellular Matrix

It has been demonstrated that the extracellular matrix proteins may be involved in the adhesion of *H. pylori* in the gastric epithelium (39, 99, 177). The binding to these ligands has been shown to be saturatable and, in some cases, at very high affinity (39). In the case of *H. pylori* adhesion to collagen and laminin it was demonstrated that this binding was non-competitive between these two ligands (165). Little else is conclusively known about the *H. pylori* adhesion to collagen, whereas there has been significant interest in the adhesion to laminin. It was demonstrated that laminin was bound by *H. pylori* via a process that involved a lectin-like protein and lipopolysaccharide (169, 170). A recent study by van Putten *et al.* (172), described above, indicated that gonococci required both fibronectin as well as heparan sulfate to interact with epithelial cell membranes via the integrin molecules. Further evidence is required to determine whether or not a similar adhesion complex is necessary in the adhesive process of *H. pylori* to the gastric epithelia cell membrane.

1.5.5.5. Lipids

Phosphatidylethanolamine (PE) was one of the first cell membrane receptors to be identified for *H. pylori* (97, 142). A 63 kDa protein was identified to be responsible for the binding of *H. pylori* to PE . The binding could be demonstrated to occur if the lipids were extracted from the cell membranes of human gastric epithelia or from other sources

(e.g. porcine erythrocytes) (97). There are still many questions to be answered about this mechanism of adhesion, as the protein identified as the adhesin also reacts with antisera against the *Pseudomonas aeruiginosa* exoenzyme S, and N terminal sequencing identified the protein as the *H. pylori* catalase protein (96). The ubiquitous expression of PE in many cell types does not explain the tissue tropism that is exhibited by *H. pylori* and if indeed this receptor is utilized *in vivo*, it may be a secondary adhesin.

1.5.5.6. Fucose-containing antigens

Tissue culture cell lines are not the ideal candidates for binding experiments as they are artificial systems and some cell types used (e.g. HEp-2) (45) are not of gastric origin. Use of gastric epithelial sections represent a more natural system which could lead to the identification of the true epithelial cell receptor (21). The idea that *H. pylori* binds to fucosylated blood group carbohydrate molecules expressed on the gastric epithelial cell surface was an attractive hypothesis (21, 48). Some investigators used these data as a way to rationalize that people of certain blood group types were more susceptible to infection by *H. pylori* due to the expression of certain Lewis antigens on the gastric epithelium (87). The gene, *babA2*, encoding the 75 kDa BabA protein was identified and isolated on the basis of Le^b binding (74). Interestingly, *babA2* has two other homologues within the genome, *babA1* and *babB* (74). The difference between the *babA* alleles is the deletion of a 10 base pair region in the 5' region of the gene which eliminated the start codon (74). The other homologue is a non-coding region which may provide genetic material for the alteration of the BabA protein expression by way of homologous recombination. A phase-variation mechanism was proposed, but not directly demonstrated, based upon the homologous recombination which would allow activation or inactivation of genes products (74). Boren *et al.* also noted that the adhesion to Le^b and the H Type I antigen (Figure 1.3) was not a universal phenotype among the *H. pylori* isolates, with only 66% of isolates demonstrating this binding (74). This Lewis binding has also been demonstrated

to be growth-phase dependent as only cultures in late logarithmic phase growth bound to the fucosylated antigens (176).

Other studies on the fucose binding capabilities of *H. pylori* indicated that Le^b is not the only fucosylated carbohydrate that *H. pylori* can adhere to. A study by Alkout *et al.* (2) has indicated that a smaller protein (61 kDa) also bound the Le^b antigen, but in contrast to Boren (21) and Ilver (74) this protein could also mediate *H. pylori* binding to Le^a and the H Type II antigen (for structures see Figure 1.3). The differences in binding specificity were determined by the use of flow cytometry to assess the binding of *H. pylori*, as well as the use of different antibodies (2).

Another study of fucose-containing receptors implicates a 16 kDa protein which can adhere to fucosylated carbohydrates, the Lewis antigens included, but this protein most avidly adhered to sulfated carbohydrates (125). This protein was identified as NapA (125). The NapA protein is homologous to a bacterioferritin-type protein and appears to be present in all isolates tested but its exact role remains unknown (125). It is hypothesized that the potential to adhere to other carbohydrate antigens, such as Le^x, would allow the activation of neutrophils via crosslinking of the surface exposed Le^x (125). One other theory is that the NapA protein may be responsible for the cross-linking of *H. pylori* Le^x contained in the LPS of some isolates to the Le^x on the gastric epithelium. Indirect evidence for a similar mechanism is presented in the study by Taylor *et al.* (160) which demonstrated, by electron microscopy, that the Le^x antigens were present at the site of interaction (adhesion pedestals) between the *H. pylori* and the gastric epithelium. It appears that some *H. pylori* cells are able to take advantage of adhesion to the fucosylated antigens on the gastric epithelial cell surface, but these mechanisms are not ubiquitous among the *H. pylori* isolates studied.

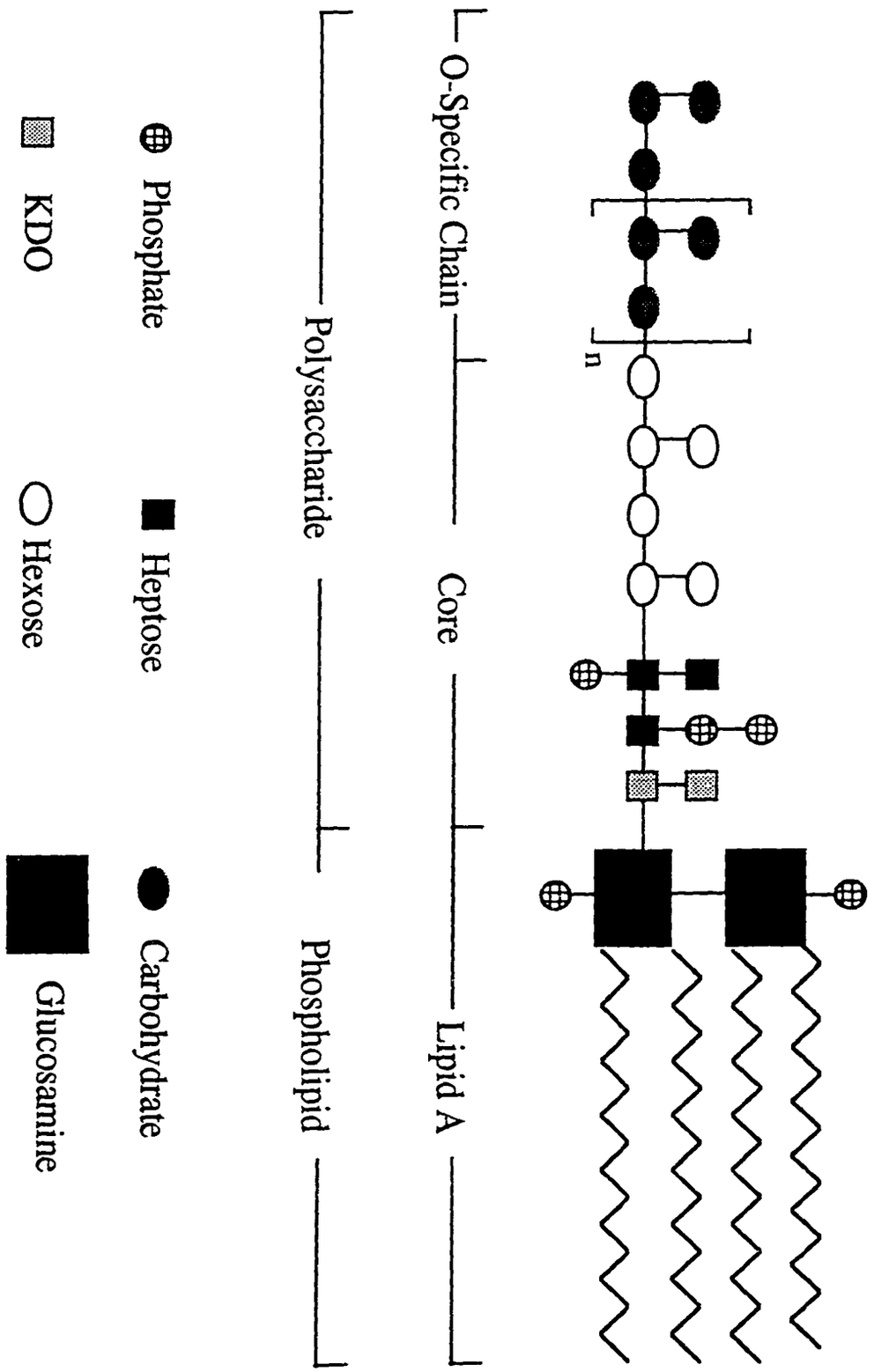
The significance of the binding to fucosylated antigens, in particular Lewis antigens, is that these antigens are expressed on the gastric epithelium. If the bacterium uses these antigens to adhere, then the individual's blood type may be an appropriate

predictor of *H. pylori* infectability. The studies discussed above have been used to rationalize other population studies which indicate that a certain blood group is more prone to *H. pylori* infection (87). Clyne and Drumm (27) demonstrated that the binding of *H. pylori* to primary gastric tissue was not affected by the gastric tissue Lewis antigen expression nor was the binding inhibited by the preincubation with anti-Lewis antigen antibodies. If the adhesion to fucose containing antigens was the primary method of *H. pylori* adhesion we would observe a distribution of infection based upon blood type (87). The proteins for adhesion to these antigens are not present in all *H. pylori* isolates (74) and the correlation between infection and blood type is weak. Therefore, it is evident that although some isolates may use these antigens as receptors, they are not the primary means of adhesion for *H. pylori.*

1.6. Lipopolysaccharide

Lipolysaccharide is an amphipathic molecule that is composed of three parts, lipid A, core oligosaccharides and O side chain saccharides (Figure 1.2) (139). The lipid A, which inserts into the membrane, is composed of fatty acids in various stages of saturation depending on the bacterial species and is usually attached to diglucosamine units (139). The lipid A has been demonstrated to be the portion of the LPS which mediates eukaryotic cell toxicity (139). Attached to the lipid A are the core oligosaccharides that are highly conserved portions of the LPS molecules within bacterial species; *E. coli* has been shown to express five core types based on carbohydrate composition (10), whereas *Salmonella* species express only two (128). *H. pylori* has only one core region identified to date (116). The O side chain is attached to the core oligosaccharides and is the highly variable portion of the LPS molecule (139). The variability of the O side chain is the basis for serotyping systems that have been developed for many organisms, as *E.coli* can express greater than 170 different O side chains (72), and *Salmonella* has greater than 2000 serotypes (128).

Figure 1.2. Diagram of Lipopolysaccharide. Three main sections of the LP molecule, lipid A, core saccharides and O side chain. The lipid A consists of the fatty acid portions as well as conserved glucosamine and phosphates. The core oligosaccharides are highly conserved within a species. The O side chain is composed of variable saccharides which determine the strain serotype or glycotype.



1.6.1. *H. pylori* Lipopolysaccharide

The lipopolysaccharide (LPS) of *H. pylori* is one of the most intensely studied molecules produced by this organism but many questions still remain about its synthesis and assembly. The LPS of *H. pylori* is unique in a number of ways. Firstly, it contains structures identical to complex human carbohydrates as well as other carbohydrates within the O side chain (116), which may or may not play a role in the direct pathogenesis of this organism. Secondly, *H. pylori* LPS has a thousand-fold decrease in toxicity when compared to the LPS of other gram-negative organisms (122). Third, the O side chain units can be produced in various states of fucosylation (116), which is not observed among the LPS O side chain of other gram-negative organisms (187). *H. pylori* LPSs have been grouped together as glycotypes based on data derived from detailed chemical analysis rather than serology (116). Eight glycotype families have been identified in *H. pylori* (116) (Table 1.3).

An early discovery about the *H. pylori* LPS was that it contained the human histo-blood group antigens (12). A discussion of the distributions of the Lewis antigens among *H. pylori* populations is provided later in this chapter (section 1.6.3). The presence of the Lewis antigens as a bacterial factor was thought to be for immune evasion via molecular mimicry (9, 120). This idea was supported by the fact that people infected with Lewis antigen containing *H. pylori* appeared to have antibodies which would react to the Lewis antigens of the LPS as well as the gastric epithelium (49, 126, 148). Although never directly demonstrated, it was assumed that the autoreactive antibodies were reacting to the Lewis blood group antigens (7). Direct investigations demonstrated that there was a high prevalence of anti-*H. pylori* LPS antibodies produced during infection, but this serum did not react with synthetic Lewis antigens (7). It was further demonstrated that the addition of human sera from infected patients decreased the interaction between the anti-Lewis monoclonal antibodies and the Lewis antigen containing *H. pylori* LPS (9). The assumption at the time was that the Lewis reactive antibodies were present but could not

Table 1.3. Lewis Antigens Expressed by *H. pylori*

Glycotype Family	O Chain	Terminus	Strain	DD-Heptan Core	LPS
A	Le^x	Le^x	NCTC11637	-	Smooth
	Le^x	Le^x	UA1182	-	
	Le^x	Sialyl-Le^x	P466	-	
B	Le^x	Le^x or Le^y	O:3	+	Smooth
C	-	Le^y	O:2	+	Smooth
D	Glc-[LacNAc]$_n$	Glc-[LacNAc]$_n$	UA861	-	Smooth
E	Gal-[LacNAc]$_n$	Gal-[LacNAc]$_n$	471	-	Smooth
F	Type I and Type II	Le^a, Type I Chain or Le^x, Type II Chain	UA948	-	Smooth
		Lewis dissacharide	UA955	-	
G	-	Le^x or Le^y or Linear B blood group or Lewis dissachride	26695	-	Semi-rough
H	-	Le^a, Le^b or H Type I	UA1111	-	Semi-rough

recognize the monomeric synthetic Lewis antigens used to determine if the sera contained reactive antibodies (9). It has since been demonstrated that the Lewis antigens are not the primary target of the human immune response but other portions of the LPS are (4, 25, 198, 199). These findings may also explain why there is a decrease in the reactivity of the anti-Lewis monoclonal antibodies to the *H. pylori* LPS when human sera was also added. The human sera may contain antibodies that recognize portions of the Lewis antigen structures. This reactivity would prevent the anti-Lewis antigen MAbs from reacting with the *H. pylori* LPS.

It has been suggested that the anti-*H. pylori* LPS antibodies produced during human infection may react to the core oligosaccharides and much of the recent data supports this hypothesis (198, 199). Mills *et al.* demonstrated that the LPS of *H. pylori* was not as antigenically reactive as the LPS from other organisms (111) and further work has suggested that there are actually three groups of *H. pylori* based upon the LPS reactivity with human sera (198, 200). The three groups consist *H. pylori* isolates that produce smooth LPS with a highly antigenic epitope, smooth LPS with a weakly antigenic epitope, or rough LPS that contains no O side chain (198). It was also demonstrated that the human immune response to the *H. pylori* LPS differed among patients with approximately 50% of patients whose sera reacted with the highly immunogenic epitope, only 10% whose sera reacted exclusively with the weakly antigenic epitope and 40% whose sera that reacted with both antigens (198). None of the reactivity of the aforementioned serum to either the strong or weak epitope could be directly correlated to the Lewis antigens contained within the LPS (198). A correlation between the reactivity of the LPS and tumor formation has been established as the *H. pylori* from patients with tumors consistently contained smooth LPS with lower antigenicity than the LPS from *H. pylori* obtained from individuals with gastritis or ulcers (200). This may be due to the inverse correlation between the decrease in the reactivity or pathogenicity of the *H. pylori* LPS with long-term carriage of the organism. It is necessary to identify the differences in

the epitopes which elicit the strong and weak reactivity as it has now been conclusively demonstrated that the LPS of *H. pylori* does elicit an immune response but the exact carbohydrate epitope remains undefined.

The lipid A portion of the *H. pylori* LPS has been demonstrated to be not as toxic and to not elicit the same immune response as the lipid A from other gram-negative pathogens (17, 122). It was demonstrated that the *H. pylori* LPS lipid A portion had a number of structural differences, when compared to the lipid A moieties of other gram-negative organisms, which are thought to be responsible for this decrease in biological activity (59, 118, 119). One difference noted was that the fatty acids of *H. pylori* LPS that extend into the outer membrane were longer than those of other gram-negative LPS (119). It had been previously demonstrated that LPS containing longer fatty acid chains have a reduced biological activity (91). Also, most gram negative bacterial LPS have two phosphate moities as part of the lipid A molecule, whereas the *H. pylori* lipid A has only a single phosphate unit (119). This difference has been demonstrated to decrease the biological activity of the LPS from other organisms (129). These differences of the *H. pylori* LPS lipid A portion are cited as major contributing factors to the decrease in the biological activity (119, 129). Of the *H. pylori* LPS analysed to date, it appears that all contain the elements that may be responsible for the decrease in biological activity.

1.6.2. LPS O Side Chain Assembly

The assembly of the LPS O side chain in other Gram-negative organisms typically follows one of three pathways (188). The three pathways involved are termed Wzy-dependent, ABC transporter dependent and synthase dependent. Important aspects to O side chain assembly are the location of O side chain polymerization, orientation of O side chain addition and protein requirement (187, 188).

Wzy-dependent assembly is the "classical" pathway of O side chain polymerization (188) and the O antigens produced by this process contain heteropolymers as well as branched structures. This pathway utilizes O side chain repeat units that are coupled to

carrier molecules (undecaprenol pyrophosphoryl linked [und-PP]). The und-PP coupled O side chain units are assembled by glycosyltransferases on the cytoplasmic face of the plasma membrane and the each is transported to the periplasmic membrane face via the Wzx protein (98), where they are polymerized to the reducing end of the growing LPS chain by the Wzy protein (187, 188). Wzy homologues have been identified in other gram-negative organisms (37, 121). This system is used by many gram-negative pathogens to produce the LPS O side chain.

The ABC transporter-dependent biosynthetic pathway is characterized by the synthesis of linear homopolymers and does not utilize the polymerase, Wzy (187, 188). In this system the O side chain is completely assembled on the cytoplasmic face of the plasma membrane and then transported across the membrane via an ABC transporter to be ligated to the lipid A and core oligosaccharides (187). The presence of this transporter precludes the requirement for the Wzx protein (187). Polymerization of this O side chain is initiated by a primer of GlcNAc linked to und-PP. Because only the completed O side chain is transported across the membrane, only a single und-PP molecule is used per O side chain (187). Sequential addition of the carbohydrate groups to the non-reducing end of the growing O side chain is determined by the specificity of the glycosyltransferase enzymes that are present during the synthesis (188).

A third method of O side chain synthesis has been described, which has only been observed in *Salmonella enterica* serovar Borreze, named synthase-dependent O side chain synthesis (85). The synthesis of this organism's O side chain does not employ a specific ABC transporter or a Wzx protein for the transport of the O side chain across the inner membrane, but uses a single enzyme, WbbF, that is responsible for the transport as well as transferase functions (85). This pathway similar to the Wzy independent pathway utilizes the GlcNAc-und-PP primer upon which the WbbF enzyme sequentially adds glycosyl groups. WbbF then transports the completed O antigen to the periplasmic face of

the membrane (85). Other polymers that are created in a similar manner by synthases with dual functions are chitin and cellulose (188).

The ligation of the O side chain polymer to the Lipid A and core oligosaccharides appears to be conserved irrespective of the synthetic pathway for the O side chain. It has become evident that the O side chain in some cases is exported to the bacterial surface without the ligation to the lipid A and becomes a capsular material (188, 189). There are also a number of genes identified, specific for each assembly process, which play a role in the determination of the length of the O side chain polymer (187, 188). The exact mechanism by which these gene products control the LPS O side chain distribution is unclear (188).

From the chemical characterization of the LPS from many *H. pylori* it has become evident that the production of the O side chain does not really conform to any of the above described pathways (116) and *H. pylori* homologues for many of the genes mentioned above have not been identified. The production of incomplete O side chain units which are transported to the bacterial surface is not common among other gram-negative organisms (144, 187, 190) and is contrary to the specific transporter and polymerase functions required in the Wzy-dependent synthesis pathway. The expression of a repeating heteropolysaccharide unit prevents the ABC transporter-dependent or synthase-dependent pathways from being a viable option for *H. pylori* LPS biosynthesis.

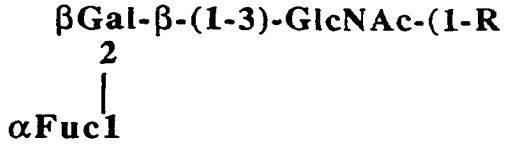
It has been noted that Neisseria species, *N. gonorrhoeae* and *N. meningitidis*, can express a variable O side chain, but this O side chain is not typical of most organisms (173). The Neisseria O side chain is short and does not contain the repeating units typical of most gram-negative organisms (173, 187). The *H. pylori* O side chain in most cases exhibits an extended heteropolysaccharide O side chain containing repeated O side chain units with differing degrees of fucosylation corresponding to the different Lewis antigenic structures (Figure 1.3) (116). The identification of the *H. pylori* LPS O side chain polymerization and assembly process may lead to the designation of a fourth assembly

Figure 1.3. Lewis Antigens. The Lewis antigens are divided into two groups based on the difference of the linkage of the backbone type I antigens have a 1-3 linkage whereas type II antigens have a 1-4 linkage. The different antigens are produced by different degrees of fucosylation.

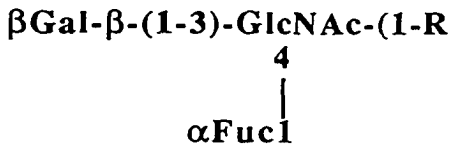
Type I Series

βGal-β-(1-3)-GlcNAc-(1-R

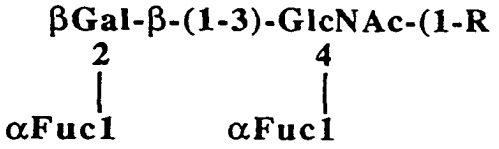
Type I Chain Precursor (Lewis C)



H Type I (Lewis D)



Lewis A

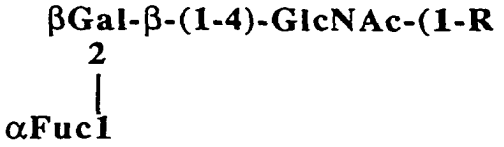


Lewis B

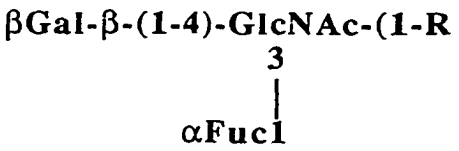
Type II Series

βGal-β-(1-4)-GlcNAc-(1-R

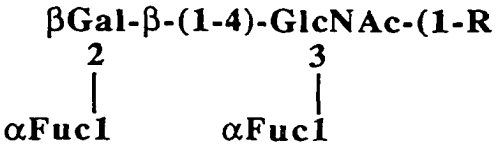
Type II Chain Precursor (LacNAc)



H Type II



Lewis X



Lewis Y

process.

It is difficult to comment on the biosynthetic pathways of the O side chains in *H. pylori* as the number of genes identified that may play a role in the assembly of the LPS is limited (3, 16, 163). One of the most likely reasons that a low number of genes required for the synthesis of the LPS have been identified is that the *H. pylori* LPS biosynthetic genes are not arranged into an operon like many other gram-negative organisms (10). Overall, less than ~25% of the genes required for the assembly of the *H. pylori* LPS have been identified, and a number of genes have been ascribed functions based upon genetic homology when no functional data exists (16). Much of what we do know and/or assume about the synthesis of the *H. pylori* LPS molecule comes from the comparison of the chemically-defined structures that have been obtained (116) as well as the few enzymes that have been cloned and characterized (180). A total of 41 genes for polysaccharide synthesis have been identified in the *H. pylori* genomes (38). The majority of the identified genes are scattered around the genome and only a few are contained within operons. There are no operons identified that are larger than three genes responsible for any aspect of *H. pylori* LPS biosynthesis (38). Fifteen of these 41 genes (~37%) have been labeled as "Lipopolysaccharide biosynthesis protein" which, according to the authors of that work, means that these proteins are involved in LPS biosynthesis but the enzyme specificity could not be determined from the nucleotide sequence homology (38). Genes that encode most of the reactions that are necessary for the production of the LPS are not identified in either of the genome projects or by any other research group. Functional determination of predicted LPS biosynthetic genes will allow for the formulation of a more complete view of the *H. pylori* LPS biosynthetic pathway.

1.6.3. Lewis antigens

The Lewis antigens are complex fucose containing carbohydrates that are used as precursor molecules in the human system for the production of the ABO blood group antigens (67, 185) (Figure 1.3). The production of the Lewis antigens in humans is

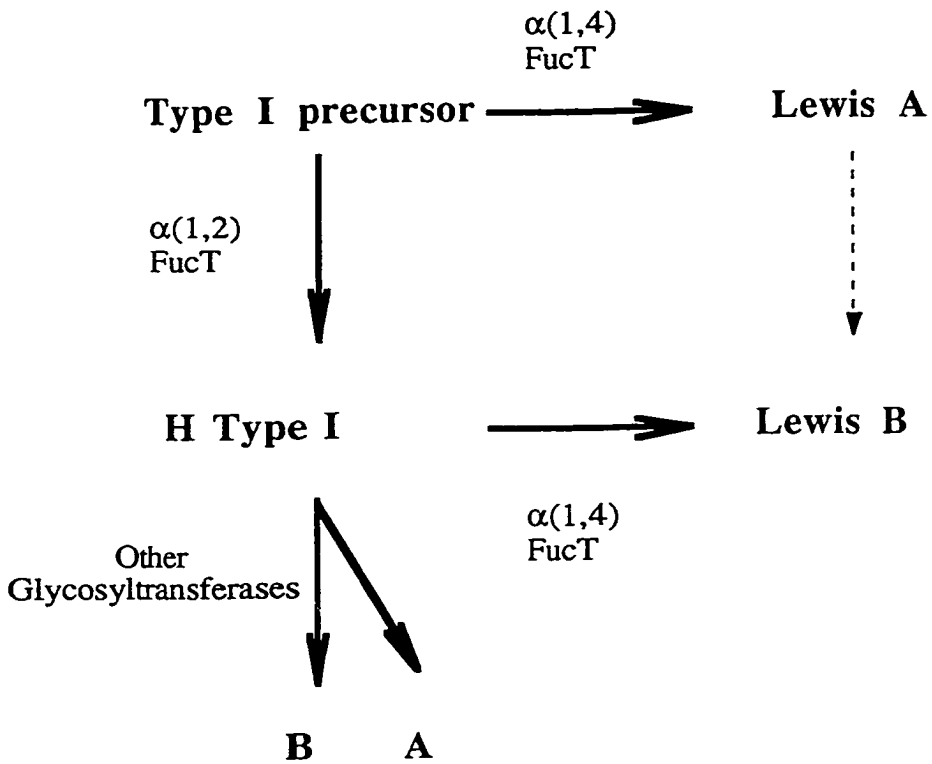
accomplished by a series of sequential carbohydrate additions that are completed by the fucosylation of the structures (67, 185). Each antigen has a tightly regulated expression which is specific and characteristic of certain tissues (185). Some of the Lewis antigens (Le^x) are regulated in their expression based upon stage of development not only location within the body (44, 185). There are multiple roles proposed for the Lewis antigens within the human body. They are believed to be used in adhesion during early development of the blastula (44) and they are also involved with the adhesion of immune cells during a localized immune response by acting as an adhesin (CD15) (100). They also prevent blood donation among individuals who are not compatible (44). The gastric epithelium has a distribution of the Lewis antigens that is distinct from other tissue types with Le^x and Le^y being expressed predominantly in the deep glands, whereas Le^a and Le^b are expressed more on the foveolar surface (28, 88, 160). While the level of expression of Lewis antigens may be different depending on the tissue type, the enzymes that are involved in creating these antigens are similar in all cases.

The most extensively studied system of Lewis antigen synthesis is the case of the histo-blood group antigens which coat the red blood cells providing people with their ABO blood type (67, 185). There are two types of complex carbohydrate molecules included in the histo-blood groups antigens the type I and type II antigens. The type I antigens consists of H Type I, Le^a and Le^b, whereas the type II antigens are H Type II, Le^x and Le^y. The main difference between the two groups of antigens is the linkage of the galactose to the *N*-acetylglucosamine which is a 1,3 linkage in type I antigens and 1,4 in type II antigens (Figure 1.3).

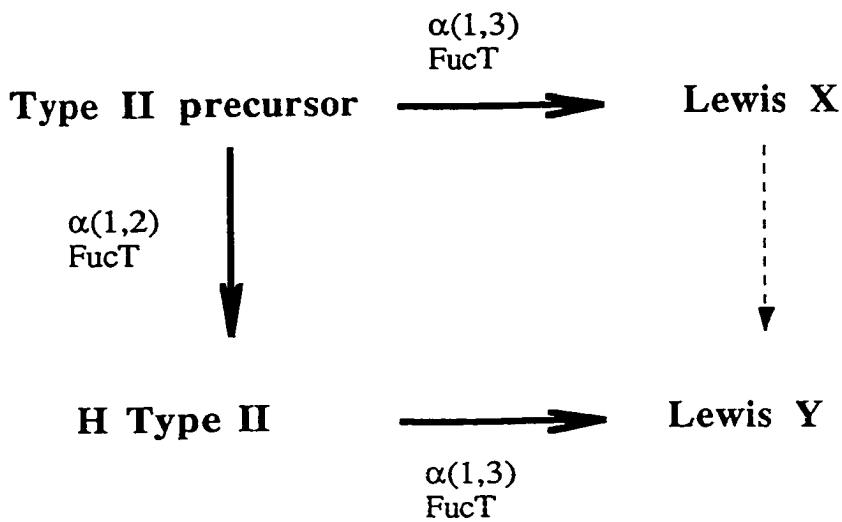
A similar pathway is utlized by human cells in the production of all of these antigens (67). Human cells make the both Le^a and Le^b antigens starting with the type I core precursor molecule (Lewis C), the Le^a antigen is produced by a fucosylation of the core by an $\alpha(1,4)$ fucosyltransferase (FucT) (Figure 1.4). The Le^a antigen in humans is not further fucosylated to produce the Le^b antigen under normal conditions (67, 185).

Figure 1.4. Human Lewis antigen synthesis. **A.** Human synthesis of the type I antigens proceeds via a fucosylation of the precursor by an α(1,4) FucT to produce the Le^a antigen. This antigen is not further fucosylated under normal circumstances. The synthesis all other type I antigens starts with the action of a an α(1,2) FucT on the Type I precursor to produce the H Type II antigen. The H Type I antigen is further fucosylated by an α(1,4) FucT to produce the Le^b antigen or glycosylated by other antigens to produce the blood group antigens A and B. **B.** Human synthesis of the type II antigens starts with the Type II precursor (also known as LacNAc) by an α(1,3) FucT to produce the Le^x antigen, or alternatively by the by an α(1,2) FucT to produce the H Type II antigen which is then further fucosylated to produce the Le^y antigen.

A.



B.



Alternatively, the Le^b antigen is produced by a terminal fucosylation of the precursor by an α(1,2) FucT to produce the H Type I antigen which is then further fucosylated at the subterminal position by the α(1,4) FucT to produce the Le^b antigen (Figure 1.4). The Le^b antigen is further processed by a galactosyltransferase to produce the B blood group antigen, or by an *N*-acetylgalactosamine transferase to produce the A blood group antigen (Figure 1.4) (67, 185). Similarly, to produce the Le^x antigen, the type II precursor chain (also known as lactosamine or LacNAc) is fucosylated at the 3 position by an α(1,3) FucT, because the 4 position of the GlcNAc is already occupied by the galactose moiety. This antigen is also not further fucosylated to create the Le^y antigen under normal conditions. The synthesis of the Le^y antigen proceeds via a terminal fucosylation of LacNAc by an α(1,2) FucT to produce the H Type II antigen which is internally fucosylated by an α(1,3) FucT to produce the Le^y antigen (Figure 1.4) (67, 185). Unlike the type I antigens, there does not appear to be any further processing of the type II antigens in humans to create blood group-like antigens.

1.6.4. *H. pylori* Lewis Antigen Distributions

The Le^x antigen was the first Lewis antigen to be identified as a component of the LPS of *H. pylori* by Aspinal *et al.* (12). A number of groups have investigated the expression of these antigens by *H. pylori* isolated from different patient groups (111, 152, 191-193) (Table 1.3). The LPS of the *H. pylori* isolates studied has been shown to contain one or more of the Lewis antigenic structures and, more recently, other non-Lewis antigens have been identified (191-193). It was determined that the Le^x and Le^y antigens were expressed by greater than 80% of the *H. pylori* isolates (152, 192, 193). It was unknown why these isolates expressed these antigens specifically and not the other Lewis antigens. An interesting hypothesis has recently been presented in which the expression of Le^x was correlated to the increased prevalence of ulcers and inflammation (66, 104). Another recent study included within this thesis (Chapters 2 and 3) has also arrived at the same conclusion, although the expression of Le^x was decreased in *H. pylori* isolates that

were obtained from asymptomatic individuals. It is prudent to note here that the study of *H. pylori* as an organism has been limited to the isolates that are obtained from people who present with disease symptoms, but most *H. pylori* infections do not result in an infection that requires medical intervention (41). Thus it is noted here that the *H. pylori* populations studied to date are not necessarily representative of the *H. pylori* that infect all people. The investigation of the *H. pylori* from asymptomatic individuals may allow the determination of the factors responsible for the development of disease states.

The expression of the type I Lewis antigens, Le^a and Le^b, in *H. pylori* LPS has been reported, but the expression is extremely limited (152, 193). It is unclear why the distributions of type I antigens is limited while expression of the type II antigens is widespread, as they are very similar antigens (Figure 1.3) A recent paper in press by Monteiro *et al.* (117) has discovered that there was a geographic distribution of the expression of the type I antigens, as *H. pylori* isolated from patients of Asian descent have a high prevalence of these antigens. There had not been the identification of any geographic distribution of the Lewis antigen expression among the previous studies (152, 192, 193). Identification of the role of the Lewis antigens during infection may come from the studying the expression the Lewis antigens by *H. pylori* isolated from asymptomatic subjects or other ethnic groups such as the patients from Asian countries who demonstrate a high level of gastric cancers.

It has recently become evident that the *in vitro* measurement of the phase variation rate may not be the same as the phase variation rate of *H. pylori* that have been recently isolated from gastric biopsy samples (60, 191) (Chapter 3). The mechanism of Lewis antigen phase variation is thought to be slip strand mispairing at the homopolymeric nucleotide repeats that are contained in the fucosyltransferase genes (3, 5, 163, 182), but this mechanism does not seem to account for the degree of variation that is observed in *H. pylori* cultured from gastric biopsy samples. There may be some type of environmental regulation of the Lewis antigen and possibly other LPS components *in vivo* that are not

duplicated *in vitro*. The continued passage of *H. pylori* on agar plates leads to a decreased expression of the Lewis antigen containing O side chain (178), whereas it appears as though the passage of *H. pylori* through a human (60, 191) or animal (78) increases the variability of the LPS O side chain profile. This indicates that *H. pylori* grown *in vitro* do not require the expression of the Lewis antigens or an O side chain whereas *in vivo* the continued variation may be a survival strategy that is also similarly exploited by other organisms (76, 173, 106, 141). The regulation of the biosynthetic enzymes and other factors may be a reason for the observed variability of the Lewis antigens. The expression of the Lewis antigenic structures has been correlated to the expression of other virulence factors such as CagA (192), which in turn can be correlated to the expression of VacA (14) and ultimately disease. The correlation of the expression of any Lewis antigen with any other virulence factor is suspect due to the apparent variability of these antigens *in vivo* (see section 1.4 and 1.5.2).

1.6.5. Roles of Lewis Antigens in *H. pylori* LPS

The most attractive hypothesis for the role of the Lewis antigen was that it was acting as a molecular mimicry of the Lewis antigens that are expressed on the gastric epithelium (9, 120). It appeared as though this was the case since a number of studies identified the reactivity of patient sera to LPS molecules. The LPS of *H. pylori* does elicit an human immune response but it is not directed against the Lewis antigens (198, 199). This epitope of the LPS that does attract immune attention has not yet been identified. The interpretation of the development of antibodies that react against the human gastric epithelia has been a major area of contention within *H. pylori* research (9).

The development of the serological response to *H. pylori* has been demonstrated to be towards multiple antigens, most notably the LPS (49, 126), but also CagA and VacA (198). There are some patients who elicit an immune response to the Lewis antigens during *H. pylori* infection, but these individuals are rare (7). The components of the gastric mucosa, such as mucin and the H^+K^+ATPase that also express complex

carbohydrates, including the Lewis antigens, were focused on as possible targets for cross-reactive epitopes (7). It was demonstrated that the H^+K^+ ATPase of human parietal cells was heavily glycosylated and expressed the Le^y epitope (6, 49). It was first assumed that the Lewis antigens of *H. pylori* induced the host response that reacted to these epitopes, whereas more recent data indicated that the anti-H^+K^+ATPase pump antibodies react to the protein components and not the carbohydrates (i.e. Lewis antigens) (25). Further investigation into the role of LPS and Lewis antigens in the development of auto-antibodies is necessary.

1.7. Fucosyltransferases

The fucosyltransferases are a group of enzymes that are present among many prokaryote (*H. pylori* and *Vibrio cholerae*) and eukaryote (*Homo sapiens* and *Rattus*) species (23, 29, 130). At of the time of writing there are 78 identified fucosyltransferases (FucTs) (30, 130). The fucosyltransferases are responsible for the transfer of fucose, usually from GDP-fucose to an acceptor molecule, creating a specific linkage. The FucTs can be characterized into three broad groups based on their enzyme specificity; α(1,2), α(1,3) and α(1,6) (130). Each linkage is specific to the group of enzymes and there are no known FucT enzymes that can catalyze the addition of fucose across these designations (130). In the α(1,3) FucT group, there are enzymes which can add fucose in both α(1,3) and α(1,4) linkages (140, 186). These enzymes are grouped together as they contain the similar motifs which are thought to be involved with the FucT activity (22, 23, 130). The α(1,6) FucT enzymes are not very widely distributed and will not be discussed in this work.

There are eight fucosyltransferases that are responsible for the production of all of the Lewis antigens within the human body (130). The differential expression of the antigens is based upon which enzymes are active in that particular cell. *FUT1* and *FUT2* encode the H and Se enzymes respectively and are responsible for the α(1,2) FucT activity (130). The specificity of these enzymes allows for the production of either Le^b or Le^y, as

well as H Type I or H Type II (section 1.6.3). There is a single human FucT which encodes an α(1,6) FucT enzyme (130). Three of the remaining *FUT* genes (*FUT4, 6* and *7*) encode α(1,3) FucTs and one gene (*FUT5*) has been definitively identified as encoding an enzyme with both α(1,3) FucT and α(1,4) FucT activity (186), while the final *FUT* gene, *FUT3,* expresses only α(1,4) FucT activity (54). The expression of the α(1,4) FucT activity allows production of the Le^a antigen, whereas the α(1,3) FucT activity is required for the synthesis of Le^x (section 1.6.3). The interest in the human blood antigens has lead to the identification of naturally occurring mutants which have guided research to identify which regions of the proteins are essential for activity (30, 127, 149, 175, 195).

A number of higher organisms as well as bacteria possess FucTs. Most create specific carbohydrate structures which are unique to that organism, although there are some examples where two species of FucT enzymes are responsible for the production of the same carbohydrate antigen. This is the case with the human and *H. pylori* FucTs (6, 12). The Lewis antigen synthesis by human enzymes was described in section 1.6.3 of this work and it was assumed that when they were first discovered the *H. pylori* antigens would be synthesized in the same manner. Only recently has this idea been challenged. The first *H. pylori* α(1,3) *fucT* was cloned simultaneously by Martin *et al.* (105) and Ge *et al.* (57). The enzyme activities from these works implied that the *H. pylori* Lewis antigens were synthesized in a similar manner to the human enzymes, as the *H. pylori* FucT enzyme could not utilize the H Type II antigen as an acceptor. Upon sequencing of the *H. pylori* 26695 genome it was discovered that there were two α(1,3) *fucT* genes present (163), but no α(1,2) *fucT* gene was identified. It was known that an α(1,2) *fucT* gene must be present because 26695 expressed Le^y, which required this enzyme to produce this structure (182). Further discussion of each enzyme's capabilities and genetic structure is included below.

1.7.1. *H. pylori* α(1,3(4)) Fucosyltransferases

It was known that *H. pylori* could express the range of Lewis antigens (Le^a, Le^b, Le^x and Le^y) and therefore enzymes for the synthesis must be produced. The identification of an α(1,3) FucT by Martin *et al.* (105) led to the further investigation of isolates that expressed Le^a to determine if, as in the human system, mutation of an existing gene was responsible for the broadening of the enzyme acceptor range. Identification of a *fucT* gene homologue encoding a single gene product that contained both enzyme activities is described in this work (Chapter 4). The identification of an *H. pylori* enzyme that contains both α(1,3) and α(1,4) FucT activity has led to difficulty with the nomenclature for this group of enzymes. In this work α(1,3/4) FucT will refer to the enzyme that contains both α(1,3) and α(1,4) FucT activity, whereas α(1,3(4)) *fucTs*/FucTs will refer to the group of genes or enzymes that may contain both α(1,3) and α(1,4) enzyme functions. No *H. pylori* enzyme has been identified which contains only α(1,4) FucT activity.

Comparison of the DNA and predicted amino acid sequences of all of the cloned α(1,3(4)) *fucTs* has lead to the identification of three interesting regions within the gene products. The 5' regions of these genes exhibits the greatest variability and contain a number of nucleotide repeat regions, a conserved internal region encodes a hypothetical catalytic domain, and the 3' region encodes a series of amino acid heptad repeats with unknown function. Based upon the 5' nucleotide sequence of the ORFs there appears to be two groups of *H. pylori* α(1,3(4)) *fucTs* (180). The members of each group and their distinctive sequences are shown in Table 1.4 and Figure 1.5. Group II contains only two members J99*fucTb* and UA948*fucTa* and is characterized by smaller polynucleotide repeats, the poly-A region is the shortest observed with only three adenines, whereas the average of the group I *fucT* homologues is six. The poly-C region is also significantly shorter in the group II homologues, containing only five cytosines each, while the group I homologues have an average cytosine tract length of 10.4, with the shortest being 8 in UA802*fucT* and UA1111*fucTa*, and the longest being 13 in the homologues from 26695

Table 1.4 Polynucleotide Tracts of the *H. plyori* α(1,3(4)) *fucTs*

Homologue	Group	Adenine	Cytosine	Reference
NCTC11637*fucT*	I	6	9[b]	105
NCTC11639*fucTa*	I	6	10	57
26695*fucTa*	I	6	13	163
26695*fucTb*	I	6	13	163
J99*fucTa*	I	9[a]	13	3
UA802*fucT*	I	7	8	Z. Ge, unpublished
[Ψ]UA1111*fucTa*	I	6	8	Rasko et al., submitted
[Ψ]UA1111*fucTb*	I	6	10	Rasko et al., submitted
UA1182*fucT*	I	6	9	Z. Ge, unpublished
J99*fucTb*	II	3	5	3
[Ψ]UA948*fucTa*	II	3	5	140

[a] during annotation of the J99 genome sequence an additional adenine was included to allow the ORF to produce a protein

[b] an additional cytosine is added to allow the ORF to produce a protein.

[Ψ] These enzymes are discussed in this thesis and are included in this table for completeness

Figure 1.5. Polynucleotide repeat region of *H. pylori* α(1,3(4)) *fucT* genes. This regions consists of an adenine and cytosine repeat tract, each gene has a specific number of nucleotides to produce a functional protein. Two groups of *fucT* genes have been identified on the nucleotide homology of this region designated *fucT* group I and *fucT* group II. Regions designated A, B and C of group II highlight regions which are divergent from group I and may indicate DNA insertions.

fucT Group	Poly A Tract	Poly C Tract
I		
NCTC11637	CATTG---**AAAAAA**TGGCCTCTAAATCT**CCCCCCCCC**----TAAAAATCGCTGTGG	
NCTC11639	CATTG---**AAAAAA**TGGCCTCTAAATCT**CCCCCCCCCC**---TAAAAATCGCTGTGG	
26695*fucTa*	CATTG---**AAAAAA**TGGCCTCTAAATCT**CCCCCCCCCCCCCC**TAAAAATCGCTGTGG	
26695*fucTb*	CATTG---**AAAAAA**TGGTCTCTAAATCT**CCCCCCCCCCCCCC**TAAAAATCGCTGTGG	
J99*fucTa*	CAATT-**AAAAAAAA**TTACTTTTAAATCT**CCCCCCCCCCCCCC**TAAAAATCGCTGTGG	
UA802	CAATT--**AAAAAAA**TG-CCTCTGAGTTA**CCCCCCCC**-----TAAAAATCGCTGTGG	
UA1111*fucTa*	CATTG---**AAAAAA**TGGCCTCTAAATCT**CCCCCCCC**-----TAAAAATCGCTGTGG	
UA1111*fucTb*	CATTG---**AAAAAA**TGGCCTCTAAATCT**CCCCCCCCCC**---TAAAAATCGCTGTGG	
UA1182	CATTG---**AAAAAA**TTACCTCTAAATCT**CCCCCCCCC**----TAAAAATCGCTGTGG	

II	A	B	C
J99*fucTb*	CATTA-GATG**AAA**CCGATTATAAG**CCCCC**ATT-AAATATAGCCCTAGCCAATTGGTGG		
UA948*fucTa*	CATTTAGATG**AAA**CAACCCATAAG**CCCCC**ATTTAAATGTAGCC-TAGCCAATTGGTGG		

and J99*fucTa* (Table 1.4). In the group II homologues there appears to have been an insertion of DNA into the poly-C tract which provides divergence at both the nucleotide and amino acid level.

There are other differences with respect to these two groupings of *fucT* genes. The regions surrounding the polynucleotide repeats also show significant divergence between the two groups whereas the regions preceding and following the sequence shown in Figure 1.5 are highly homologous (Appendix 1A). Prior to the poly A region in the group II homologues, there appears to have been an insertion of 6-7 nucleotides that is not present in the group I genes (Figure 1.5A). The regions designated as B and C in Figure 1.5 show further divergent regions between the two groups. Following the poly-C regions in the group II genes there appears to be another insertion of DNA, possibly to compensate for the decrease in length of the polynucleotide regions. The role of these variable regions are not clear. This region of diversity is not directly responsible for the change in acceptor specificity as both UA1111*fucTa* and UA1111*fucTb*, group I genes, as well as UA948*fucTa* a group II gene, all contain α(1,3/4)FucT activity ((140) Chapter 5).

Appelmelk *et al.* (5) demonstrated that the variability of this polynucleotide region is responsible for some of the antigenic phase variation that is observed during *in vitro* growth. A change in the poly C length of a certain *fucT* gene created an isolate that lacked expression of the fucosylated antigen (5). Upon sequencing the *fucT* genes from that isolate, it was discovered that a deletion of a single cytosine was responsible for the loss of protein function and antigen expression by that isolate. This was the first definitive evidence of a phase variation mechanism in *H. pylori* based upon slipped strand mispairing (5). It must be noted that in the study mentioned above the phase-variation was based strictly on the poly C region of the *fucT* gene. We have found examples that demonstrate that the poly C or even poly A region is not the only region which can affect the expression of functional FucT protein (Chapter 5). J99*fucTb* contains a frameshift mutation caused by the lack of an adenine in the poly A tract, but for annotation purposes

Alm *et al.* (3) added an additional adenine to the poly A region of the gene to produce a compete ORF.

The catalytic domain of the *H. pylori* α(1,3(4)) FucT proteins resides in an internal highly conserved region (Appendix 1A and 1B). There is little divergence in this region of the 11 identified *H. pylori* FucT proteins, regardless of enzyme activity or expression level. The differences observed among the *H. pylori* enzymes are considered to be conservative substitutions. By computer analysis of eukaryotic and prokaryotic FucT proteins, two conserved catalytic domains exist in all α(1,3(4)) FucT proteins investigated including *H. pylori* FucTs (Figure 1.6) (130). Domain I is poorly conserved among the FucT proteins compared, with only two of the 19 amino acids being conserved across the species, whereas domain II has 13 of 35 conserved amino acids in this domain. Mutations in this region of the human enzymes have been demonstrated to prevent fucose transfer but not fucose binding (149). It is interesting that the region for GDP-fucose interaction is not conserved as all compared FucT proteins are hypothesized to utilize GDP-fucose as the fucose donor (29, 130).

One other conserved feature of the *H. pylori* α(1,3(4)) FucTs is the presence of a series of heptad repeats located in the carboxyl terminus of the protein (Appendix 1B and Table 1.5). These heptad repeats are variable in both the number that are present, ranging from 2-11 repeats, as well as in sequence, even within the homologues of the same isolate (Table 1.5). These heptad repeats contain a conserved leucine residue that is in the position to function as a leucine zipper (57, 105). The leucine zipper is hypothesized to mediate the dimerization between proteins that contain this motif to form homodimers (93), or heterodimers (167). These dimerized proteins usually then bind to promoter regions of the DNA and thus constitute a type of transcriptional control (93, 167). It has not been demonstrated conclusively if the leucine zippers contained within the *H. pylori* FucT proteins are responsible for dimerization or transcriptional control, but the deletion of this region does create an *H. pylori* FucT protein that is inactive as a fucosyltransferase (57).

Figure 1.6. Conserved hypothetical catalytic domain of α(1,3) FucTs. Alignment of a series of known α(1,3) FucTs from different species. The shaded boxes indicates amino acids that are conserved across the species. This region has been shown in humans, but not in other species, to be responsible for fucose transfer but not binding of the substrates.

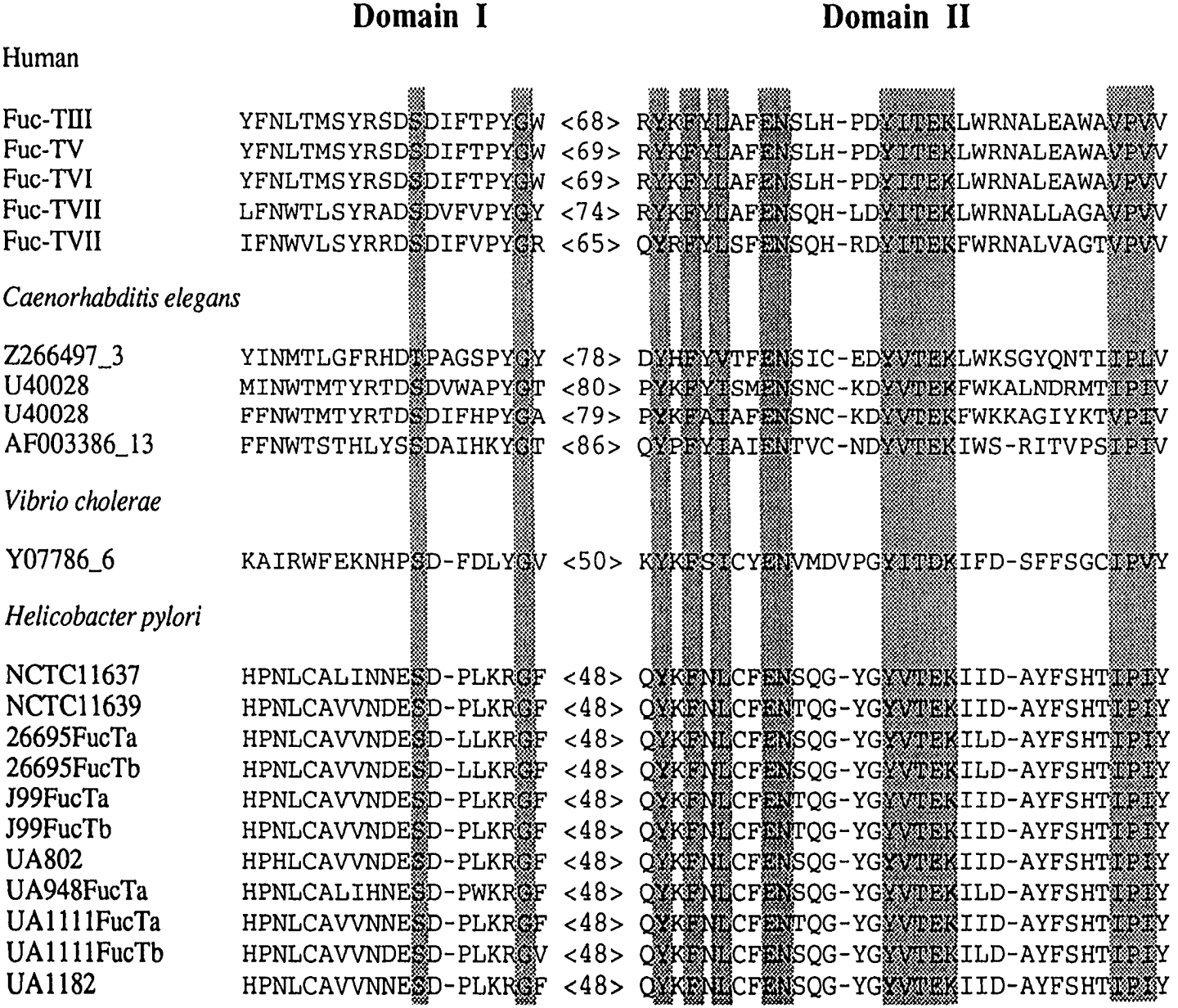


Table 1.5. Amino Acid Sequence of the Heptad Repeats of the *H. pylori* α(1,3(4)) FucTs

Homologue	Number of Repeats	Sequence of Repeat	Reference
NCTC11637FucT	7	D(D/N)LR(V/I)NY[a]	105
NCTC11639FucTa	10	DDLR(V/I)NY[b]	57
26695FucTa	2	DDLRVNY	163
26695FucTb	10	DDLRVNY	163
J99FucTa	4	DDLRVNY	3
UA802FucT	5	(D/N)(D/N)LRADY[c]	Z. Ge, unpublished
[Ψ]UA1111FucTa	4	D(D/G)LRVNY[c]	Rasko et al., submitted
[Ψ]UA1111FucTb	6	DDLRVNY	Rasko et al., submitted
UA1182FucT	11	DDLRVNY	Z. Ge, unpublished
J99FucTb	7	DDLRVNY	3
[Ψ]UA948FucTa	8	DDLR(V/R)(N/D)(Y/H/R)[d]	140

[Ψ] These enzymes were identified in this thesis and are included here for completeness

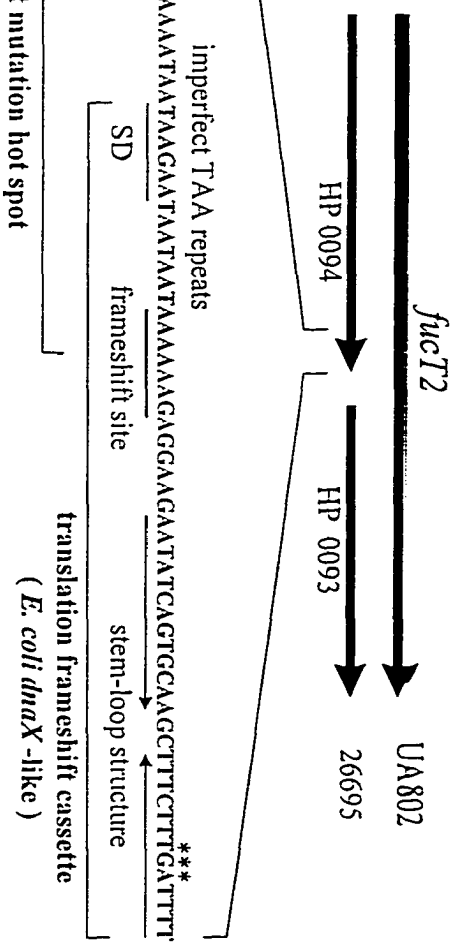
It is not clear why dimerization would be necessary for fucosyltransferase activity as no other fucosyltransferase appears to require dimerization prior to activity (30, 130). Further work is needed on this area to determine the exact role of the heptad repeats in the *H. pylori* FucTs.

1.7.2. *H. pylori* α(1,2) Fucosyltransferase

Serological as well as chemical analysis of *H. pylori* had indicated that *H. pylori* must possess an enzyme that contained α(1,2) FucT activity (152, 192). As was mentioned previously upon release of the genome sequence of 26695, no α(1,2) *fucT* gene was identified (163). It was proposed by Berg *et al.* (16) that the α(1,2) *fucT* gene was actually truncated and comprised of two smaller open reading frames. Identification of the *H. pylori* α(1,2) *fucT* gene and demonstration of α(1,2) FucT function from that gene provided the enzyme responsible for the final step in production of the Le^y and Le^b antigens (182). The *H. pylori* α(1,2) FucT was the first α(1,2) FucT of bacterial origin identified (182) and it contained an interesting genetic element, a translational frameshift cassette (TFC), that allowed production of functional protein from the two identified ORFs (182).

The identification of the translational frameshift cassette was possibly the most significant finding with respect to these genes (182) (Figure 1.7). A similar cassette is present in the production of the DNA polymerase subunits of the *E. coli*, that allows the production of the two separate polymerase subunits from the same coding sequence (53). The *H. pylori* translational frameshifting cassette is comprised of a series of imperfect TAA repeats, which contain a Shine Delgarno site, a frameshift site containing a poly A tract, and a section of DNA which can form a stem loop (see Figure 1.7) (182). The ribosomal complex is halted by the formation of a stem loop structure upon which it can shift back from the underlined codon, AAA AAA G, to the AAA AAA G codon. One reason for the occurrence of this frameshift is that the affinity for the latter codon is higher in *H. pylori*, as there is only aminoacyl tRNA for lysine, AAA, and not AAG (3, 163,

Figure 1.7. Translational frameshift cassette of *H. pylori* α(1,2) *fucT*. Comparison of UA802, containing a full length *fucT2* ORF, and 26695 which contains two ORFs (HP0093 and HP0094) with the translational frameshift cassette. The translational frameshift cassette is preceeded by a poly C tract and contains a series of imperfect TAA repeats which compose a Shine Delgarno site, followed by the frameshift site and the stem loop structure. The poly C tract and the imperfect TAA repeats are hotspots for frameshift mutations. *** denotes the stop codon contained in 26695 ORF HP0094.



182). Thus, the ribosome shifts the reading frame back by one base and continues to read in a -1 frame that no longer contains a stop codon (designated by *** in Figure 1.7) but produces functional α(1,2) FucT protein (182). It is hypothesized that the translational frameshifting cassette is active approximately 50% of the time to produce active α(1,2) FucT protein (182). In the *E. coli* situation the TFC is also active 40-50% of the time leading to two separate subunits that are essential for the activity of the DNA polymerase (53).

The sequences of many *H. pylori fucT2* genes have been determined and UA802*fucT2* has been designated as the prototypical gene. Many variations of this gene have been identified among other isolates (179). Within the *H. pylori* α(1,2) *fucT* genes there is a higher level of identity than is observed among the α(1,3(4)) *fucT* genes. The corresponding amino acids of the α(1,2) FucT proteins are greater than 95% identical, whereas the α(1,3(4)) FucTs identity is less than 75%. The translational frameshifting cassette area provides the greatest degree of variation within these proteins (179). Preceding and within the translation frameshift cassette there are polynucleotide regions as well as imperfect TAA repeat regions that allow for a high degree of variation much in the same manner as the α(1,3(4)) *fucT* polynucleotide tracts allow for the increase in the mutation rate of those proteins (5). Mutations of this gene have been noted in one or both of these regions causing a decrease in the difucosylated Lewis antigen expression of some isolates (Table 1.6) (179). What is evident from this list of mutations is that there is no one conserved mutation which causes protein inactivation. Also, because of the translational frameshift cassette the functional state of the protein cannot be predicted directly from the nucleotide sequence. Only measurement of the difucosylated antigens or heterologous enzyme expression can determine the status of these genes.

The enzyme activity of the *H. pylori* α(1,2) FucT proteins is significantly different from the human homologues (179). The human enzymes *FUT1* (H enzyme) and *FUT2* (Se enzyme) act only on the unfucosylated carbohydrate chains to produce either the H

Table 1.6. Mutations in *H.pylori fucT2* Genes

Isolate	Poly-C	Poly-A	Imperfect TAA Repeat	Status
26695	+ 2C	-	-	On
UA802	-	-	-	On
UA1174	+ 2C	+2A	-	Off
UA1182	- 1C	-	-	On
UA1207	-	-	-(TAA)	Off
UA1210	- 1C	+1A	-	Off
UA1218	+ 1C	-	- (AATA)	Off
UA1234	-	-	-(TAA)	On

type I or H type II antigens (30, 67) which are then further fucosylated to produce the other antigens. The ability of the *H. pylori* FucT2 enzymes to add fucose to both the unfucosylated as well as the monofucosylated antigens, Le^a and Le^x, allows for the production of the H antigens as well as Le^b and Le^y by the same enzyme (179). Discussion of the synthesis of the Lewis antigens by *H. pylori* is included in chapters 4-6 of this work.

1.8. Rationale for Experiments

The goal of this work was to investigate the distribution of the Lewis antigens among the *H. pylori* isolates of Alberta to determine if there were any geographic differences when compared to previous studies. The study of the Lewis antigen distribution identified *H. pylori* that expressed alternative Lewis antigens. Through cloning, expression and enzymatic analysis of the fucosyltransferase enzymes of these isolates it was hoped that a greater understanding of the *H. pylori* Lewis antigen and LPS biosynthetic pathways could be obtained.

1.9. Bibliography.

1. **Akopyants, N. S., Clifton, S. W., Kersulyte, D., Crabtree, J. E., Youree, B. E., Reece, C. A., Bukanov, N. O., Drazek, E. S., Roe, B. A. and Berg, D. E.** 1998. Analyses of the *cag* pathogenicity island of *Helicobacter pylori*. Mol. Microbiol. **28**: 37-53.

2. **Alkout, A. M., Blackwell, C. C., Weir, D. M., Poxton, I. R., Elton, R. A., Luman, W. and Palmer, K.** 1997. Isolation of a cell surface component of *Helicobacter pylori* that binds H Type 2, Lewis A, and Lewis B antigens. Gastroenterology. **112**: 1179-1187.

3. **Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F. and Trust, T. J.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. **397**: 176-180.

4. **Amano, K., Hayashi, S., Kubota, T., Fijii, N. and Yokata, S.** 1997. Reactivities of Lewis monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. Clin. Diag. Lab. Immunol. **4**: 540-544.

5. **Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M. J. E. and Kusters, J. G.** 1999. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α3-fucosyltransferase genes. Infect. Immun. **67**: 5361-5366.

6. **Appelmelk, B. J., Negrini, N., Moran, A. P. and Kuipers, E. J.** 1997. Molecular mimicry between *Helicobacter pylori* and the host. Trend. Microbiol. **5**: 70-73.

7. **Appelmelk, B. J. and Negrini, R.** 1997. *Helicobacter pylori* and gastric autoimmunity. Curr. Op. Gastroenterol. **13**: 31-34.

8. **Appelmelk, B. J., Shiberu, B., Trinks, C., Tapsi, N., Zheng, P. Y., Verboom, T., Maaskant, J., Hokke, C. H., Schiphorst, W., Blanchard, D., Simoons-Smit, I. M., van den Eijnden, D. H. and Vandenbroucke-Grauls, C.** 1998. Phase variation in *Helicobacter pylori* lipopolysaccharide. Infect. Immun. **66**: 70-76.

9. **Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinal, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A. and Degraaff, J.** 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun. **64**: 2031-2040.

10. **Amor, K., Heinrichs, D. E., Frirdich, E., Ziebell, K., Johnson, R. P. and Whitfield, C.** 2000. Distribution of core oligosaccharide types in lipopolysaccharides from *Escherichia coli*. Infect. Immun. **68**: 1116-1124.

11. **Asashi, M., Azuma, T., Ito, S., Ito, Y., Suto, H., Nagai, Y., Tsubokawa, M., Tohyama, Y., Maeda, S., Omata, M., Suzuki, T. and Sasakawa, C.** 2000. *Helicobacter pylori* CagA protein can be tyrosine phosphorylated in gastric epithelial cells. J. Exp. Med. **191**: 593-602.

12. **Aspinall, G., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1994. O antigen chains in the lipopolysaccharide of *Helicobacter pylori* NCTC11637. Carbohydr. Lett. **1**: 151-156.

13. **Atherton, J. C., Cao, P., Peek, R. M., Tummuru, M. K. R., Blaser, M. J. and Cover, T. L.** 1995. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*; association of specific *vacA* types with cytotoxin production and peptic ulceration. J. Biol. Chem. **270**: 17771-17777.

14. **Atherton, J. C., Peek, R. M., Tham, K. T., Cover, T. L. and Blaser, M. J.** 1997. Clinical and pathological importance of heterogeneity In *vacA*, the vacuolating cytotoxin gene of *Helicobacter pylori*. Gastroenterology. **112**: 92-99.

15. **Balfour, A.** 1906. A haemogregarine of mammals and some notes on trypanosomiasis in Anglo-Egyptian Sudan. J. Trop. Med. **9**: 81-92.

16. **Berg, D. E., Hoffman, P. S., Appelmelk, B. J. and Kusters, J. G.** 1997. The *Helicobacter pylori* genome:genetic factors for a long life in the gastric mucosa. Trend. Microbiol. **5**: 468-474.

17. **Birkholz, S., Knipp, U., Nietzki, C., Adamek, R. J. and Opferkuch, W.** 1993. Immunological activity of lipopolysaccharide of *Helicobacter pylori* on human peripheral mononuclear blood cells in comparison to lipopolysaccharides of other intestinal bacteria. FEMS Immunol. Med. Microbiol. **6**: 317-324.

18. **Blaser, M. J.** 1990. *Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation. J. Infect. Dis. **161**: 626-633.

19. **Blaser, M. J.** 1996. The bacteria behind ulcers. Scientific American. **274**: 104-107.

20. **Blaser, M. J. and Parsonnet, J.** 1994. Parasitism by the "slow" bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. J. Clin. Invest. **94**: 4-8.

21. **Boren, T., Falk, P., Kevin, A. R., Larson, G. and Normark, S.** 1993. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. **262**: 1892-1895.

22. **Breton, C., Oriol, R. and Imberty, A.** 1996. Sequence alignment and fold recognition of fucosyltransferases. Glycobiology. **6**: vii-xii.

23. **Breton, C., Oriol, R. and Imberty, A.** 1998. Conserved structural features in eukaryotic and prokaryotic fucosyltransferases. Glycobiology. **8**: 87-94.

24. **Censini, S., Lange, C., Xiang, Z. Y., Crabtree, J. E., Ghiara, P., Borodovsky, M., Rappuoli, R. and Covacci, A.** 1996. *cag*, a pathogenicity island of *Helicobacter pylori*, encodes Type I specific and disease associated virulence factors. Proc. Natl. Acad. Sci. USA. **93**: 14648-14653.

25. **Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R. and Kirchner, T.** 1998. The gastric H+, K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. Gastroenterology. **115**: 340-347.

26. **Clyne, M. and Drumm, B.** 1996. Cell envelope characteristics of *Helicobacter pylori*: their role in adherence to mucosal surfaces and virulence. FEMS Immunol. Med. Microbiol. **16**: 141-155.

27. **Clyne, M. and Drumm, B.** 1997. Absence of effect of Lewis A and Lewis B expression on adherence of *Helicobacter pylori* to human gastric cells. Gastroenterology. **113**: 72-80.

28. **Cordon-Cardo, C., Lloyd, K. O., Sakamoto, J., McGroarty, M. E., Old, L. J. and Melamed, M. R.** 1986. Immunohistologic expression of blood group antigens in normal human gastrointestinal tract and colonic carcinoma. Int. J. Cancer. **37**: 667-676.

29. **Costache, M., Apoil, P.-A., Cailleau, A., Elmgren, A., Larson, G., Henry, S., Blancher, A., Iordachescu, D., Oriol, R. and Mollicone, R.** 1997. Evolution of fucosyltransferase genes in vertebrates. J. Biol. Chem. **272**: 29721-29728.

30. **Costache, M., Calleau, A., Fernandez-Mateos, P., Oriol, R. and Mollicone, R.** 1997. Advances in molecular genetics of α-2 and α-3/4-fucosyltransferases. Transfus. Clin. Biol. **4**: 367-382.

31. **Covacci, A., Cesini, C., Bugoli, M., Petracca, R., Burroni, D., Macchia, G., Massone, A., Papini, E., Xiang, Z. Y. and Figura, N.** 1993. Molecular characterization of the 128 kDa immunodominant antigen of *Helicobacter pylori* associted with cytotoxicity and duodenal ulcer. Proc. Natl. Acad. Sci. USA. **90**: 5791-5795.

32. **Covacci, A., Telford, J. L., Del Giudice, G., Parsonnet, J. and Rappuoli, R.** 1999. *Helicobacter pylori* virulence and genetic geography. Science. **284**: 1328-1333.

33. **Cover, T. L.** 1996. The vacuolating cytotoxin of *Helicobacter pylori*. Mol. Microbiol. **20**: 241-246.

34. **Cover, T. L. and Blaser, M. J.** 1992. Purification and characterization of the vacuolating toxin from *Helicobacter pylori*. J. Biol. Chem. **267**: 10570-10575.

35. **Cover, T. L., Dooley, C. P. and Blaser, M. J.** 1990. Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. Infect. Immun. **58**: 603-610.

36. **Cover, T. L., Tummuru, M. K., Cao, P., Thompson, S. A. and Blaser, M. J.** 1994. Divergence of genetic sequences for the vacuolating cytotoxin among *Helicobacter pylori* strains. J. Biol. Chem. **269**: 10566-10573.

37. **Coyne, M. J. J. and Goldberg, J. B.** 1995. Cloning and characterization of the gene (*rfc*) encoding O-antigen polymerase of *Pseudomonas aeruginosa* PAO1. Gene. **167**: 81-86.

38. **Doig, P., de Jonge, B. L., Alm, R. A., Brown, E. D., Uria-Nickelsen, M., Noonan, B., Mills, S. D., Tummino, P., Carmel, G., Guild, B. C., Moir, D. T., Vovis, G. F. and Trust, T. J.** 1999. *Helicobacter pylori* physiology predicted from genomic comparison of two strains. Microbiol. Mol. Biol. Rev. **63**: 675-707.

39. **Doig, P. and Trust, T. J.** The molecular basis for *H. pylori* adherence and colonization. 1997. in *The Immunobiology of H. pylori: from pathogenesis to prevention* P. B. Ernst, P. Michetti, P. D. Smith, Eds. Lippincott-Raven Publishers, Philadelphia PA. pages 231-276.

40. **Dorrell, N. and Wren, B. W.** 1997. From genes to genome biology: a new era in *Helicobacter pylori* research. Gut. **39**: 451-453.

41. **Dunn, B. E., Cohen, H. and Blaser, M. J.** 1997. *Helicobacter pylori.* Clin. Microbiol. Rev. **10**: 720-741.

42. **Eaton, K. A. and Krakowka, S.** 1994. Effect of gastric pH on urease dependent colonization of gnotobiotic piglets by *Helicobacter pylori.* Infect. Immun. **62**: 3604-3607.

43. **Eaton, K. A., Suerbaum, S., Josenhans, C. and Krakowka, S.** 1996. Colonization of gnotobiotic piglets by *Helicobacter pylori* deficient in two flagellin genes. Infect. Immun. **64**: 2445-2448.

44. **Eggens, I., Fenderson, B., Toyokuni, T., Dean, B., Stroud, M. and Hakomori, S. I.** 1989. Specific interaction between Lewis X and Lewis X determinants. J. Biol. Chem. **264**: 9476-9484.

45. **Evans, D. G., Evans, D. J. and Graham, D. Y.** 1989. Receptor-mediated adherence of *Campylobacter pylori* to mouse Y-1 adrenal gland monolayers. Infect. Immun. **57**: 2272-2278.

46. **Evans, D. G., Evans Jr., D. J., Moulds, J. J. and Graham, D. Y.** 1988. N-acetylneuraminyllactose-binding fimbrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor. Infect. Immun. **56**: 2896-2906.

47. **Evans, D. G., Karjalainen, T. K., Evans Jr., D. J., Graham, D. Y. and Lee, C. H.** 1993. Cloning nucleotide sequence and expression of a gene encoding an adhesion subunit protein of *Helicobacter pylori.* J. Bacteriol. **175**: 674-683.

48. **Falk, P., Roth, K. A., Boren, T., Westblom, T. U., Gordon, J. I. and Normark, S.** 1993. An *in vitro* adherence assay reveals that *Helicobacter pylori* exhibits cell-lineage specific tropism in human gastric epithelium. Proc. Natl. Acad. Sci. **90**: 2035-2039.

49. **Faller, G., Steininger, H., Eck, M., Hensen, J., Hahn, E. G. and Kirchner, T.** 1996. Antigastric autoantibodies in *Helicobacter pylori* gastritis: prevalence, in-situ binding sites and clues for clinical relevance. Virch. Arch. Int. J. Pathol. **427**: 483-486.

50. **Fauchere, J. L. and Blaser, M. J.** 1990. Adherence of *Helicobacter pylori* cells and their surface components to HeLa cell membranes. Microbiol. Pathog. **9**: 427-429.

51. **Ferrero, R. L.** 1992. Immune responses to mucosal infection: the *Helicobacter pylori* paradigm. Res. Immunol. **148**: 91-107.

52. **Finlay, B. B. and Falkow, S.** 1997. Common themes in microbial pathogenecity revisited. Microbiol. Mol. Biol. Rev. **61**: 136-169.

53. **Flower, A. M. and McHenry, C. S.** 1990. The γ subunit of DNA polymerase III holoenzyme of *Escherichia coli* is produced by ribosomal frameshifting. Proc. Natl. Acad. Sci. USA. **87**: 3713-3717.

54. **Gallet, P. F., Vaujour, H., Petit, J.-M., Maftah, A., Oulmouden, A., Oriol, R., Le Narvor, C., Guiloton, M. and Julien, R.** 1998. Heterologous expression of an engineered truncated form of human Lewis fucosyltransferase (FucT-III) by the methylotrophic yeast *Pichia pastoris*. Glycobiology. **8**: 919-925.

55. **Garner, J. A. and Coer, T. L.** 1996. Binding and internalization of the *Helicobacter pylori* vacuolating cytotoxin by epithelial cells. Infect. Immun. **64**: 4197-4203.

56. **Garner, R. M., Fulkerson, J. J. and Mobley, H. L. T.** 1998. *Helicobacter pylori* glutamine synthetase lack features associated with transcriptional and posttranscriptional regulation. Infect. Immun. **66**: 1839-1847.

57. **Ge, Z., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1997. Cloning and heterologous expression of an α1,3 fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. J. Biol. Chem. **272**: 21357-21363.

58. **Ge, Z. and Taylor, D. E.** 1999. Contributions of genome sequencing to understanding the biology of *Helicobacter pylori* . Ann. Rev. Microbiol. **53**: 353-387.

59. **Geis, G., Leying, H., Suerbaum, S. and Opferkuch, W.** 1990. Unusual fatty acid substitution in lipids and lipopolysaccharides of *Helicobacter pylori*. J. Clin. Microbiol. **28**: 930-932.

60. **Gibson, J. R., Chart, H. and Owen, R. J.** 1998. Intra-strain variation in expression of lipopolysaccharide by *Helicobacter pylori*. Lett. Appl. Microbiol. **26**: 399-403.

61. **Gold, B. D. and Marty, A.** 1998. *Helicobacter pylori*, in *Pathology of Emerging Infections* C. R. Horsburgh, A. M. Nelson, Eds. ASM Press, Washington D.C.,pages 225-241.

62. **Guruge, J. L., Falk, P. G., Lorenz, R. G., Dans, M., Wirth, H. P., Blaser, M. J., Berg, D. E. and Gordon, J. I.** 1998. Epithelial attachment alters the outcome of *Helicobacter pylori* infection. Proc. Natl. Acad. Sci. USA. **95**: 3925-3930.

63. **Hacker, J., Blum-Oehler, G., Muhldorfer, I. and Tschape, H.** 1997. Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. Mol. Microbiol. **23**: 1089-1097.

64. **Hancock, R. E. W., Alm, R., Bina, J. and Trust, T.** 1998. *Helicobacter pylori*: A surprisingly conserved bacterium. Nature Biotech. **16 (3)**: 216-217.

65. **Hazell, S. L., Lee, A., Brady, L. and Hennessy, W.** 1986. *Campylobacter pyloridis* and gastritis: association with intercellular spaces and adaptation to environmental mucus as important factors in colonization of the gastric epithelium. J. Infect. Dis. **153**: 658-663.

66. **Heneghan, M. A., McCarthy, C. F. and Moran, A. P.** 2000. Relationship of blood group determinants on *Helicobacter pylori* lipopolysaccharide with host Lewis phenotype and inflammatory response. Infect. Immun. **68**: 937-941.

67. **Henry, S., Oriol, R. and Samuelsson, B.** 1995. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang. **69**: 166-182.

68. **Hoffman, P. S., Goodwin, A., Johnsen, J., Magee, K., Veldhuyzen, S. J. O. and Van Zanten, S.** 1996. Metabolic activities of metronidazole-sensitive and -resistant strains of *Helicobacter pylori*: Repression of pyruvate oxidoreductase and expression of isocitrate lyase activity correlate with resistance. J. Bacteriol. **178**: 4822-4829.

69. **Hofreuter, D., Odenbreit, S., Henke, G. and Haas, R.** 1998. Natural competence for DNA transformation in *Helicobacter pylori*: identification and genetic characterization of the *comB* locus. Mol. Microbiol. **28**: 1027-1038.

70. **Hopkins, R. J., Morris, J. G., Papadimitriou, J. C., Drachenberg, C., Smoot, D. T., James, S. P. and Panigrahi, P.** 1996. Loss of *Helicobacter pylori* hemagglutination with serial laboratory passage and correlation of hemagglutination with gastric epithelial cell adherence. Pathobiology. **64**: 247-254.

71. **Hu, L. T. and Mobley, H. L. T.** 1990. Purification and N terminal analysis of urease from *Helicobacter pylori*. Infect. Immun. **58**: 992-998.

72. **Hull, S.** 1997 *Escherichia coli* lipopolysaccharide in pathogenesis and virulence in *Escherichia coli:* mechanisms of virulence M. Sussman, Ed., Cambridge University Press, Cambridge. pp. 145-167.

73. **Hynes, R. O.** 1987. Integrins: a family of cell surface receptors. Cell. **48**: 549-554.

74. **Ilver, D., Arnqvist, A., Ogren, J., Frick, I. M., Kersulyte, D., Incecik, E. T., Berg, D. E., Covacci, A., Engstrand, L. and Boren, T.** 1998. Helicobacter pylori adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. **279 (5349)**: 373-377.

75. International Agency for Research on Cancer. 1994. Shistosomes, liver flukes and *Helicobacter pylori*. WHO Monograph 61. Lyon, France:IARC, pp. 177-240.

76. **Inzana, T. J., Hensley, J., McQuiston, J., Lesse, A. J., Campagnari, A. A., Boyle, S. M. and Apicella, M. A.** 1997. Phase variation and conservation of lipopolysaccharide epitopes in *Haemophilus somnus*. Infect. Immun. **65**: 4675-4681.

77. **Ito, Y., Azuma, T., Ito, S., Miyaji, H., Hirai, M., Yamazaki, Y., Sato, F., Kato, T., Y., K. and Kuriyama, M.** 1997. Analysis and typing of the *vacA* gene from *cagA*-positive strains of *Helicobacter pylori* isolated in Japan. J. Clin. Microbiol. **35**: 1710-1735.

78. **Janvier, B., Grignon, B., Audibert, C., Pezennec, L. and Fauchere, J. L.** 1999. Phenotypic changes of *Helicobacter pylori* components during an experimental infection in mice. FEMS Immunol. Med. Microbiol. **24**: 27-33.

79. **Jiang, Q., Hiratsuka, K. and Taylor, D. E.** 1996. Variability of gene order in different *Helicobacter pylori* strains contributes to genome diversity. Mol. Microbiol. **20**: 833-842.

80. **Jones, A. C., Logan, R. P. H., Foynes, S., Cockayne, A., Wren, B. W. and Penn, C. W.** 1997. A flagellar sheath protein of *Helicobacter pylori* is identical to HpaA, a putative *N*-acetylneuraminyllactose-binding hemagglutinin, but is not an adhesin for AGS cells. J. Bacteriol. **179**: 5643-5647.

81. **Josenhans, C., Labigne, A. and Suerbaum, S.** 1995. Comparative ultrastructural and functional studies of *Helicobacter pylori* and *Helicobacter mustelae* flagellin mutants: both flagellin subunits, FlaA and FlaB, are necessary for full motility in *Helicobacter* species. J. Bacteriol. **177**: 3010-3020.

82. **Josenhans, C., Labigne, A. and Suerbaum, S.** 1995. Reporter gene analyses show that expression of both *H. pylori* flagellins are dependent on the growth phase. Gut. **37 (Suppl. 1)**: A62 (Abstract).

83. **Kadam, S. K., Rehemtulla, A. and Sanderson, K. E.** 1985. Cloning of *rfaG, B, I* and *J* genes for glycosyltransferase enzymes for synthesis of the lipopolysaccharide core of *Salmonella typhimurium*. J. Bacteriol. **161**: 277-284.

84. **Karjalainen, T. K., Evans, D. G., So, M. and Lee, C. H.** 1989. Molecular cloning and nucleotide sequence of the colonization factor I gene of *Escherichia coli*. Infect. Immun. **57**: 1126-1130.

85. **Keenleyside, W. J. and Whitfield, C.** 1996. A novel pathway for O-polysaccharide biosynthesis in *Salmonella enterica* serovar Borreze. J. Biol. Chem. **271**: 28581-28592.

86. **Kenny, B., DeVinney, R., Stein, M., Reinscheid, D. J., Frey, E. A. and Finlay, B. B.** 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell. **91**: 511-520.

87. **Klaamas, K., Kurtenkov, O., Ellamaa, M. and Wadstrom, T.** 1997. The *Helicobacter pylori* seroprevalence in blood donors related to Lewis (A,B) histo blood group phenotype. Eur. J. Gastroenterol. Hepatol. **9**: 367-370.

88. **Kobyashi, K., Sakamoto, J., Kito, T., Yamamura, Y., Koshikawa, T., Fujita, M., Watananbe, T. and Nakazato, H.** 1993. Lewis blood group related antigen expression in normal gastric epithelium, intestinal metaplasia, gastric adenoma and gastric carcinoma. Am. J. Gastroenterol. **88**: 919-924.

89. **Krishnamurthy, P., Parlow, M., Ziter, J. B., Vakil, N. B. and Mobley, H. L. T.** 1998. *Helicobacter pylori* containing only cytoplasmic urease is susceptible to acid. Infect. Immun. **66**: 5060-5066.

90. **Kuipers, E. J., Israel, D. A., Kusters, J. G. and Blaser, M. J.** 1998. Evidence for a conjugation-like mechanism of DNA transfer in *Helicobacter pylori*. J. Bacteriol. **180**: 2901-2905.

91. **Kumazawa, Y., Nakatsuka, N., Takimoto, H., Furuya, T., Nagumo, T., Yamamoto, A., Homma, J. Y., Inada, K., Yoshida, M., Kiso, M. and Hasegawa, A.** 1988. Importance of fatty acid substituents of chemically synthesized lipid A-subunit analogs in the expression of immunopharmacological activity. Infect. Immun. **56**: 149-155.

92. **Kusters, J. G., Gerrits, M. M., van Strijp, J. A. G. and Vandenbroucke-Grauls, C. M. J. E.** 1997. Coccoid forms of *Helicobacter pylori* are the morphological manifestation of cell death. Infect. Immun. **65**: 3672-3679.

93. **Lau, P. C. K., Wang, Y., Patel, A., Labbe, D., Bergeron, H., Brousseau, R., Konishi, Y. and Rawlings, M.** 1997. A bacterial basic region leucine zipper histidine kinase regulating toluene degradation. Proc. Natl. Acad. Sci. U.S.A. **94**: 1453-1458.

94. **Lelwala-Guruge, J., Ljungh, A. and Wadstrom, T.** 1992. Haemagglutination patterns of *Helicobacter pylori*: frequency of sialic acid specific and non-sialic acid specific haemagglutinins. APMIS. **100**: 908-913.

95. **Leying, H., Suerbaum, S., Geis, G. and Haas, R.** 1992. Cloning and genetic characterization of a *Helicobacter pylori* flagellin gene. Mol. Microbiol. **6**: 2863-2874.

96. **Lingwood, C. A., Huseca, M. and Kuksis, A.** 1992. The glycerolipid receptor for *Helicobacter pylori* (and exoenzyme s) is phosphatidylethanolamine. Infect. Immun. **60**: 2470-2474.

97. **Lingwood, C. A., Law, H., Pellizzari, A., Sherman, P. and Drumm, B.** 1989. Gastric glycerolipid as a receptor for *Campylobacter pylori*. Lancet. **ii**. 241-246.

98. **Liu, D., Cole, R. and Reeves, P. R.** 1996. An O antigen processing function for Wzx: a promising candidate for O-unit flippase. J. Bacteriol. **178**: 2102-2107.

99. **Ljungh, A., Moran, A. P. and Wadstrom, T.** 1996. Interactions of bacterial adhesins with extracellular matrix and plasma proteins: pathogenic implications and therapeutic possibilities. FEMS Immunol. Med. Microbiol. **16**: 117-126.

100. **Lo, S. K., Golenbock, D. T., Sass, P. M., Maskati, A., Xu, H. and Silverstein, R. L.** 1997. Engagement of the Lewis X antigen (CD15) results in monocyte activation. Blood. **89**: 307-314.

101. **Lupetti, P., Heuser, J. E., Manetti, R., Massari, P. and Lanzavecchia, S.** 1996. Oligomeric and subunit structure of the *Helicobacter pylori* vacuolating toxin. J. Cell Biol. **133**: 801-807.

102. **Marais, A., Mendz, G. L., Hazell, S. L. and Megraud, F.** 1999. Metabolism and genetics of *Helicobacter pylori*: the genome era. Microbiol. Mol. Biol. Rev. **63**: 642-674.

103. **Marshall, D. G., Dundon, W. G., Beesley, S. M. and Smyth, C. J.** 1998. *Helicobacter pylori* - a conundrum of genetic diversity. Microbiology. **144**: 2925-2939.

104. **Marshall, D. G., Hynes, S. O., Coleman, D. C., O'Morain, C. A., Smyth, C. J. and Moran, A. P.** 1999. Lack of a relationship between Lewis antigen expression and *cagA*, Cag, *vacA*, and VacA status of Irish *Helicobacter pylori* isolates. FEMS Immunol. Med. Microbiol. **24**: 79-90.

105. **Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. and Bird, M. I.** 1997. Lewis X biosynthesis in *Helicobacter pylori*. molecular cloning of an α(1,3) fucosyltransferase gene. J. Biol. Chem. **272**: 21349-21356.

106. **Maskell, D. J., Szabo, M. J., Butler, P. D., Williams, A. E. and Moxon, E. R.** 1991. Phase variation of lipopolysaccharide in *Haemophilus influenzae*. Res. Microbiol. **142**: 719-724.

107. **Megraud, F.** 1995. Transmission of *Helicobacter pylori*: faecal-oral versus oral-oral route. Aliment. Pharmacol. Ther. **9(suppl. 2)**: 85-91.

108. **Miehlke, S., Kibler, K., Kim, J. G., Figura, N., Small, S. M., Graham, D. Y. and Go, M. F.** 1996. Allelic variation in the *cagA* gene of

Helicobacter pylori obtained from Korea compared to the United States. Am. J. Gastroenterol. **91**: 1322-1325.

109. **Miller-Podroza, H., Bergstrom, J., Teneberg, S., Milh, M. A., Longard, M., Olsson, B. M., Uggla, L. and Karlsson, K. A.** 1999. *Helicobacter pylori* and neutrophils: sialic acid-dependent binding to various isolated glycoconjugates. Infect. Immun. **67**: 6309-6313.

110. **Miller-Podroza, H., Milh, M. A., Teneberg, S. and Karlsson, K. A.** 1997. Binding of *Helicobacter pylori* to sialic acid containing glycolipids of various origins separated on thin layer chromatograms. Infect. Immun. **65**: 2480-2482.

111. **Mills, S. D., Kurjanczyk, L. A. and Penner, J. L.** 1992. Antigenicity of *Helicobacter pylori* lipopolysaccharides. J. Clin. Microbiol. **30**: 3175-3180.

112. **Mizote, T., Yoshiyama, H. and Nakazawa, T.** 1997. Urease-dependent chemotactic responses of *Helicobacter pylori* to urea, urease inhibitors, and sodium bicarbonate. Infect. Immun. **65**: 1519-1521.

113. **Mobley, H. L. T.** 1996. The role of *Helicobacter pylori* urease in the pathogenesis of gastritis and peptic ulceration. Alim. Pharmacol. Therap. **10**: 57-64.

114. **Mobley, H. L. T., Fulkerson, J. J., Hendricks, J. K. and R.M., G.,** 1997. in *Pathogenesis and Host Response in Helicobacter pylori* A. P. Moran, C. A. O'Morain, Eds. Normed Verlag, Englewood, NJ, pages 58-66.

115. **Molinari, M., Galli, C., Norais, N., Telford, J. L., Rappuoli, R., Luzio, J. P. and Montecucco, C.** 1998. Vacuoles induced by *Helicobacter pylori* toxin contain both late endosomal and lysosomal markers. J. Biol. Chem. **273** : 25339-25344.

116. **Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Moran, A. P., Hynes, S. O., McLean, L. L., Chan, K. H., Michael, F. S., Logan, S. M., O'Rourke, J., Lee, A., Taylor, D. E. and Perry, M. B.** 2000. Lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, mouse model *H. pylori* Sydney strain, *H. pylori* P466 carrying sialyl Lewis X, and *H. pylori* UA915 expressing Lewis B: Classification of *H. pylori* lipopolysaccharides into Glycotype families. Eur. J. Biochem. **276**: 305-320.

117. **Monteiro, M. A., Zheng, P. Y., Ho, B., Yokota, S. I., Amano, K. I., Pan, Z. J., Berg, D. E., Chan, K. H., MacLean, L. L. and Perry, M. B.** 2000. Expression of histo-blood group antigens by lipopolysaccharides of *Helicobacter pylori* strains from Asian hosts: the propensity to express type 1 blood-group antigens. Glycobiology. **In Press.**

118. **Moran, A. P., Helander, I. M. and Kosunen, T. U.** 1992. Compositional analysis of *Helicobacter pylori* rough-form lipopolysaccharides. J. Bacteriol. **174**: 1370-1377.

119. **Moran, A. P., Lindner, B. and Walsh, E. J.** 1997. Structural characterization of the Lipid A component of *Helicobacter pylori* rough and smooth form lipopolysaccharides. J. Bacteriol. **179**: 6453-6463.

120. **Moran, A. P., Prendergast, M. M. and Appelmelk, B. J.** 1996. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. FEMS Immunol. Med. Microbiol. **16**: 105-115.

121. **Morona, R., Mavris, M., Fallarino, A. and Manning, P. A.** 1994. Characterization of the *rfc* region of *Shigella flexneri*. J. Bacteriol. **176**: 733-747.

122. **Muotiala, A., Helander, I. M., Pyhala, L., Kosunen, T. U. and Moran, A. P.** 1992. Low biological activity of *Helicobacter pylori* lipopolysaccharide. Infect. Immun. **60**: 1714-1716.

123. **Nakamura, H., Yoshiyama, H., Takeuchi, H., Mizote, T., Okita, K. and Nakazawa, T.** 1998. Urease plays an important role in the chemotactic motility of *Helicobacter pylori* in a viscous environment. Infect. Immun. **66**: 4832-4837.

124. **Nakazawa, T., Ishibashi, M., Konishi, H., Takemoto, T., Shigeeda, M. and Kochiyama, T.** 1991. Hemagglutination activity of *Campylobacter pylori*. Infect. Immun. **57**: 989-991.

125. **Namavar, F., Sparrius, M., Veerman, E. C. I., Appelmelk, B. J. and VandenbrouckeGrauls, C.** 1998. Neutrophil-activating protein mediates adhesion of Helicobacter pylori to sulfated carbohydrates on high-molecular-weight salivary mucin. Infect. Immun. **66**: 444-447.

126. **Negrini, R., Savio, A., Poiesi, C., Appelmelk, B. J., Buffoli, F., Paterlini, A., Cesari, P., Graffeo, M., Vaira, D. and Franzin, G.** 1996. Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. Gastroenterology. **111**: 655-665.

127. **Nguyen, A. T., Holmes, E. H., Whitaker, J. M., Ho, S., Shetterly, S. and Macher, B. A.** 1998. Human α1,3/4-Fucosyltransferases: I Identification of amino acids involved in acceptor substrate binding by site-directed mutagenesis. J. Biol. Chem. **273**: 25244-25249.

128. **Nnalue, N. A.** 1999. All accessible epitopes in the *Salmonella* lipopolysaccharide core are associated with branch residues. Infect. Immun. **67**: 998-1003.

129. **Ogawa, T., Suda, Y., Kashihara, W., Hayashi, T., Shimoyama, T., Kusumoto, S. and Tamura, T.** 1997. Immunobiological activities of chemically defined Lipid A from *Helicobacter pylori* LPS in comparison with *Porphyromonas gingivalis* Lipid A and *Escherichia coli* type synthetic Lipid A (Compound 506). Vaccine. **15**: 1598-1605.

130. **Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L. and Breton, C.** 1999. Divergent evolution of fucosyltransferase genes from vertebrates, invertebrates, and bacteria. Glycobiology. **9**: 323-334.

131. **Osaki, T., Yamaguchi, H., Taguchi, H., Kumada, J., Ogata, S. and Kamiya, S.** 1997. Studies on the relationship between adhesive activity and haemagglutination by *Helicobacter pylori*. J. Med. Microbiol. **46**: 117-121.

132. **Pagliaccia, C., de Bernard, M., Lupetti, P., Ji, X., Burroni, D., Cover, T. L., Papini, E., Rappuoli, R., Telford, J. L. and Reyrat, J. M.** 1998. The m2 form of the *Helicobacter pylori* cytotoxin has cell type specific vacuolating activity. Proc. Natl. Acad. Sci. USA. **95**: 10212-10217.

133. **Papini, E., Bugnoli, M., de Bernard, M., Figura, N., Rappuoli, R. and Montecucco, C.** 1994. Cellular vacuoles induced by *Helicobacter pylori* originate from late endosomal compartments. Proc. Natl. Acad. Sci. USA. **91**: 9720-9724.

134. **Parkkinen, J., Rogers, G. N., Korhonen, T., Dahr, W. and Finne, J.** 1986. Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors fro S-fimbriated *Escherichia coli*. Infect. Immun. **54**: 37-42.

135. **Parsonnet, J.** 1996. *Helicobacter pylori* in the stomach - A paradox unmasked. N. Engl. J. Med. **335**: 278-280.

136. **Parsonnet, J., Friedman, G. D., Orentreich, N. and Vogelman, H.** 1997. Risk for gastric cancer in people with *cagA* positive or *CagA* negative *Helicobacter pylori* infection. Gut. **40**: 297-301.

137. **Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N. and Sibley, R. K.** 1991. *Helicobacter pylori* infection and the risk of gastric cancer. N. Engl. J. Med. **325**: 1127-1131.

138. **Penn, C. W. and Luke, C. J.** 1992. Bacterial flagellar diversity and significance in pathogenesis. FEMS Microbiol. Lett. **79**: 421-426.

139. **Raetz, C. R. H.** 1996. Bacterial lipopolysaccharides: a remarkable family of bioactive macroamphiphiles in *Escherichia coli and Salmonella* F. C. Niedhardt, *et al.*, Eds. ASM Press, Washington. pages 1035-1063.

140. **Rasko, D. A., Wang, G., Palcic, M. M. and Taylor, D. E.** 2000. Cloning and characterization of the α1,3/4 fucosyltransferase of *Helicobacter pylori*. J. Biol. Chem. **276**: 4988-4994.

141. **Roche, R. J. and Moxon, E. R.** 1995. Phenotypic variation of carbohydrate surface antigens and the pathogenesis of *Haemophilus influenzae* infections. Trend. Microbiol. **3**: 304-309.

142. **Saitoh, T., Natomi, H., Zhao, W., Okuzumi, K., Sugano, K., Iwamori, M. and Nagai, Y.** 1991. Identification of glycolipid receptors for *Helicobacter pylori* by TLC-immunostaining. FEBS Lett. **282**: 385-387.

143. **Saunders, N. J., Peden, J. F., Hood, D. W. and Moxon, E. R.** 1998. Simple sequence repeats in the *Helicobacter pylori* genome. Mol. Microbiol. **27**: 1091-1098.

144. **Schnaitman, C. A. and Klena, J. D.** 1993. Genetics of lipopolysaccharide biosynthesis in enteric bacteria. Microbiol. Rev. **57**: 655-682.

145. **Segal, E. D.** 1997. Consequences of attachment of *Helicobacter pylori* to gastric cells. Biomedicine And Pharmacotherapy. **51**: 5-12.

146. **Segal, E. D., Cha, J., Lo, J., Falkow, S. and Tompkins, L. S.** 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA. **96**: 14559-14564.

147. **Segal, E. D., Falkow, S. and Tompkins, L. S.** 1996. *Helicobacter pylori* attachment to gastric cells induces cytoskeletal rearrangements and tyrosine phosphorylation of host cell proteins. Proc. Natl. Acad. Sci. USA. **93**: 1259-1264.

148. **Sherburne, R. and Taylor, D. E.** 1995. *Helicobacter pylori* expresses a complex surface carbohydrate, Lewis X. Infect. Immun. **63**: 4564-4568.

149. **Sherwood, A. L., Nguyen, A. T., Whitaker, J. M., Macher, B. A., Stroud, M. R. and Holmes, E. H.** 1998. Human α1,3/4-Fucosyltransferases: III A lys/arg residue located within the 1,3-FucT motif is required for activity but not for substrate binding. J. Biol. Chem. **273**: 25256-25260.

150. **Shortridge, V. D., Stone, G. G., Flamm, R. K., Beyer, J., Versalovic, J., Graham, D. W. and Tanaka, S. K.** 1997. Molecular typing of *Helicobacter pylori* isolates from a multicenter US clinical trial by *ureC* restriction fragment length polymorphism. J. Clin. Microbiol. **35**: 471-473.

151. **Simon, P. M., Goode, P. L., Mobasseri, A. and Zopf, D.** 1997. Inhibition of *Helicobacter pylori* binding to gastrointestinal epithelial cells by sialic acid containing oligosaccharides. Infect. Immun. **65**: 750-757.

152. **Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B. S., Rudnica, W., Savio, A. and De Graaff, J.** 1996. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol. **34**: 2196-2200.

153. **NIH Consensus Statement.** 1994. *Helicobacter pylori* in peptic ulcer disease. NIH Consensus Statement. **12**: 1-22.

154. **Stein, M., Rappuoli, R. and Covacci, A.** 2000. Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after *cag*-driven host cell translocation. Proc. Natl. Acad. Sci. USA. **97**: 1263-1268.

155. **Strobel, S., Bereswill, S., Balig, P., Allgaier, P., Sonntag, H.-G. and Kist, M.** 1998. Identification and analysis of a new *vacA* genotype variant of *Helicobacter pylori* in different patient groups in Germany. J. Clin. Microbiol. **36**: 1285-1289.

156. **Su, B., Johansson, S., Fallman, M., Patarroyo, M., Granstrom, M. and Normark, S.** 1999. Signal transduction-mediated adherence and entry of *Helicobacter pylori* in cultured cells. Gastroenterology. **117**: 595-604.

157. **Suerbaum, S., Smith, J. M., Bapumia, K., Morelli, G., Smith, N. H., Kunstman, E., Dyrek, I. and Achtman, M.** 1998. Free recombination within *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA. **95**: 12619-12624.

158. **Szabo, I., Brutsche, S., Tombola, F., Moshioni, M., Satin, B., Telford, J. L., Rappuoli, R., Montecucco, C., Papini, E. and Zoratti, M.** 1999. Formation of anion selective channels in the cell plasma membrane by the toxin VacA of *Helicobacter pylori* is required for biological activity. The EMBO Journal. **18**: 5517-5527.

159. **Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W. and Jewell, L. D.** 1987. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. Am. J. Clin. Pathol. **87**: 49-54.

160. **Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C. and Jewel, L. D.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

161. **Telford, J. L., Ghiara, P., Dell'Orco, M., Comanducci, M., Burroni, D., Bugnoli, M., Tecce, M. F., Cesini, S., Covacci, A., Xiang, Z., Papini, E., Montecucco, C., Parente, L. and Rappuoli, R.** 1994. Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. J. Exp. Med. **179**: 1653-1658.

162. **Thomas, J. E., Gibson, G. R., Darboe, M. K., Dale, A. and Weaver, L. T.** 1992. Isolation of *Helicobacter pylori* from human faeces. Lancet. **340**: 1194-1195.

163. **Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L. X., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M. and Venter, J. C.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. **388**: 539-547.

164. **Tombola, F., Carlesso, C., Szabo, I., de Bernard, M., Reyrat, J. M., Telford, J. L., Rappuoli, R., Montecucco, C., Papini, E. and Zoratti, M.** 1999. *Helicobacter pylori* vacuolating toxin forms anion-selective channels in planar lipid bilayers: possible implications for the mechanism of cellular vacuolation. Biophys. J. **76**: 1401-1409.

165. **Trust, T., Doig, P., Emdy, L., Kienle, Z., Wadstrom, T. and O'Toole, P.** 1991. High-affinity binding of the basement membrane proteins collagen type IV and laminin to the gastric pathogen *Helicobacter pylori*. Infect. Immun. **59**: 4398-4404.

166. **Tsuda, M., Karita, M., Morshed, M. G., Okita, K. and Nakazawa, T.** 1994. A urease negative mutant of *Helicobacter pylori* constructed by allelic

exchange mutagenesis lacks the ability to colonize the nude mouse stomach. Infect. Immun. **62**: 3586-3589.

167. **Turner, R. and Tjian, R.** 1989. Leucine repeats and an adjacent DNA binding domain mediate the formation of functional cFos-cJun heterodimers. Science. **243**: 1689-1694.

168. **Utt, M. and Wadstrom, T.** 1997. Identification of hepran sulfate binding surface proteins of *Helicobacter pylori*: inhibition of hepran sulfate binding with sulfated carbohydrate polymers. J. Med. Microbiol. **46**: 541-546.

169. **Valkonen, K. H., Wadstrom, T. and Moran, A. P.** 1994. Interaction of lipopolysaccharides of *Helicobacter pylori* with basement membrane protein laminin. Infect. Immun. **62**: 3640-3648.

170. **Valkonen, K. H., Wadstrom, T. and Moran, A. P.** 1997. Identification of the N-acetylneuraminyllactose specific laminin binding protein of *Helicobacter pylori*. Infect. Immun. **65**: 916-923.

171. **van Doorn, L.-J., Figueiredo, C., Sanna, R., Pena, S., Midolo, P., Ng, E. K. W., Atherton, J. C., Blaser, M. J. and Quint, W. G. V.** 1998. Expanding allelic diversity of *Helicobacter pylori vacA*. J. Clin. Microbiol. **36**: 2597-2603.

172. **van Putten, J. P. M., Duensing, T. D. and Cole, R. L.** 1998. Entry of OpaA + gonococci into HEp-2 cells requires concerted action of glycosaminoglycans, fibronectin and integrin receptors. Mol. Microbiol. **29**: 369-379.

173. **van Putten, J. P. M. and Robertson, B. D.** 1995. Molecular mechanisms and implications for infection of lipopolysaccharide variation in *Neisseria*. Mol. Microbiol. **16**: 847-853.

174. **Vanet, A. and Labigne, A.** 1998. Evidence for specific secretion rather than autolysis in the release of some Helicobacter pylori proteins. Infect. Immun. **66**: 1023-1027.

175. **Vo, L., Lee, S., Marcinko, M. C., Holmes, E. H. and Macher, B. A.** 1998. Human α1,3/4-Fucosyltransferases: A single amino acid at the COOH terminus of FucT III and V alters their kinetic properties. J. Biol. Chem. **273**: 25250-25255.

176. **Wadstrom, T., Hirmo, S. and Boren, T.** 1996. Biochemical aspects of *Helicobacter pylori* colonization of the human gastric mucosa. Alim. Pharmacol. Therap. **10**: 17-27.

177. **Wadstrom, T., Hirmo, S. and Nilsson, B.** 1997. Biochemical aspects of *H. Pylori* adhesion. J. Physiol. Pharmacol. **48**: 325-331.

178. **Walsh, E. J. and Moran, A. P.** 1997. Influence of medium composition on the growth and antigen expression of *Helicobacter pylori*. J. Appl. Microbiol. **83**: 67-75.

179. **Wang, G., Boulton, P. G., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1999. Novel *Helicobacter pylori* α1,2-fucosyltransferase, a key enzyme in the synthesis of Lewis antigens. Microbiology. **145**: 3245-3253.

180. **Wang, G., Ge, Z., Rasko, D. A. and Taylor, D. E.** Submitted. Lewis antigens in *Helicobacter pylori*:biosynthesis and phase variation. Mol. Microbiol.

181. **Wang, G., Humayun, M. Z. and Taylor, D. E.** 1999. Mutation as an origin of genetic variability in *Helicobacter pylori*. Trend. Microbiol. **7**: 488-493.

182. **Wang, G., Rasko, D. A., Sherburne, R. and Taylor, D. E.** 1999. Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori* : analysis of the α(1-2) fucosyltransferase gene. Mol. Microbiol. **31**: 1265-1274.

183. **Wang, Y., Roos, K. P. and Taylor, D. E.** 1993. Transformation of *Helicobacter pylori* by chromosomal metronidazole resistance and by a plasmid with selectable chloramphenicol resistance. J. Gen. Microbiol. **139**: 2485-2493.

184. **Warren, J. R. and Marshall, B. J.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. **1**: 1273-1275.

185. **Watkins, W. M., Greenwell, P., Yates, A. D. and Johnson, P. H.** 1988. Regulation of expression of carbohydrate blood group antigens. Biochimie. **70**: 1597-1611.

186. **Weston, B. W., Nair, R. P., Larsen, R. D. and Lowe, J. B.** 1992. Isolation of a novel human α(1,3) fucosyltransferase gene and molecular comparison to the human Lewis blood group α (1,3/1,4) fucosyltransferase gene. J. Biol. Chem. **267**: 4152-4160.

187. **Whitfield, C.** 1995. Biosynthesis of lipopolysaccharide O antigens. Trend. Microbiol. **3**: 178-185.

188. **Whitfield, C., Amor, P. A. and Koplin, R.** 1997. Modulation of the surface architecture of Gram-negative bacteria by the action of surface polymer:lipid A-core ligase and by determinants of the polymer chain length. Mol. Microbiol. **23**: 629-638.

189. **Whitfield, C. and Roberts, I. S.** 1999. Structure, assembly and regulation of capsules in *Escherichia coli*. Mol. Microbiol. **31**: 1307-1319.

190. **Whitfield, C. and Valvano, M. A.**, 1993. Biosynthesis and expression of cell surface polysaccharides in gram negative bacteria in *Advances in Microbial Physiology* A. H. Rose, Ed. Academic Press, San Diego, CA. vol. 35, pp. 135-246.

191. **Wirth, H.-P., Yang, M., Peek, R. M., Hook-Nikanne, J., Fried, M. and Blaser, M. J.** 1999. Phenotypic diversity in Lewis expression of *Helicobacter pylori* isolates from the same host. J. Lab. Clin. Med. **133**: 488-500.

192. **Wirth, H. P., Yang, M. Q., Karita, M. and Blaser, M. J.** 1996. Expression of the human cell surface glycoconjugates Lewis X and Lewis Y by *Helicobacter pylori* isolates is related to CagA status. Infect. Immun. **64**: 4598-4605.

193. **Wirth, H. P., Yang, M. Q., Peek, R. M., Tham, K. T. and Blaser, M. J.** 1997. *Helicobacter pylori* Lewis expression is related to the host Lewis phenotype. Gastroenterology. **113**: 1091-1098.

194. **Worku, M. L., Sidebotham, R. L., Walker, M. M., Keshavarz, T. and Karim, Q. N.** 1999. The relationship between *Helicobacter pylori* motility, morphology and phase of growth: implications for gastric colonization and pathology. Microbiology. **145**: 2803-2811.

195. **Xu, Z., Vo, L. and Macher, B. A.** 1996. Structure-function analysis of human α1,3-fucosyltransferase: amino acids involved in acceptor substrate specificity. J. Biol. Chem. **271**: 8818-8823.

196. **Yamaoka, Y., Kodama, T., Kashima, K., Graham, D. Y. and Sepulveda, A. R.** 1998. Variants of the 3` region of the *cagA* gene in *Helicobacter pylori* isolates from patients with different *H. pylori*-associated diseases. J. Clin. Microbiol. **36**: 2258-2263.

197. **Ye, D., Willhite, D. C. and Blanke, S. R.** 1999. Identification of the minimal intracellular vacuolating domain of the *Helicobacter pylori* vacuolating toxin. J. Biol. Chem. **274**: 9277-9282.

198. **Yokota, S., Amano, K., Shibata, Y., Nakajima, M., Suzuki, M., Hayashi, S., Fujii, N. and Yokochi, T.** 2000. Two distinct antigenic types of polysaccharide chains of *Helicobacter pylori* lipopolysaccharides characterized by reactivity with sera from humans with natural infection. Infect. Immun. **68**: 151-159.

199. **Yokota, S.-I., Amano, K.-I., Hayashi, S., Kubota, T., Fujii, N. and Yokochi, T.** 1998. Human antibody response to *Helicobacter pylori* lipopolysaccharide: presence of an immunodominant epitope in the polysaccharide chain of lipopolysaccharide. Infect. Immun. **66**: 3006-3011.

200. **Yokota, S. I., Amano, K. I., Hayashi, S. and Fujii, N.** 1997. Low antigenicity of the polysaccharide region of *Helicobacter pylori* lipopolysaccharides derived from tumors of patients with gastric cancer. Infect. Immun. **65**: 3509-3512.

201. **Yoshiyama, H., Nakamura, H., Kimoto, M., Okita, K. and Nakazawa, T.** 1999. Chemotaxis and motility of *Helicobacter pylori* in a viscous environment. J. Gastroenterol. **34 (Suppl. 11)**:18-23.

Chapter 2

Expression of Lewis Antigens by *Helicobacter pylori*

Portions of this work have been part of the following publications:

D. A. Rasko, C. M. Szymanski, G. D. Armstrong and D. E. Taylor. 1997 Binding of *Helicobacter* pylori to Lewis Antigens. Irish Journal of Medical Science. Vol.116(Suppl.3) :25(P40).

Monteiro, M. A., Chan, K. H. N., **Rasko, D. A.,** Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P. and Perry, M. B. 1998. Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem. **273**: 11533-11543.

Taylor, D. E., **Rasko, D. A.,** Sherburne, R., Ho, C. and Jewel, L. D. 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

Monteiro, M. A., Appelmelk, B. J., **Rasko, D. A.**, Moran, A. P., Hynes, S. O., McLean, L. L., Chan, K. H., Michael, F. S., Logan, S. M., O'Rourke, J., Lee, A., Taylor, D. E. and Perry, M. B. 2000. Lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, mouse model *H. pylori* Sydney strain, *H. pylori* P466 carrying sialyl Lewis X, and *H. pylori* UA915 expressing Lewis B: Classification of *H. pylori* lipopolysaccharides into Glycotype families. Eur. J. Biochem. **276**: 305-320.

2. Preface

The discovery that the gastric pathogen *H. pylori* expressed Lewis antigens in its lipopolysaccharide, led to detailed investigations regarding the distribution of these structures among *H. pylori* populations and the role that they play in the infectious process. The synthesis and production of the Lewis antigens by *H. pylori* is a process that is not clearly understood. A study of the Lewis antigens was carried out to determine if their expression is growth phase dependent. A thorough study of the distribution of the Lewis antigens among *H. pylori* isolates obtained from patients and subjects at the University of Alberta and other sources was conducted to determine differences among isolates obtained from symptomatic and asymptomatic individuals. The observed differences in the expression of the Lewis antigens among isolates from asymptomatic individuals when compared to *H. pylori* isolates from symptomatic patients indicated that these antigens may play some role during infection.

2.1 Introduction

The gastric pathogen *Helicobacter pylori* infects approximately 50% of the world's population (15). Of the people that are infected, all experience some degree of superficial gastritis, yet many do not seek medical treatment and thus the infection is not diagnosed and would be considered asymptomatic. It has been reported that approximately 15-20% of people infected with *H. pylori* progress to the more serious pathologies such as chronic gastritis, gastric ulcer, duodenal ulcer, lymphoma or gastric cancer in correlation with *H. pylori* infection (13). This association has lead to the assignment of the class I carcinogen status for *H. pylori*, the only bacterium to have been given such a designation (21). The risk factors which are associated with the progression from gastritis to the more serious pathologies are unknown.

The expression of Lewis antigens by *H. pylori* is considered by to be a major factor in the ability of this organism to cause chronic disease in humans (4, 25). The first reported identification of the Le^x antigen in the LPS of the *H. pylori* was by Aspinall *et al.* (5). Since then a number of studies have documented the distribution of Lewis antigens among particular study groups (28, 38, 39). The expression of the Lewis antigens by *H. pylori* has been correlated to the CagA status of the *H. pylori* isolate (38) as well as with the patient's ABO blood type (39). A more recent study demonstrated that there was no correlation between the Lewis antigens produced by the *H. pylori* isolate and the expression of antigens on the patients gastric epithelium (31). Recent work by Marshall *et al.* (23) and Heneghan *et al.* (18) supports the conclusion that the *H. pylori* Lewis antigens were not correlated to the gastric epithelial Lewis antigen expression but an association was identified between the expression of Le^x by *H. pylori* and increased gastric inflammation. The pathogenic role for these antigens remains unidentified.

Recent reports demonstrate that the Lewis antigens in the LPS of *H. pylori* may not be a primary antigenic target for the immune system. The antibody response of the host was not directed toward the Lewis antigens but rather at another as yet unidentified

carbohydrate epitope of the LPS (1, 12, 40, 41). The *in vivo* stability of the Lewis antigens has also recently been brought into question as the LPS profile of the Sydney strain (SS1) was altered by passage within a mouse (22), and multiple isolates obtained from a primary gastric biopsy have been demonstrated to be phenotypically distinct but genetically identical (16, 37). This evidence indicates that the expression of the Lewis antigens may be more variable *in vivo* when compared to the measured *in vitro* mutation rate of 0.2-0.5% (3).

A number of studies have also initiated investigations into the biosynthetic process by which the Lewis antigens are produced by *H. pylori*. The fucosyltransferases of *H. pylori* are the best characterized enzymes of the *H. pylori* LPS O side chain biosynthetic system. Appelmelk *et al.* (2) demonstrated that phase variation can be due to slipped strand mispairing within the polynucleotide tracts of the α(1,3(4)) fucosyltransferase genes of these enzymes. Although not demonstrated directly, the *H. pylori* α(1,2) *fucT* gene contains similar polynucleotide tracts which may also be responsible for phase variation of these gene products (35). These genes also appear to utilize a translational frameshifting mechanism to overcome negative effects of slipped strand mispairing mutations at these sites in certain isolates (36).

This chapter examines the stability of the Lewis antigen expression during the growth phases of *H. pylori* and compares the expression of Lewis antigens from the *H. pylori* isolates obtained from symptomatic and asymptomatic individuals.

2.2 Experimental Procedures

2.2.1. *H. pylori* Isolation

Strains were cultured by the standard methods described by Taylor *et al.* (30). Biopsy samples were plated out on brain heart infusion agar plates (BHI-YE agar) [3.7% BHI (Difco), 0.5% yeast extract, 15 μg/ml of both vancomycin and amphoterocin B, 50 ml/L of fetal bovine serum]. These plates were incubated at 37°C under microaerobic

conditions for 2-4 days. Positive *H. pylori* cultures were confirmed by urease test, and light microscopy. Isolates from symptomatic patients were routinely collected at the University of Alberta in the Department of Gastroenterology (1983-present). *H. pylori* isolates were obtained from asymptomatic subjects as part of the Neose phase II clinical trial on the anti-adhesive carbohydrate therapy 3'SL (26), as well as from asymptomatic subjects at the University of Alberta. All patients and subjects provided informed consent. Designation between patient and subject is necessary due to the implication that a patient has disease symptoms whereas the asymptomatic subjects presented no signs of disease induced by *H. pylori* infection.

2.2.2. ELISA for Lewis Antigens

Isolated *H. pylori* were examined for Lewis antigenic expression patterns. The conditions for the ELISA were previously described (24, 38). The primary antibodies used were anti-Le^a (MAb BG-5, clone T174), anti-Le^b (MAb BG-6, clone T218), anti-Le^x (MAb BG-7, clone P12), anti-Le^y (MAb BG-8, clone F3), anti-H Type I antigen (MAb BG-4) and anti-sialyl Le^x (MAb BG-9) from Signet Laboratories Inc. (Dedham, MA, USA). The primary antibodies were diluted 1:100 while the secondary antibody, goat anti-mouse IgG + IgM conjugated to horseradish peroxidase (HRP) (Biocan #115 035 068, Mississauga, Ontario, CAN), was diluted 1:2000. The absorbance was recorded at 405 nm using a Titretek Multiscan MC (Helsinki, Finland) microtitre plate reader. Absorbance values were an average of triplicate wells with blanks subtracted. Values below 0.1 absorbance units were considered negative. Strains found not to express Lewis antigens were assayed on two separate occasions to confirm their status.

2.2.3. Polyacrylamide Gel Electrophoresis and Immunoblots

Whole cell extracts of the *H. pylori* strains were treated with proteinase K and processed as described by Hitchcock and Brown (20). For the analysis of the LPS, a 15% polyacrylamide separating gel containing urea and a 5% polyacrylamide stacking gel was used. Electrophoresis was conducted with a constant current of 35 mA for 1 hour. These

gels were either stained with zinc imidazole, according to the method of Hardy *et al.* (17) or transferred to nitrocellulose membrane (Micron Separations Inc. Westboro MA, USA poresize 0.22μm) according to the methods described by Towbin *et al.* (32). Nitrocellulose membranes, with transferred LPS, were probed with the primary antibodies described above, diluted 1:500 and bound antibodies detected with goat anti-mouse antibodies conjugated to HRP (24). Blots were developed using an enhanced chemiluminescence kit (Amersham Life Sciences, Canada) according to the manufacturer's specifications, and images were visualized on BioMax BM film (Kodak, Rochester NY, USA).

2.2.4. Growth Curve Determinations.

Isolates were selected based upon the primary examination of the Lewis antigen status from agar growth for their expression of a single or multiple Lewis antigens. *H. pylori* from frozen stock were grown as described above and emulsified from an agar plate into BHI broth (3.7% BHI [Difco], 0.5% yeast extract, 15 μg/ml of both vancomycin and amphoterocin B, 50 ml/L of fetal bovine serum) and used to inoculate 25 ml volumes of BHI broth. The cultures were allowed to grow for 48-72 hours on a shaking incubator at 37°C until a turbid culture was observed (>0.2 ODU at 600_{nm} (optical density units)). The optical density of the cultures were adjusted to 0.2 ODU at 600_{nm} by dilution in BHI broth corresponding to a cell density of approximately 10^6 cfu/ml. This culture was then diluted (1:100) in fresh 25 ml BHI broths. Cultures were then monitored for growth by an increase in the optical density. Aliquots were removed at each time point and frozen until the completion of the experiment when the *H. pylori* cells were removed by centrifugation (5000 g for 8 minutes), resuspended in 0.9% saline and adjusted to a protein concentration of 10 μg/ml as determined by BCA protein assay according to manufacturer's instructions (Pierce Inc). The Lewis antigen expression was then analyzed by ELISA.

2.2.5. Analysis of *H. pylori* Supernatants for Lewis Antigens.

Equal volumes (10μl) of supernatant material were spotted onto nitrocellulose sheets and allowed to dry overnight at 37°C. Resulting membranes were probed in a similar manner to the immunoblots described above using anti-Lewis antigens MAbs as the primary antibody and goat anti mouse IgG + IgM conjugated to HRP as the secondary antibody. The concentrations of antibodies used were identical to those used with the immunoblots described above. Blots were developed using an enhanced chemiluminescence kit (Amersham Life Sciences, Canada) according to the manufacturer's specifications, and images were visualized on BioMax BM film (Kodak, Rochester NY, USA). The difference of the intensity of the staining of the supernatant was scored visually.

2.2.6. Statistical Analysis.

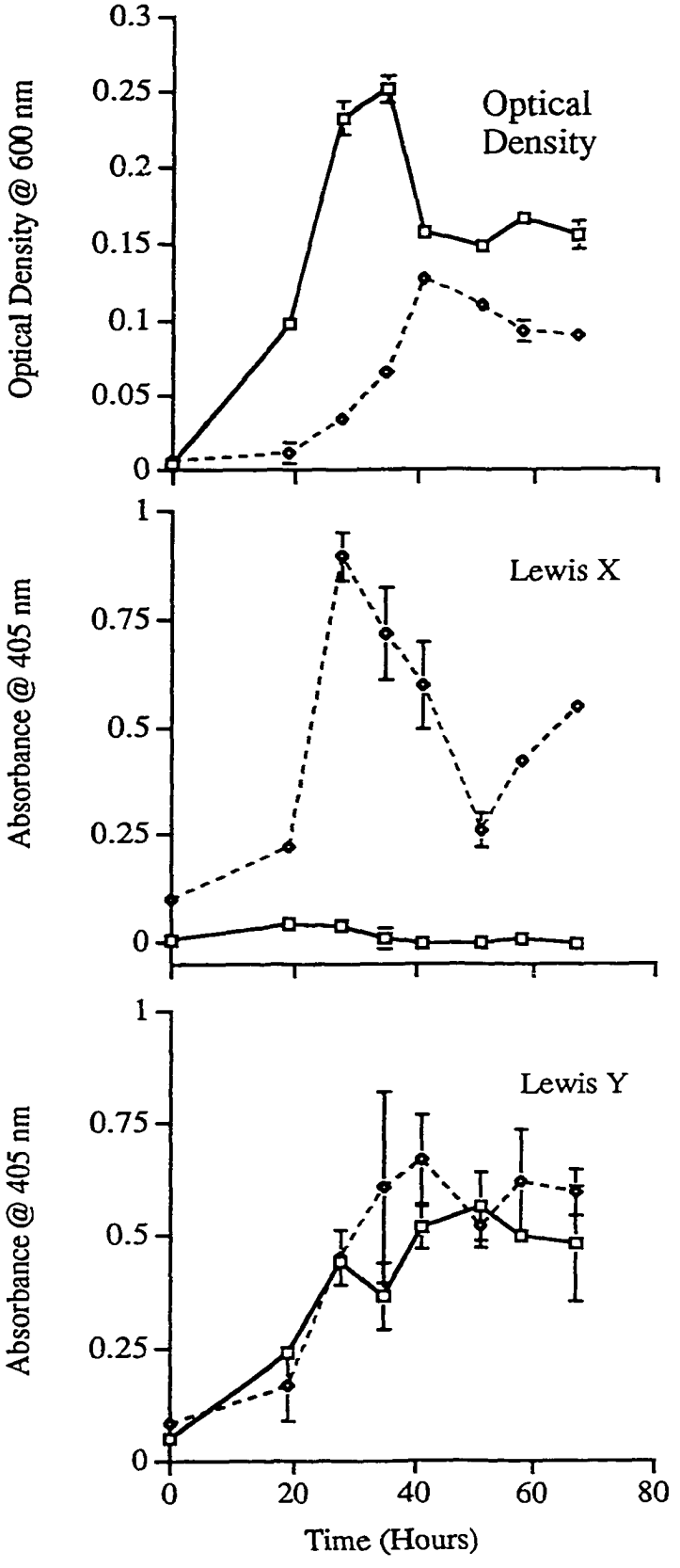
Proportions were compared using the χ^2 test. All P values were calculated for significance levels and values of <0.001 were considered significant.

2.3. Results

2.3.1. Lewis Antigen Expression by *H. pylori* During Growth.

Isolates were chosen to examine the stability of the Lewis antigen expression of *H. pylori* based on the antigen profile of agar grown biomass. UA802 demonstrated an exclusively Le^y phenotype whereas UA1182 expressed both Le^x and Le^y simultaneously from both agar grown (not shown) or liquid grown cells (Figure 2.1). The expression of the Lewis antigens per microgram of protein over a period of growth does not appear to be stable. As the *H. pylori* population passed through the logarithmic phase of growth (~45 hours) there was an increase in the amount of Lewis antigen expressed per microgram of protein (Figure 2.1). The expression of a single or more than one Lewis antigen did not affect this result as, UA802, expressing only Le^y has the same general expression profile as UA1182 which expresses both Le^x and Le^y. In UA1182 the level of the Le^x antigen

Figure 2.1. Growth phase dependent expression of Lewis antigens by *H. pylori* in BHI broth at 37°C. UA802 (squares and solid lines) and UA1182 (diamonds and dashed lines) were followed over a cycle of growth monitored by optical density. Growth phase dependent expression is observed by *H. pylori* with a single antigen (UA802 - Le^y) or two antigens (UA1182 - Le^x and Le^y). The greatest expression occurs in the logarithmic growth phase for the Le^x antigen (UA1182) and decreases significantly in the stationary phase. The expression of the Le^y antigen by both isolates increases expression in the logarithmic phase of growth and plateaus in the stationary phase.



reaches maximal expression before the Le^y antigen. This trend was also evident among other isolates investigated (data not shown) and is in agreement with the proposed enzymatic synthesis of these antigens (36). There was a decrease in the amount of Lewis antigens expressed as the *H. pylori* cells enter stationary phase growth and the expression level plateaus during this phase of growth.

2.3.2. Analysis of *H. pylori* Supernatant for Lewis Antigens.

The examination of the supernatant material revealed that an increasing amount of Lewis antigens are present in the culture supernatant as the *H. pylori* reached stationary phase (Table 2.1). Suggesting that increasing amounts of Lewis antigens were being shed into the media as growth proceeded. This observation also does not appear to be specific to any one Lewis antigen as both the UA802 (Le^y only) and UA1182 (Le^x and Le^y simultaneously) exhibit this behaviour (Table 2.1).

2.3.3. Lewis Antigen Distribution of *H. pylori* Isolates from Symptomatic and Asymptomatic Individuals.

It was hoped that the comparison of the Lewis antigen expression of the *H. pylori* from symptomatic patients and asymptomatic subjects would provide possible insight into the disease process.

The expression of Le^a and Le^b was rare among *H. pylori* isolates. However, in this study only 4.8% (19/156) of the *H. pylori* isolates from symptomatic patients exhibited expression of these antigens, whereas none of the isolates obtained from asymptomatic subjects exhibited these phenotypes (Table 2.2). The proportion of Le^b expressing isolates is artificially increased due to reaction of the monoclonal antibodies with Le^b precursor structures providing a false positive signal (24). Because of this increased cross-reactivity of the Le^b MAb we assumed that even the precursor structures for the type I antigens, unfucosylated backbone or the H Type II antigen (Figure 1.3) are not present in any *H. pylori* isolate from the asymptomatic subjects. Only one isolate was

Table 2.1 : Expression of Lewis Antigens in Culture Supernatants

Growth Phase	UA802		UA1182	
	Lewis X	Lewis Y	Lewis X	Lewis Y
Lag	-	+/-	+/-	+/-
Logarithmic	-	+	+	++
Stationary	-	++	+	++

Table 2.2:Incidence of Lewis Antigen Expression by *H. pylori*

Antigen	Symptomatic (n=156)		Asymptomatic (n=159)	
	Number of Isolates	Percent	Number of Isolates	Percent
Lewis A	4	1.6	0	0
Lewis B	15[a]	3.2	0	0
Lewis X[b]	96	58.1	63	39.6
Lewis Y	118	71	102	64.2
NonTypable[c]	3	4.8	47	30
Lewis X and Lewis Y	72	41.9	53	33.3
Lewis X only	19	14.5	10	6.2
Lewis Y only	38	30.6	57	35.8

[a] Isolates were identified on the basis of ELISA, which has shown to not be reliable in the identification due to cross reaction of the MAb (24).

[b] The numbers of isolates with Le^x expression is significantly different between the two groups (χ^2 value 15.25, $P<0.001$).

[c] The numbers of isolates with the NTHp phenotype are significantly different between these two groups (χ^2 value 43.04, $P<0.001$).

identified by ELISA to express both Le^a and Le^b simultaneously. When the proportion of Le^a and Le^b expressing *H. pylori* are examined, a significant difference between the two study groups is observed ($\chi^2 = 20.64$, $P<0.001$).

The type II carbohydrate antigens (Le^x and Le^y) were reported to be expressed by greater than 80% of *H. pylori* symptomatic isolates (28, 38, 39), but a comparison of the expression of these antigens between isolates from symptomatic and asymptomatic individuals has not been reported previously. We observed that 71% of the *H. pylori* isolates from symptomatic patients expressed Le^y, whereas 64.2% of the isolates from asymptomatic subjects express this phenotype. The proportion of *H. pylori* isolates expressing only the Le^y antigen is slightly increased among the isolates from asymptomatic subjects. Among the *H. pylori* from the asymptomatic individuals 35.8% demonstrated this phenotype, whereas 30.6% of the symptomatic isolates expressed only Le^y (Table 2.1). None of the differences noted among the distribution of the Le^y antigen are statistically significant.

The distribution of the Le^x antigen is significantly altered when the *H. pylori* from the two subject groups are compared (Table 2.2). *H. pylori* Le^x expression decreases significantly from 58.1% among the isolates from symptomatic individuals to 39.6% of the *H. pylori* obtained from the asymptomatic group ($\chi^2 = 15.25$, $P<0.001$). The proportion of isolates exclusively displaying the Le^x antigen is also decreased among *H. pylori* from the asymptomatic subjects. Among the *H. pylori* from symptomatic patients 14.5% expressed this phenotype, whereas only 6.2% of isolates from the asymptomatic subjects displayed only the Le^x antigen (Table 2.1). This difference was determined not to be significant by the level of confidence used in this study. As would also be expected from the decrease in overall Le^x expression, the expression of Le^x and Le^y simultaneously was also decreased among the asymptomatic subjects' isolates, but not by a significant amount.

The most striking difference between these two groups of individuals is in the proportion of *H. pylori* isolates that do not express any of the Lewis antigens and thus are designated non-typable *H. pylori* (NTHp) {previously referred to as Penner serotype O:2 (28)}. The proportion of isolates exhibiting this phenotype increases from 4.8% of the *H. pylori* from symptomatic individuals to 30% of the asymptomatic subjects isolates (χ^2 = 43.04, P<0.001). The difference between the proportions of this phenotype among the two groups examined is the largest observed in this study.

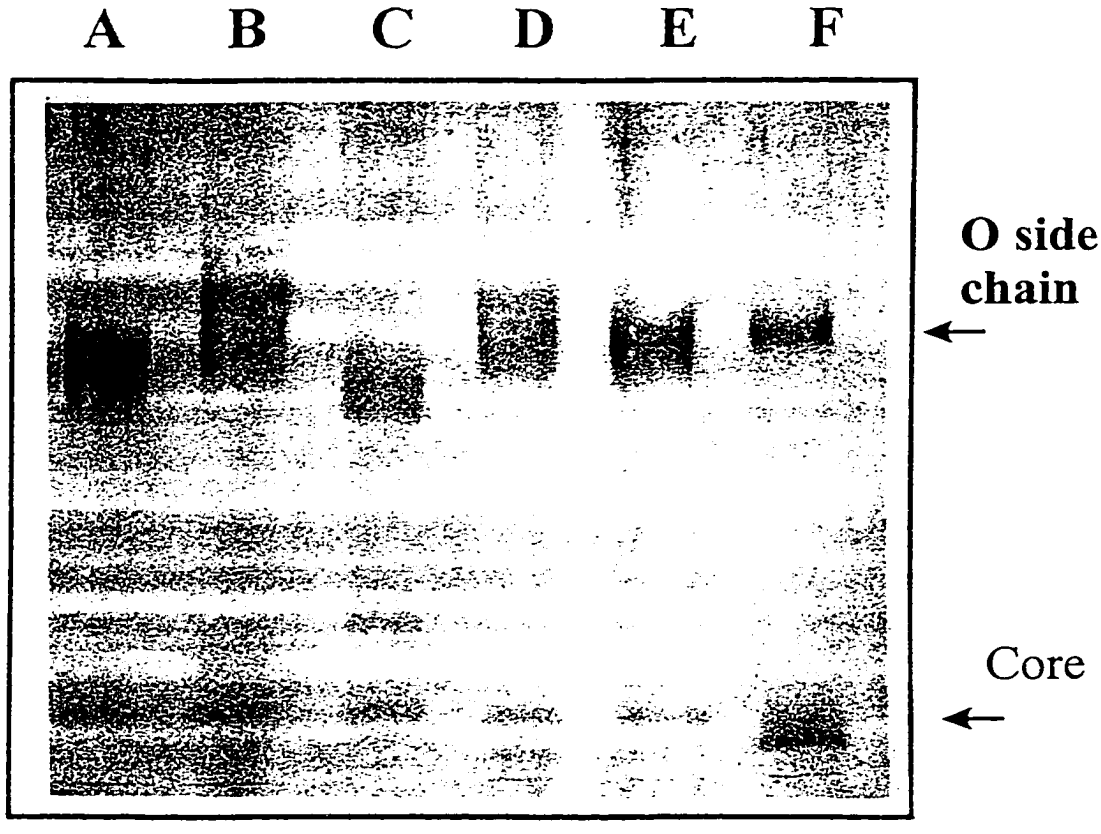
2.3.4. Expression of Lewis Antigens Investigated by Immunoblot

LPS was prepared from a selected group of isolates that expressed both typical (Le^x and Le^y) and atypical (Le^a, Le^b and NTHp) antigens as measured by ELISA. Figure 2.2 is an example of a typical immunoblot which contains isolates that have reacted with the Le^a, Le^x and Le^y monoclonal antibodies. Reactions similar to the ones observed in Figure 2.2 were typical for all other isolates tested. A positive immunoblot reaction for the Le^b antigen was not observed until recently (see Chapter 5). The LPS from one isolate that exhibited a positive reaction for Le^b by ELISA was also investigated by immunoblot, but did not provide a positive reaction (Figure 2.2 lane B), but the ELISA results were usually confirmed by immunoblotting.

NTHp isolates were also examined by polyacrylamide gel electrophoresis and immunoblot to determine if these isolates expressed an LPS O side chain and were capable of Lewis antigen expression. A selection of the LPS of six NTHp isolates is shown in Figure 2.3 demonstrating that the NTHp do express an O side chain, but immunoblots confirmed that these O side chains do not contain Lewis antigens (not shown). To date there has been only one NTHp isolate identified that lacked O side chain expression (Chapter 3, Figure 3.2). Interestingly, we observed that the LPS from the NTHp did not all have the same gel mobility. This may indicate that the LPS carbohydrate composition is also variable. There is also another band with similar mobility to the core carbohydrates

Figure 2.2. Immunoblot of *H. pylori* LPS samples demonstrating reactivity with anti-Lewis MAbs. Lanes A -UA948, Lanes B - UA955, Lanes C - UA1182. UA948 and UA955 both expressed Le^x, UA948 also expressed Le^a. By ELISA UA955 expressed Le^b, no reactivity is demonstrated by immunoblot. Both UA955 and UA1182 expressed Le^y.

Figure 2.3. Analysis of NTHp Isolates. Proteinase-K treated whole cell lysates of six isolates which were determined to be NTHp by ELISA. All demonstrate the presence of an O side chain by staining with Zn-Imidazole, but none reacted by immunoblot (data not shown). Isolates are as follows A - 1C, B - 7A, C - 12C, D - 62A , E - 75A , F - 77C from the culture collection maintained at the University of Alberta.



that is variable among the LPS of these isolates. The identity of this band has not been determined.

2.4. Discussion

This work has demonstrated that the expression of the Lewis antigens by *H. pylori* is not constant during the growth phases of the organism (Figure 2.1). The antigen expression reached a maximum in the logarithmic phase of growth and decreased or plateuas as the *H. pylori* reach stationary phase. It has been previously observed that the membrane characteristics of an *H. pylori* cell change upon entry into the coccoid phase (9, 29). It may be that the decrease in the Lewis antigen expression is related to these membrane alterations resulting in a loss of membrane integrity. When supernatant material was tested for reactivity with the anti-Lewis antigen MAbs the reaction was intensified in the supernatant of the later growth period samples, indicating that more Lewis antigens were being shed into the supernatant during the stationary phase of growth (Table 2.1). It is possible that when the *H. pylori* enter the stationary phase growth they release pieces of membrane containing the Lewis antigens which may act as an immunological decoys, thus protecting the remaining organisms.

What role this growth phase dependent expression of these antigens plays is still under investigation. It has been noted by Dr. Simala-Grant (in Dr. Taylor's Lab) that newly divided *H. pylori* cells have close to a 100% adhesion rate to AGS cells (gastric carcinoma cell line) whereas only ~10% of a culture obtained from agar grown *H. pylori* adhere to the same cells (Dr. Simala-Grant, personal communication). The expression of the Lewis antigens on the LPS is developmentally regulated. Adherence of other antigens to a specific factor early in the *H. pylori* life cycle may be compromised by the Lewis antigens. It is possible that the "early" receptor is also carbohydrate based and the expression of the Lewis antigens inhibits efficient adhesion to these factors during later growth phases. A number of carbohydrate adhesins have been proposed for *H. pylori* such as Le^b (11) and sialic acid containing carbohydrates (14, 27) that may be inhibited by

Lewis antigen expression. It has also been noted by Valkonen *et al.* (33) that the LPS plays a role in the adhesion of *H. pylori* to the gastric epithelium. It was not determined which portion of the LPS was responsible for adhesion, but it was observed that a 25 kDa protein was also involved in the adhesive process (33). This protein associated with the LPS in a lectin like process and it was this complex that is believed to be responsible for adhesion to laminin (33). The developmental regulation of the Lewis antigens may allow for adhesion of new progeny to certain receptors while the expression of the LPS-lectin like complex directs adherence to other areas or niches within the stomach. Further work is needed to examine the role of these antigens in adhesion.

Comparison of the Lewis antigen expression of the *H. pylori* isolates from symptomatic and asymptomatic individuals demonstrates an important antigenic difference between these two groups of *H. pylori* isolates. Implicating the Lewis antigens in the disease causing process. One point to mention is the definition of an asymptomatic versus symptomatic infection. There are individuals who get infected with *H. pylori* and choose to "live with" the symptoms and not seek medical treatment, but there are also the individuals who are truly asymptomatic. In this work subjects were deemed asymptomatic if they were not exhibiting any overt disease symptoms. Three significant differences were noted between the two groups of *H. pylori* isolates. The first difference is that type I antigen expression is absent among *H. pylori* from asymptomatic subjects. The second difference is a decrease in Le^x-expressing *H. pylori* obtained from asymptomatic individuals and finally the observation of a significant increase in the proportion of the NTHp among *H. pylori* isolated from asymptomatic subjects (Table 2.2).

The absence of *H. pylori* isolates that express the type I carbohydrate antigens in asymptomatic individuals indicates that expression of the type I antigens by the isolate may predispose the individual to develop a more severe disease. The type I antigens may provide a stronger immunological target than do Le^x or Le^y which results in a more severe pathology caused in part by the host immune response. The proportion of isolates

displaying this phenotype was very low among isolates from symptomatic individuals (<5%) and it may be that the level of these antigens among the *H. pylori* from asymptomatic subjects is so low that it is not identified in this study, but that in itself indicates that a difference does exist. The role of the LPS antigens during infection is not clear but the inclusion of isolates that express these alternative antigens in animal model studies may provide clues as to why this LPS glycotype family is not represented in *H. pylori* from asymptomatic subjects.

It has been previously noted that Le^x-expressing isolates were identified more often from patients who had peptic ulcer disease (19, 23) and more recently a direct link between expression of Le^x and increased pathology has been reported (18). It is now clear that the expression of Le^x is linked to an increased inflammatory response and thus also linked to increased pathology. We observed a lower than expected proportion of Le^x-expressing *H. pylori* obtained from individuals with no disease symptoms (Table 2.2), providing additional confirmation that the Le^x antigen is correlated with the disease states. The selection over time of *H. pylori* isolates with decreased potential to cause disease may have lead to the development of the asymptomatic infection. A symbiosis may have developed in which the *H. pylori* do not cause a severe disease and the host does not clear the *H. pylori* with a self damaging, potentially autoimmune, response. It is estimated that greater than 80% of the *H. pylori* infected people in the world have an asymptomatic infection (13), leading to the conclusion that in most cases a symbiosis has been achieved and only in the cases where the immune response is overactive or bacterial factors are damaging does the pathology occur.

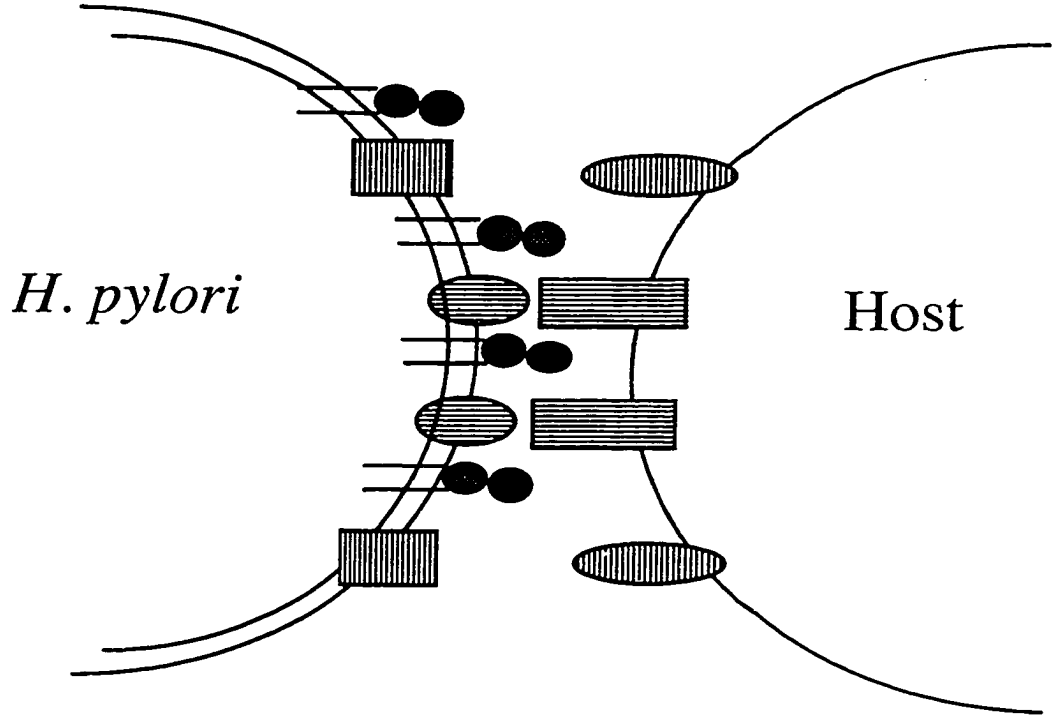
The increased proportion of NTHp identified among the *H. pylori* from asymptomatic subjects may also be an evolutionary trend toward decreased immune responses. If the host immune response is attracted to the Lewis antigens contained in the LPS the best way of preventing that response and possibly establishing a chronic disease state is to not produce those antigens. The studies of inflammatory responses in relation to

Lewis antigens did not include any NTHp isolates (18, 19, 23). It will be necessary to investigate the immune response, colonization and inflammation capabilities of the isolates that contain these LPS antigens in both *in vitro* and *in vivo* model systems to see if any differences are evident.

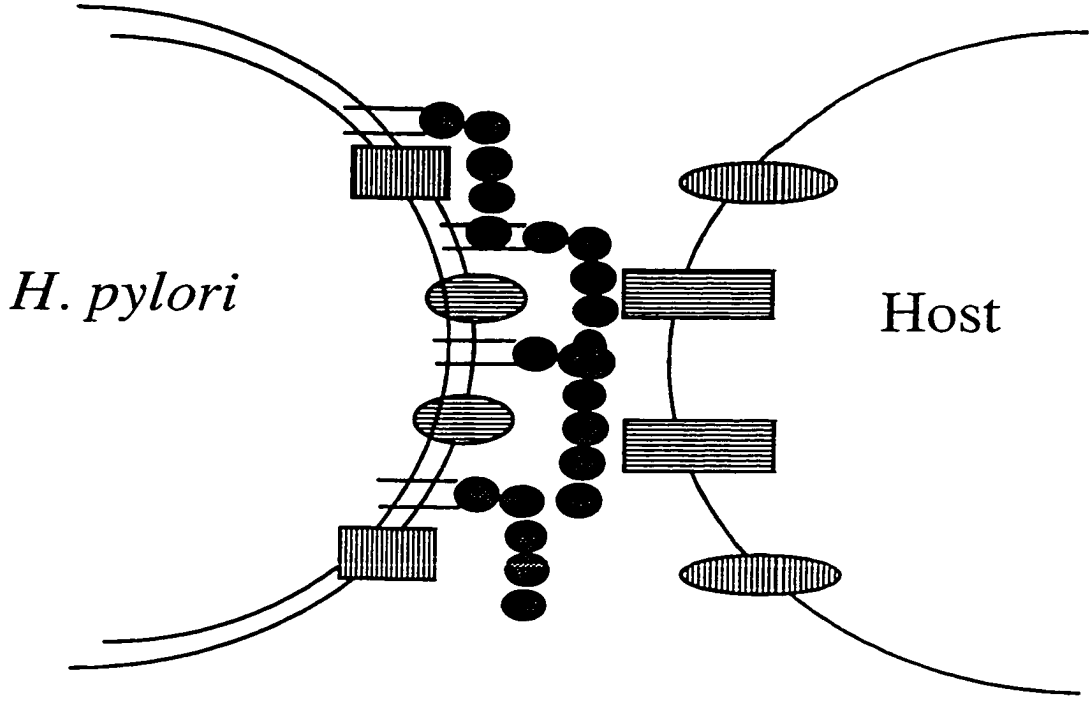
In the present study, and all others to date, the isolates that do not express the Lewis antigens are classified together into a single group (28). Preliminary detailed chemical analyses have revealed the carbohydrate composition of the LPS produced by the NTHp to be non-identical among this group. Some of the NTHp LPS carbohydrate structures are similar to the Lewis antigens but they do not contain the complete antigenic structures and thus do not react with the MAbs used in this study (Monteiro and Rasko unpublished). The LPS of one asymptomatic isolate, MO19, has been chemically characterized and shown to contain the Le^y antigen (6). The one significant difference in the LPS composition of this isolate was that it contained DD-heptan polymer as a part of the O side chain of LPS (6). This region was termed the intervening region because it was between a typical Lewis antigen containing O side chain and an *H. pylori* LPS core structure that was identical to the ones previously characterized (7). This region has also been found in the LPS other isolates designated O:3 and O:6 in the Penner serotyping system (8) which both express Lewis antigens. The linkage in this region could not be conclusively determined, but the presence of this region provides increased length and possibly increased flexibility to the LPS O-side chain. Interestingly, four of the seven isolates displayed in Figure 2.3 also express the DD-heptan intervening region (unpublished data). It has been suggested that LPS does not always extend away from the bacterial cell membrane but can bend to cover the bacterial surface and interfere with the interactions of bacterial virulence factors and the epithelium of host cells (10, 34). If this intervening region of the *H. pylori* LPS increases flexibility it may allow the LPS to mask other antigens on the bacterial surface which could cause symptomatic infection (Figure 2.4). Further work must be done to characterize the antigens expressed by these

Figure 2.4. Possible mechanisms for LPS O side chain interactions with other bacterial factors to determine the outcome of infection. **A.** short chain LPS (hatched circles) allows the interaction of *H. pylori* factors with host factors (horizontal and vertical lined ovals and rectangles), possibly leading to a symptomatic infection. **B.** Longer O side chain containing LPS may interfere with the interactions of host and pathogen factors. The LPS O side chain may play a role in the adhesion and colonization, but block further progression to symptomatic disease.

A.



B.



NTHp isolates and to determine if these LPS antigens play a direct role in the lack of disease symptoms in those individuals.

Investigations of the distribution of Lewis antigens has lead to conclusion that the pathology caused by an *H. pylori* isolate may, in part, be linked to the Lewis antigens it expresses. A direct relationship appears to exist between the expression of the LPS antigens by *H. pylori* and the disease states (18), but it is not clear what role the LPS antigens play. A significant proportion of the isolates from asymptomatic individuals still expressed Le^x (39.6%), and yet caused no disease, while NTHp have been isolated from individuals who do exhibit disease symptoms (28). In summary, this indicates that the Lewis antigens contained within the LPS are not the only factor responsible for the symptoms observed during infection. The relative level of the Lewis antigen expression varies during the growth of the organism potentially allowing the exploitation of variable adhesive mechanisms during growth. Further work into the role of LPS antigens in the disease process is required.

2.5. Bibliography

1. **Amano, K., Hayashi, S., Kubota, T., Fijii, N. and Yokata, S.** 1997. Reactivities of Lewis monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. Clin. Diag. Lab. Immunol. **4**: 540-544.

2. **Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M. J. E. and Kusters, J. G.** 1999. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α3-fucosyltransferase genes. Infect. Immun. **67**: 5361-5366.

3. **Appelmelk, B. J., Shiberu, B., Trinks, C., Tapsi, N., Zheng, P. Y., Verboom, T., Maaskant, J., Hokke, C. H., Schiphorst, W., Blanchard, D., Simoons-Smit, I. M., van den Eijnden, D. H. and VandenbrouckeGrauls, C.** 1998. Phase variation in *Helicobacter pylori* lipopolysaccharide. Infect. Immun. **66**: 70-76.

4. **Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A. and Degraaff, J.** 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun. **64**: 2031-2040.

5. **Aspinall, G., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1994. O antigen chains in the lipopolysaccharide of *Helicobacter pylori* NCTC11637. Carbohydr. Lett. **1**: 151-156.

6. **Aspinall, G. O. and Monteiro, M. A.** 1996. Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. Biochem. **35**: 2498-2504.

7. **Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1996. Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. Biochem. **35**: 2489-2497.

8. **Aspinall, G. O., Monteiro, M. A., Shaver, R. T., Kurjanczyk, L. A. and Penner, J. A.** 1997. Lipopolysaccharides of *Helicobacter pylori* serogroups O:3 and O:6; Structures of a class of lipopoligosaccharides with reference to location of oligomeric units of D-glycero-α-D-*manno*-heptose residues. Eur. J. Biochem. **248**: 592-601.

9. **Benaissa, M., Babin, P., Quellard, N., Pezennec, L., Cenatiempo, Y. and Fauchere, J. L.** 1996. Changes in *Helicobacter pylori* ultrastructure and antigens during conversion from the bacillary to the coccoid form. Infect. Immun. **64**: 2331-2335.

10. **Bilge, S. S., Vary, J. C., Dowell, S. F. and Tarr, P. I.** 1996. Role of the *Escherichia coli* O157:H7 O side chain in adherence and analysis of the *rfb* locus. Infect. Immun. **64**: 4795-4801.

11. **Boren, T., Falk, P., Kevin, A. R., Larson, G. and Normark, S.** 1993. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. **262**: 1892-1895.

12. **Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R. and Kirchner, T.** 1998. The gastric H+,K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. Gastroenterology. **115**: 340-347.

13. **Dunn, B. E., Cohen, H. and Blaser, M. J.** 1997. *Helicobacter Pylori.* Clin. Microbiol. Rev. **10**: 720-741

14. **Evans, D. G., Evans Jr., D. J., Moulds, J. J. and Graham, D. Y.** 1988. N-acetylneuraminyllactose-binding fimbrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor. Infect. Immun. **56**: 2896-2906.

15. **Ge, Z. and Taylor, D. E.,** 1999. Contributions of genome sequencing to the understanding of the biology of *Helicobacter pylori* in Ann. Rev. Microbiol. **53**:353-387.

16. **Gibson, J. R., Chart, H. and Owen, R. J.** 1998. Intra-strain variation in expression of lipopolysaccharide by *Helicobacter pylori.* Lett. Appl. Microbiol. **26**: 399-403.

17. **Hardy, E., Pupo, E., Castellanos-Serra, L., Reyes, J. and Fernandez-Patron, C.** 1997. Sensitive reverse staining of bacterial lipopolysaccharides on polyacrylamide gels by using zinc and imidazole salts. Anal. Biochem. **244**: 28-32.

18. **Heneghan, M. A., McCarthy, C. F. and Moran, A. P.** 2000. Relationship of blood group determinants on *Helicobacter pylori* lipopolysaccharide with host Lewis phenotype and inflammatory response. Infect. Immun. **68**: 937-941.

19. **Heneghan, M. A., Moran, A. P., Feeley, K. M., Egan, E. L., Goulding, J., Connolly, C. E. and McCarthy, C. F.** 1998. Effect of host Lewis and ABO blood group antigen expression on *Helicobacter pylori* colonization density and the consequent inflammatory response. FEMS Immunol. Med. Microbiol. **20**: 257-266.

20. **Hitchcock, P. J. and Brown, T. M.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver stained polyacylamide gels. J. Bacteriol. **154**: 269-277.

21. International Agency for Research on Cancer. 1994. Shistosomes, liver flukes and *Helicobacter pylori.* WHO Monograph 61. Lyon, France:IARC, pp. 177-240.

22. **Janvier, B., Grignon, B., Audibert, C., Pezennec, L. and Fauchere, J. L.** 1999. Phenotypic changes of *Helicobacter pylori* components during an experimental infection in mice. FEMS Immunol. Med. Microbiol. **24**: 27-33.

23. **Marshall, D. G., Hynes, S. O., Coleman, D. C., O'Morain, C. A., Smyth, C. J. and Moran, A. P.** 1999. Lack of a relationship between Lewis antigen expression and *cagA*, Cag, *vacA*, and VacA status of Irish *Helicobacter pylori* isolates. FEMS Immunol. Med. Microbiol. **24**: 79-90.

24. **Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P. and Perry, M. B.** 1998. Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem. **273**: 11533-11543.

25. **Moran, A. P., Prendergast, M. M. and Appelmelk, B. J.** 1996. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. FEMS Immunol. Med. Microbiol. **16**: 105-115.

26. **Rasko, D. A., Wilson, T. J. M., Zopf, D. and Taylor, D. E.** 2000. Lewis antigen expression and stability in *Helicobacter pylori* isolated from serial gastric biopsies. J. Infect. Dis. **181:** 1189-1195.

27. **Simon, P. M., Goode, P. L., Mobasseri, A. and Zopf, D.** 1997. Inhibition of *Helicobacter pylori* binding to gastrointestinal epithelial cells by sialic acid containing oligosaccharides. Infect. Immun. **65**: 750-757.

28. **Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B. S., Rudnica, W., Savio, A. and De Graaff, J.** 1996. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol. **34**: 2196-2200.

29. **Sorberg, M., Nilsson, M., Hanberger, H. and Nilsson, L. E.** 1996. Morphologic conversion of *Helicobacter pylori* from bacillary to coccoid form. Eur. J. Clin. Microbiol. Infect. Dis. **15**: 216-219.

30. **Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W. and Jewell, L. D.** 1987. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. Am. J. Clin. Path. **87**: 49-54.

31. **Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C. and Jewel, L. D.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

32. **Towbin, H., Staehelin, T. and Gordon, J.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. **76**: 4350-4354.

33. **Valkonen, K. H., Wadstrom, T. and Moran, A. P.** 1997. Identification of the N-acetylneuraminyllactose specific laminin binding protein of *Helicobacter pylori*. Infect. Immun. **65**: 916-923.

34. **Voorhis, D. L., Dillon, S., Formal, S. B. and Isberg, R. R.** 1991. An O antigen can interfere with the function of the *Yersinia pseudotuberculosis* invasin protein. Mol. Microbiol. **5**: 317-325.

35. **Wang, G., Boulton, P. G., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1999. Novel *Helicobacter pylori* α1,2-fucosyltransferase, a key enzyme in the synthesis of Lewis antigens. Microbiology. **145**: 3245-3253.

36. **Wang, G., Rasko, D. A., Sherburne, R. and Taylor, D. E.** 1999. Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori* : analysis of the α(1-2) fucosyltransferase gene. Mol. Microbiol. **31**: 1265-1274.

37. **Wirth, H.-P., Yang, M., Peek, R. M., Hook-Nikanne, J., Fried, M. and Blaser, M. J.** 1999. Phenotypic diversity in Lewis expression of *Helicobacter pylori* isolates from the same host. J. Lab. Clin. Med. **133**: 488-500.

38. **Wirth, H. P., Yang, M. Q., Karita, M. and Blaser, M. J.** 1996. Expression of the human cell surface glycoconjugates Lewis X and Lewis Y by *Helicobacter pylori* isolates is related to CagA status. Infect. Immun. **64**: 4598-4605.

39. **Wirth, H. P., Yang, M. Q., Peek, R. M., Tham, K. T. and Blaser, M. J.** 1997. *Helicobacter pylori* Lewis expression is related to the host Lewis phenotype. Gastroenterology. **113**: 1091-1098.

40. **Yokota, S., Amano, K., Shibata, Y., Nakajima, M., Suzuki, M., Hayashi, S., Fujii, N. and Yokochi, T.** 2000. Two distinct antigenic types of polysaccharide chains of *Helicobacter pylori* lipopolysacchairdes characterized by reactivity with sera from humans with natural infection. Infect. Immun. **68**: 151-159.

41. **Yokota, S.-I., Amano, K.-I., Hayashi, S., Kubota, T., Fujii, N. and Yokochi, T.** 1998. Human antibody response to *Helicobacter pylori* lipopolysaccharide: presence of an immunodominant epitope in the polysaccharide chain of lipopolysaccharide. Infect. Immun. **66**: 3006-3011.

Chapter 3

Lewis Antigen Expression and Stability in *Helicobacter pylori* Isolated From Serial Gastric Biopsies

A version of this work has been published previously:
Rasko, D. A., Wilson, T. J. M., Zopf, D. and Taylor, D. E. 2000. Lewis antigen expression and stability in *Helicobacter pylori* isolated from serial gastric biopsies. J. Infect. Dis. **181:** 1189-1195.

3. Preface

The isolates investigated in this study were obtained as part of the phase II trial of the *H. pylori* anti-adhesive carbohydrate therapy (Neose Technologies Inc). The isolates were obtained from asymptomatic subjects enrolled in the study over the 86 day study period. The observations made over even this short period of time may provide insight into *H. pylori* pathogenic processes. Isolation of *H. pylori* from the same patient over a short period of time allowed observation of the stability of the Lewis antigens expressed by these isolates. This situation is rarely observed in the clinical laboratory, because if a second or third biopsy is taken, it is usually many years after the primary isolate and reinfection by *H. pylori* has been demonstrated to occur. I used ELISA and immunoblot techniques described in Chapter 2 to investigate Lewis antigen expression. Recent reports of isolation of multiple colonies from the primary culture plate have demonstrated that the LPS structures vary greatly from apparently genetically identical isolates and thus the genetic relatedness of the isolates was investigated by RAPD-PCR. The work included in this chapter demonstrates that the *H. pylori* Lewis antigen expression is variable during infection.

3.1. Introduction

Chronic infection with *Helicobacter pylori* is the most common cause of recurrent gastroduodenal inflammatory disease, including gastric and duodenal ulcers (19, 32, 45). *H. pylori* is now recognized as one of the most ubiquitous infectious organisms with greater than 50% of the worlds' population being infected (39). A causative role for *H. pylori* in the development of both gastric adenocarcinoma (14, 31) and lymphoma of mucosa-associated lymphoid tissue (48) has been identified, leading to the assignment of a class 1 carcinogen status to this bacterium (22). Extensive studies of this organism in recent years have yielded significant information concerning the infectious process, especially Lewis antigen expression and their role (7, 17).

Lipopolysaccharide (LPS) is an essential component of the gram-negative cell envelope. LPS plays a critical role in the structure and function of the outer membrane (30), as well as functioning as a toxin and it has been shown that *H. pylori* LPS contains the Lewis antigenic structures (8-10, 27). The Lewis structures within the LPS are thought to provide a mechanism by which the *H. pylori* can evade the immune system by mimicking the Lewis antigens expressed on the gastric epithelium (5, 29). Simoons-Smit *et al.* (35) have identified a small population of *H. pylori* isolates that did not express Lewis antigens as a component of the LPS, yet still caused infection. It has been recently demonstrated that multiple isolates obtained from a single gastric biopsy can yield different LPS profiles (18, 46) indicating that the LPS antigen expression is variable after *in vivo* growth.

When grown *in vitro*, the rate of phase variation of the Lewis antigens in *H. pylori* has been demonstrated to be 0.2-0.5% (6). The fucosyltransferase (*fucT*) genes are responsible for the addition of fucose to the carbohydrate backbone of the O side chain, which is the final step in Lewis antigen production (15, 25). A proposed mechanism of antigenic variation has recently been shown to involve slip strand mispairing during replication at the polynucleotide tracts contained in the *fucT* genes (4). A mechanism

which can overcome the deleterious effects of the slip strand mispairing has recently been identified (44). The identification of a translational frameshift cassette in the α(1,2) *fucT* gene of *H. pylori* allows the production of functional protein from a truncated open reading frame containing a stop codon caused by slip strand mispairing (44). A definite biological role for antigenic variation of the Lewis antigens has not yet been identified, although the antigenic variation in other organisms allows increased persistence and/or pathogenicity (29).

3'sialyllactose (3'SL) was previously demonstrated to be an receptor for *H. pylori* on the epithelial cell surface (12, 13). A further study provided direct evidence that the free oligosaccharide, 3'SL, could inhibit *H. pylori* adhesion to tissue culture cells *in vitro* (34). Recently, a human clinical trial was undertaken to evaluate the safety and efficacy of orally administered 3'SL on gastric *H. pylori* colonization in *H. pylori*-positive asymptomatic adults. In this report we describe the variability of *H. pylori* Lewis antigens among genetically identical serial *H. pylori* isolates obtained during the 86 day sampling period.

3.2. Experimental Procedures

3.2.1. Study Subjects

The study subjects were non-related, asymptomatic adults (ages 22-65 years) who scored positive on a ^{13}C-urea breath test (Meretek, Houston TX) at screening and were shown by subsequent exploratory endoscopy to be free of gastric or duodenal ulcer disease. Two biopsies taken at endoscopy - one from the antrum and the other from the greater curvature of the corpus - were submitted for histology and culture, and subjects were enrolled in the study if either test was positive. Demographic characteristics of the 26 study subjects are shown in Table 3.1. Subjects were randomized to be mock treated, receiving microcrystalline cellulose as a placebo (six subjects), or 3'SL in doses of 1 gram (six subjects) or 2 grams (seven subjects) four times per day given with meals and with an

Table 3.1.

Characteristics of Study Subject Population

	Male	Female
Number	14	12
Mean age (range)	45 (22-65)	51 (35-65)
Hispanic	7	3
African-American	1	2
Caucasian	6	7

evening snack for 56 days. The remaining seven subjects received 5 grams of 3'SL four times per day for 28 days.

Subjects in the 56-day dosing groups were monitored with urea breath tests on study days 0, 14, 27, 42, 55 and 86 (30 days post-treatment), and endoscopic biopsies were performed on days 0, 28, 56, and 86. Subjects in the 28-day dosing group were monitored with urea breath tests on days 0, 14, 27 and 55, with endoscopic biopsies on days 0, 28 and 56. Incomplete data is included from one subject in the 5 gram dosing group who withdrew for personal reasons, but returned to the clinic for a follow-up endoscopic biopsy. Biopsy samples taken from the 2 distant sites within the stomach were sent to the D. E. Taylor Laboratory at the University of Alberta for *H. pylori* culture.

3.2.2. *H. pylori* Isolation

Strains were cultured by standard methods described by Taylor *et al.* (36). Biopsy samples were plated out on brain heart infusion agar plates (BHI-YE agar) containing 0.5% yeast extract, 15 μg/ml of both vancomycin and amphoterocin B, as well as 50 ml/L of fetal bovine serum. These plates were incubated at 37°C under microaerobic conditions for 2-4 days. Positive *H. pylori* cultures were confirmed by urease test, and light microscopy. A total of 127 culture-positive biopsy samples were identified from the 194 samples obtained.

3.2.3. ELISA for Lewis Antigens

Isolated *H. pylori* were examined for Lewis antigenic expression patterns. The conditions for the ELISA were previously described (27, 47). The primary antibodies used were anti-Le^a (MAb BG-5, clone T174), anti-Le^b (MAb BG-6, clone T218), anti-Le^x (MAb BG-7, clone P12), anti-Le^y (MAb BG-8, clone F3), and anti-sialyl Le^x (MAb BG-9) from Signet Laboratories Inc. (Dedham, MA, USA). The primary antibodies were diluted 1:100 while the secondary antibody, goat anti-mouse IgG + IgM conjugated to horseradish peroxidase (HRP) (Biocan #115 035 068, Mississauga, Ontario, CAN), was diluted 1:2000. The absorbance was recorded at 405 nm using a Titretek Multiscan MC

(Helsinki, Finland) microtitre plate reader. Absorbance values are an average of triplicate wells with blanks subtracted. Values below 0.1 absorbance units were considered negative. Strains found not to express Lewis antigens were assayed on two separate occasions to confirm their status.

3.2.4. Statistical Analysis

Statistical analysis was carried out using Fisher's exact test comparing the significance of proportions. A probability value of $p<0.05$ was considered significant.

3.2.5. Polyacrylamide Gel Electrophoresis and Immunoblots

Whole cell extracts of the *H. pylori* strains were treated with Proteinase K and processed as described by Hitchcock and Brown (21). For the analysis of the LPS, a 15% polyacrylamide separating gel containing urea and a 5% polyacrylamide stacking gel was used. Electrophoresis was conducted on a mini-gel apparatus (BioRad) with a constant current of 35 mA for 1 hour. These gels were either stained with zinc imidazole, according to the method of Hardy *et al.* (20), or transferred to nitrocellulose membrane (Micron Separations Inc. Westboro MA, USA poresize 0.22μm) according to the methods described by Towbin *et al.* (41). Nitrocellulose membranes, with transferred LPS, were probed with the primary antibodies described above diluted 1:500 and bound antibodies detected with goat anti-mouse antibodies conjugated to HRP. Blots were developed using an enhanced chemiluminescence kit (Amersham Life Sciences, Canada) according to the manufacturer's specifications, and images were visualized on BioMax BM film (Kodak, Rochester NY, USA).

3.2.6. Genomic Analysis

Genomic DNA was isolated from *H. pylori* strains by the method of Ge and Taylor (16). A Perkin-Elmer DNA Thermal Cycler 480 was used for 35 cycles of amplification under the following conditions: 95°C for 1 minute, 36°C for 1 minute and 72°C for 2 minutes. The reaction conditions used were previously described by Taylor *et al.* (38). The four random primers used were: Primer 1, 5`-AAGAGCCCGT; Primer 2, 5`-

CCGCAGCCAA, Primer 3, 5`-AACGCGCAAC, Primer 4, 5`-GCGATCCCCA. These primers were developed by Akopyants *et al.* (1). PCR products were subjected to electrophoresis on 1% agarose gels with 1X Tris acetate running buffer and photographed under UV light (33). Isolates with no more than one band difference were considered to be identical as per Akopyants *et al.* (1).

3.3. Results

3.3.1. Lewis Antigen Expression of *H. pylori* Isolates

H. pylori strains were isolated from 127 endoscopic biopsy samples. Isolates were limited to a single passage on BHI-YE agar and then the LPS expression was maximized by growth in liquid media before Lewis antigen status was determined (37, 43). The Lewis antigens expressed by each *H. pylori* clinical isolate were determined by ELISA (Table 3.2.). Le^y was expressed by 69.3% (88/127) of isolates and was the only Lewis antigen expressed by 31.5% (40/127). Le^x was produced by 40.2% (51/127) of isolates and was the only Lewis antigen expressed by 2.4% (3/127). No Lewis antigen could be identified on 28.3% (36/127) of *H. pylori* isolates. No isolate was identified that expressed Le^a, Le^b or sialyl-Le^x.

The distribution of Lewis antigen expression by *H. pylori* changes slightly when examined on a per subject basis. The Le^y antigen was detected on at least one serial isolate from 73.1% (19/26) of subjects and was the only antigen detected in isolates from 7.7% (2/26) of subjects. Le^x was present on at least one of the serial isolates from 61.5% (16/26) of subjects, but no subject produced isolates expressing Le^x exclusively. At least one serial isolate with no detectable Lewis antigens was obtained from 38.5% (10/26) of the study subjects, and 23% (6/26) of subjects produced isolates that were consistently negative for Lewis antigen expression.

All the subjects in this study had *H. pylori* infections at pretreatment screening, as determined by positive urea breath test plus histology or culture of endoscopic biopsies,

Table 3.2.

Percent Expression of Lewis Antigens in *H. pylori* Clinical Isolates

	Biopsies (n=127)		Subjects (n=26)	
	Antigen Present	**Sole antigen**	**Antigen Present**	**Sole antigen**
Lewis X	40.2 (51)	2.4 (3)	61.5 (16)	0 (0)
Lewis Y	69.3 (88)	31.5 (40)	73.1 (19)	7.7 (2)
Neither Antigen	28.3 (36)	-	38.5 (10)	23.0 (6)*

*None of the *H. pylori* isolates from these subjects expressed any of the Lewis antigens screened for during the course of the study.

and remained positive at the end of the treatment period and 30 day post-treatment evaluation period. A formal report of clinical results will be presented elsewhere.

3.3.2. Stability of Lewis Antigen Expression

A switch in antigen expression is defined as an alteration of the Lewis antigen expression pattern (i.e. a change from Le^x alone to Le^x and Le^y together) of the *H. pylori* isolate when compared to a previous isolate from the same subject at the same gastric site, i.e. corpus or antrum, as measured by ELISA. In our study population, Lewis antigen expression frequently differed between *H. pylori* isolates cultured from the two distant biopsy sites, but no trend was observed between the expression of any one antigen and the gastric location. We also did not detect a bias toward expression of specific Lewis antigens by *H. pylori*, as measured by ELISA, with respect to gender, age or ethnic origin of subjects from which the organisms were isolated. We did observe single subjects expressing the Le^y antigen exclusively in both the 1 gram and 5 gram dosing groups whereas, the expression of the Le^x and Le^y antigens simultaneously was observed on isolates from single subjects in the placebo, 2 gram and 5 gram groups. Two subjects' isolates that were consistently negative for the Lewis antigens tested were observed in the placebo group, whereas three were identified in the 1 gram group and one subject was identified in the 2 gram group. There were three subjects with isolates containing switches in the placebo group, two subjects in the 1 gram group, and 5 subjects with this phenotype in the 2 and 5 gram groups. Although we observed an increase in the number of strains switching antigens with the increased 3'SL dose, no statistically significant trend could be identified (using Fisher's exact test $p > 0.3$).

3.3.3. Immunoblot Analysis of Lewis Antigen Expression

Immunoblots of proteinase K-treated whole cell lysates were carried out for all *H. pylori* strains that had undergone an antigenic switch. The staining patterns confirmed the results of the Lewis antigen expression profile obtained by ELISA and revealed gel mobility changes of the *H. pylori* LPS in serial isolates that had undergone an antigenic

switch (Figure 3.1.). One subject's isolates demonstrate the most extreme example of this variation (Figure 3.1. lanes 1-3). The first isolate from this subject does not express an O side chain and thus it does not have the potential to express the Lewis antigens (Figure 3.1.A., lane 1). The second isolate from this subject expresses an O side chain that contains Le^y (Figure 3.1.A and B., lane 2), whereas, the final isolate from this subject expresses an O side chain which expresses both Le^x and Le^y (Figure 3.1.A, B, and C., lane 3). Most of the switches (11/15) involved a variation of the Lewis status of the isolate and not a switch to non-Lewis antigen production. The Lewis status switches also demonstrate some minor changes in the gel mobility of the LPS (Figure 3.1.A., lane 4 and 5). Similar LPS gel mobility shifts were observed on immunoblots of strains from all 15 subjects in which an antigenic switch occurred (data not shown). No Le^a, Le^b, or sialyl-Le^x were detected on any isolate by immunoblot.

3.3.4. Analysis of Non-Typable *H. pylori* Strains

Ten of the 26 subjects (38.5%) yielded at least one *H. pylori* isolate, during the sampling period, that did not express any of the Lewis antigens screened for. These isolates were designated non-typable *H. pylori* (NTHp). A greater proportion of NTHp isolates, within this study population, was identified when compared to previous reports (35, 47). A NTHp *H. pylori* LPS sample from each of the 10 subjects is shown in Figure 3.2.. The results of the immunoblots (not shown) confirmed the results obtained by ELISA, that the LPS from these isolates do not contain any Lewis antigens. All NTHp strains investigated, except one (Figure 3.2., lane 5), expressed LPS containing an O-side chain which may contain alternate carbohydrate structures or precursors to Lewis antigens. It should be noted that not all of the LPS examined from the isolates designated NTHp were of the same mobility, indicating that although grouped together in this and other studies, the LPS from these isolates most likely varies in carbohydrate composition.

Figure 3.1. Zinc-imidazole stain and immunoblot of proteinase-K-digested whole *H. pylori* cell lysates. Lanes 1-3 are samples from *H. pylori* isolates of one subject while lanes 4 and 5 are from another, lane 6 contains strain 26695, which expresses Le^x and Le^y (44). Panel A is a zinc-imidazole stained gel of the LPS from the *H. pylori* isolates. Panel B is an immunoblot of the same samples probed with anti-Le^y MAb. Panel C is an immunoblot of the same samples probed with anti-Le^x MAb.

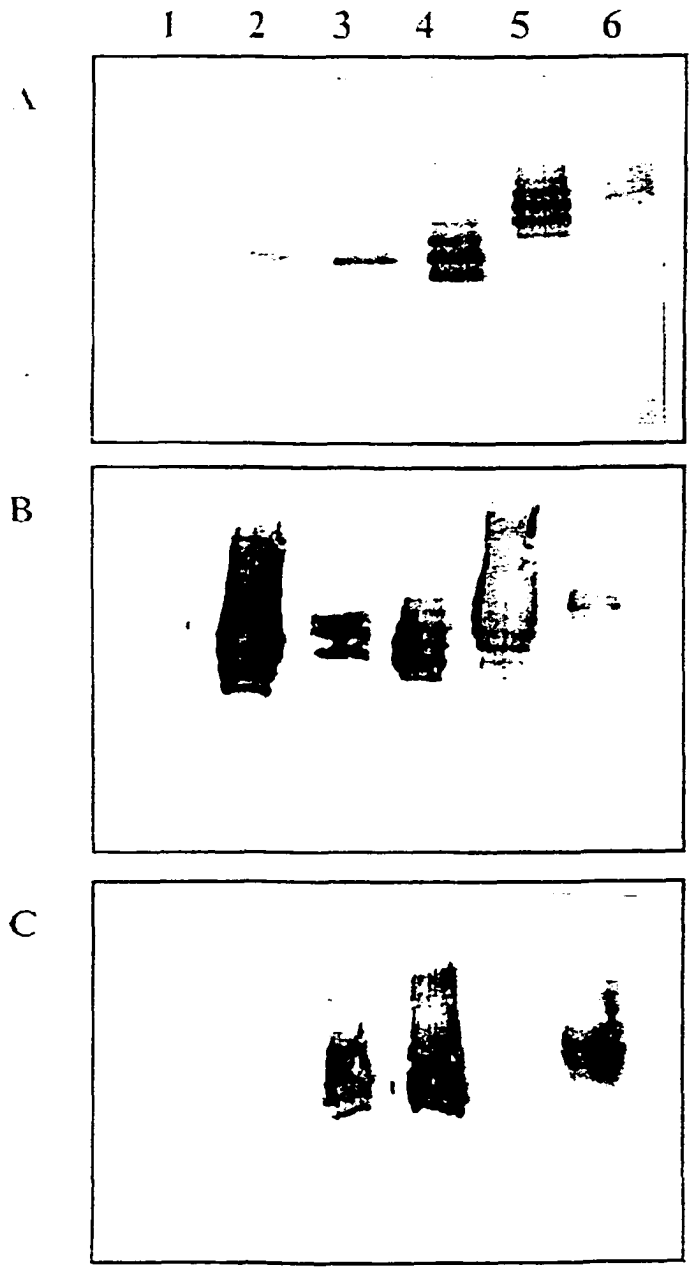
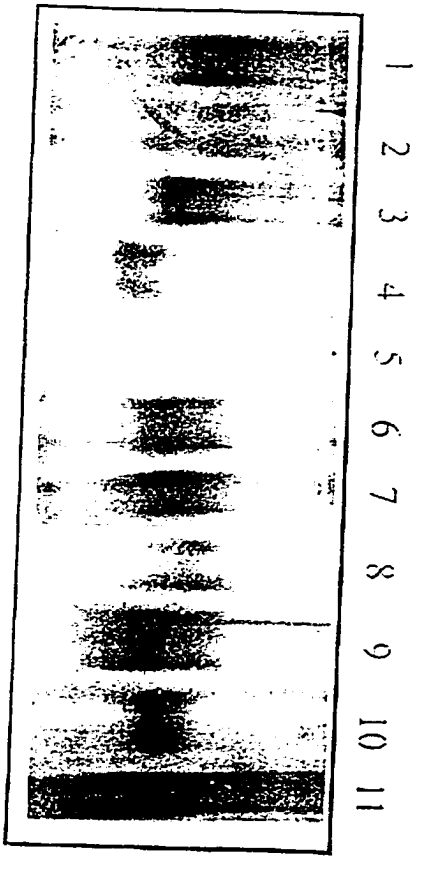


Figure 3.2. Zinc-imidazole stained 15% polyacrylamide gel of Proteinase-K-treated whole cell lysates. A representative gel of one *H. pylori* sample from each of the subjects containing a non-Lewis antigen expressing strain (NTHp). All samples except one (lane 5) express smooth O side chains.



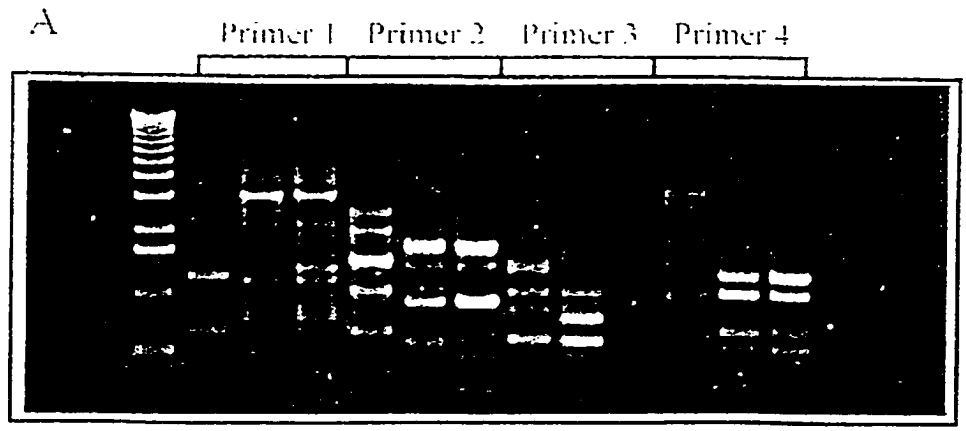
3.3.5. Genomic Analysis of *H. pylori* Isolates

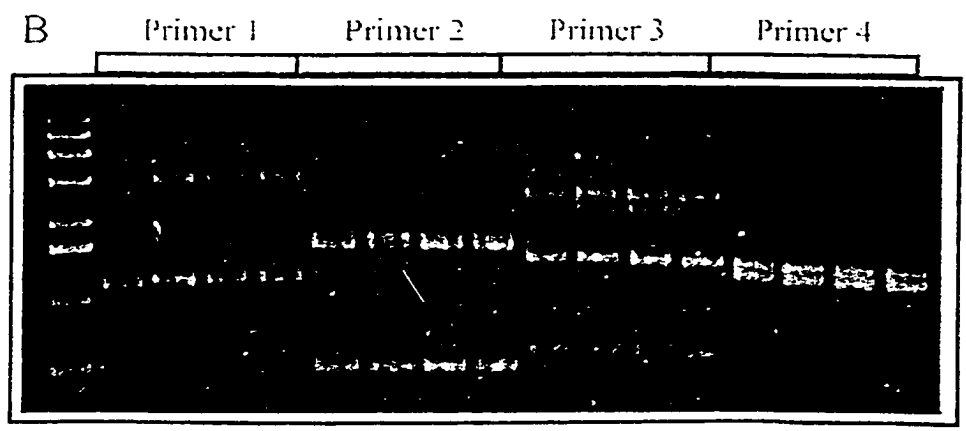
To investigate the possibility that individual subjects may have been colonized by more than one *H. pylori* strain, and that the presence of a mixture of *H. pylori* strains in the biopsies could explain the changes in Lewis antigen expression, we performed genomic analyses on the serial isolates obtained. We observed an antigenic switch in *H. pylori* serial isolates from 15 of 26 subjects (57.7 %). Genomic DNA was isolated from all *H. pylori* serial isolates from any subject expressing an antigenic switch - a total of 78 *H. pylori* DNA samples. In addition, DNA samples were obtained from several randomly chosen isolates in which expression of LPS antigens ($Le^{x/y}$, Le^{y} or NTHp) were stable over the sampling period. The DNA from these strains was investigated by randomly amplified polymorphic DNA (RAPD) PCR. The RAPD-PCR pattern obtained from fourteen of the fifteen sets of *H. pylori* serial isolates that exhibited an antigenic switch, and all serial isolates with stable LPS antigen expression demonstrated that each group of serial isolates was genetically identical, irrespective of gastric site of isolation (an example is shown in Figure 3.3.B). Additionally, each group of serial isolates produced a RAPD-PCR pattern that was distinct from the RAPD-PCR patterns of all other isolates from different subjects (data not shown). Only one subject had evidence for co-colonization with more than one *H. pylori* strain, as the RAPD-PCR pattern between isolates was different (Figure 3.3.A). These observations imply that each subject, with one exception, was colonized throughout the entire gastric mucosa with a single, genetically distinct *H. pylori* strain, each with the potential to express variable LPS antigens.

3.4. Discussion

In the present study, 3'SL was administered to *H. pylori*-positive asymptomatic subjects to evaluate safety, tolerance and efficacy of the drug when administered over a range of doses and using a variety of regimens. Two potentially significant differences must be noted when comparing this study with previous studies examining the expression of Lewis antigens. First, the subjects involved in this study were asymptomatic, providing

Figure 3.3. 1% Agarose gel of RAPD-PCR samples of *H. pylori* strains isolated from two subjects. Panel A shows RAPD-PCR samples from the *H. pylori* isolates of one subject with a mixed infection. Panel B shows four serial isolates which were obtained from a single subject which appear to be identical and are representative of the pattern observed for the other serial isolates from subjects.





an opportunity to identify possible alterations in the distribution of *H. pylori* Lewis antigens among this population. Secondly, in contrast to most previous studies where *H. pylori* clinical isolates were obtained from a single endoscopic biopsy per subject, this study provided an opportunity to examine multiple biopsy isolates obtained over a short period of time (86 days) from two distinct gastric sites.

When examining the *H. pylori* isolates obtained from the endoscopic biopsies we noted an unexpectedly high rate of switching of Lewis antigen expression. This antigenic switch occurred in 57.7% (15/26) of the subjects investigated. The effect was evident in both placebo and dosing groups and no statistically significant difference could be identified ($p>0.3$). Co-colonization with two genetically distinct *H. pylori* strains was observed in only one of 26 study subjects as determined by RAPD-PCR. The two distinct isolates were present at an early time point (day 0), but only one of these strains could be isolated later in the study (day 86). It is not apparent if one strain emerged as a consequence of natural competition between the *H. pylori* isolates or if one strain may have been eliminated due to the effect of the carbohydrate treatment. In any case, results from analysis of genomic DNA from serial *H. pylori* isolates indicate that a large proportion of *H. pylori* strains may switch LPS phenotype during the course of infection while maintaining the same genetic profile.

Some bacterial species have been shown to undergo LPS antigenic switching at rates as high as 12-16% (23, 26), whereas the phase variation rate of the LPS in most other microorganisms is more commonly reported to be approximately 1% (42). Appelmelk *et al.* have demonstrated that lab-adapted isolates of *H. pylori* can alter their Lewis antigen phenotype at a rate of 0.2-0.5% (6). It should be noted that this rate was measured with a laboratory adapted culture and may not accurately depict what occurs after growth *in vivo*. In fact, if the phenotypic switch frequency calculated by Appelmelk *et al.* (6) were operative, one could have expected to observe only a single antigenic switch among the isolates obtained from the 26 subjects sampled in this study, rather than the 15

switches (57.7%) that were actually observed. Interestingly, two groups have recently demonstrated differences in LPS phenotype depending upon which primary colony of *H. pylori* was chosen for investigation, indicating variability of recently isolated *H. pylori* (18, 46). Both groups noted that the gel mobility, staining characteristics (18) and Lewis antigen expression (46) of LPS obtained from different *H. pylori* isolates from the same gastric biopsy sample were variable. Also, Janvier *et al.* (24) demonstrated that the LPS profile of the Sydney strain (SS1), adapted for mouse colonization, was altered by passage through mice. All of the studies mentioned characterized the LPS from organisms cultured *in vitro* after growth *in vivo*. We may have identified a similar phenomenon by isolating multiple "variants" of the same strain over a period of time rather than from a single primary culture plate. Phase variation appears to be a common mechanism used by all *H. pylori* during colonization or under certain conditions and it appears as though these *in vivo* conditions or signals are not duplicated by *in vitro* growth conditions.

The fucosyltransferase *(fucT)* genes encode enzymes responsible for the final steps in the production of Lewis antigens in *H. pylori*. All *fucT* genes contain polynucleotide tracts and imperfect repeats, of different size and sequence depending on the copy of the gene and strain it is isolated from (2, 17, 40). It has recently been demonstrated by Appelmelk *et al.* (4) that the phase variation of some *H. pylori* Lewis antigens can be directly attributed to slip strand mispairing at *fucT* polynucleotide tracts. A recent study by Wang *et al.* (44) has also identified a translational frameshift mechanism that can overcome apparent stop codons, in the α(1,2) *fucT* gene, caused by slip strand mispairing to produce functional protein. This mechanism prevents the accurate determination of the status of the α(1,2) FucT enzyme activity directly from the nucleotide sequence, as a sequence that does not contain a full length open reading frame may still produce functional protein. Thus, in these studies we have not assessed glycosyltransferase activity by sequence analysis, but instead by measuring the end product of these enzymes, the Lewis antigens. The strains which express the Lewis antigens must contain functional

levels of glycosyltransferases that synthesize the lipopolysaccharide core structures as well as the FucTs that form the Le^x and Le^y antigens. In the strains where the Lewis antigen expression is altered, we infer that the FucT enzyme levels are also altered. The increased rate of antigenic variation observed after *in vivo* passage suggests that control of the *H. pylori fucTs* may not be entirely under slip strand mispairing control, but may also be subject to control by environmental signal(s) and/or conditions which contribute to the variability of the Lewis antigens.

The present study identified a larger proportion of NTHp and a smaller proportion of *H. pylori* isolates expressing Le^x than previously identified (35, 47). In a study by Wirth *et al.* (47) the incidence of strains expressing Le^x, was 70%, and the incidence of Le^x expressed as the only antigen was 19%. Similarly, Simoons-Smit *et al.* (35) found Le^x expressed by 77.1% of isolates and solely by 9.9%. In contrast, in the present study we observed that 40.2% of isolates express Le^x and it is exclusively expressed by only 2.4% of isolates. If a subject's set of isolates is treated as a single isolate and categorized as Le^x and/or Le^y and/or NTHp as if there was only a single biopsy, a difference in Le^x expression is still evident with only 61.5% of the subjects yielding at least one isolate which expressed Le^x, and none of the subjects produced serial isolates that exclusively expressed the Le^x antigen. The overall population expression value is more similar to previous studies described above for the of Le^x antigen, but the proportion of isolates expressing Le^x exclusively is still decreased.

There were 28.3% (36/127) of *H. pylori* isolates that were designated NTHp in this study, while 38.5% (10/26) of subjects yielded at least one NTHp isolate, and 23.0% (6/26) of the subjects harbored *H. pylori* isolates that expressed only this phenotype. Simoons-Smit *et al.* (35) reported a NTHp prevalence of 15.1% and also noted that most of the non-typable isolates were from subjects of Chinese origin. The subjects that harbored the NTHp strains in our study did not belong to any single ethnic, gender or age group.

The relatively high frequency of NTHp strains observed in this study calls into question the biological importance of the Lewis antigens in the *H. pylori* LPS during infection, as these antigens are thought to be beneficial for evading the immune system by molecular mimicry (7, 28). Clinical prevalence and persistence of NTHp strains demonstrate that there is no absolute requirement for LPS containing Lewis antigens during human infection. In fact, recent work by Claeys *et al.* (11) and Amano *et al.* (3) have demonstrated that the role of Lewis antigens in *H. pylori* infection may not be critical. Both studies demonstrate that auto-reactive antibodies produced during *H. pylori* infection are not directed toward the Lewis antigens but, toward the core LPS oligosaccharide. It is possible that the pathobiology of *H. pylori* infection in asymptomatic individuals differs from that in symptomatic patients. If so, the increased NTHp prevalence and even the decrease in the number of strains expressing Le^x observed in this study may represent adaptations by *H. pylori* to the host in whom disease symptoms do not develop. Conversely, the Lewis phenotypes may be characteristic of *H. pylori* that colonize patients who become symptomatic. A thorough understanding of the incidence and biological significance of phenotypic switching of Lewis antigens in various human hosts awaits further study.

In summary, our results indicate that the rate of change of expression of LPS antigens after growth in human subjects appears to be higher than estimated from previous studies (6) and clinical reports (35, 47). The increased rate of switching of Lewis antigens and NTHp that was observed does not appear to be an effect of drug treatment, but may be the result of exposure to as yet unidentified environmental signal(s) encountered during human infection. This study provides the first definite evidence that a single strain of *H. pylori* may alter its LPS antigenic phenotype during the course of infection.

3.5. Bibliography

1. **Akopyants, N. S., Eaton, K. A. and Berg, D. E.** 1995. Adaptive mutation and cocolonization during *Helicobacter pylori* infection of gnotobiotic piglets. Infect. Immun. **63**: 116-121.

2. **Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F. and Trust, T. J.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. **397**: 176-180.

3. **Amano, K., Hayashi, S., Kubota, T., Fijii, N. and Yokata, S.** 1997. Reactivities of Lewis monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. Clin. Diag. Lab. Immunol. **4**: 540-544.

4. **Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M. J. E. and Kusters, J. G.** 1999. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α3-fucosyltransferase genes. Infect. Immun. **67**: 5361-5366.

5. **Appelmelk, B. J. and Negrini, R.** 1997. *Helicobacter pylori* and gastric autoimmunity. Curr. Op. Gastroenterol. **13**: 31-34.

6. **Appelmelk, B. J., Shiberu, B., Trinks, C., Tapsi, N., Zheng, P. Y., Verboom, T., Maaskant, J., Hokke, C. H., Schiphorst, W., Blanchard, D., Simoons-Smit, I. M., van den Eijnden, D. H. and Vandenbroucke-Grauls, C.** 1998. Phase variation in *Helicobacter pylori* lipopolysaccharide. Infect. Immun. **66**: 70-76.

7. **Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A. and Degraaff, J.** 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun. **64**: 2031-2040.

8. **Aspinall, G., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1994. O antigen chains in the lipopolysaccharide of *Helicobacter pylori* NCTC11637. Carbohydr. Lett. **1**: 151-156.

9. **Aspinall, G. O. and Monteiro, M. A.** 1996. Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. Biochem. **35**: 2498-2504.

10. **Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1996. Lipopolysaccharide of the *Helicobacter pylori* type strain

NCTC11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. Biochem. **35**: 2489-2497.

11. **Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R. and Kirchner, T.** 1998. The gastric H+, K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. Gastroenterology. **115**: 340-347.

12. **Evans, D. G., Evans, D. J. and Graham, D. Y.** 1989. Receptor-mediated adherence of *Campylobacter pylori* to mouse Y-1 adrenal gland monolayers. Infect. Immun. **57**: 2272-2278.

13. **Evans, D. G., Evans Jr., D. J., Moulds, J. J. and Graham, D. Y.** 1988. N-acetylneuraminyllactose-binding fimbrillar hemagglutinin of *Campylobacter pylori*: a putative colonization factor. Infect. Immun. **56**: 2896-2906.

14. **Forman, D., Webb, P., Newell, D. G., Coleman, M., Palli, D., Maller, H., Hengels, J., Elder, J. and DeBacker, G.** 1993. An international association between *Helicobacter pylori* infection and gastric cancer. Lancet. **341**: 1359-1362.

15. **Ge, Z., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1997. Cloning and heterologous expression of an α1,3 fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. J. Biol. Chem. **272**: 21357-21363.

16. **Ge, Z. and Taylor, D. E.** 1992. *H. pylori* DNA transformation by natural competence and electroporation in *Helicobacter pylori Protocols* C. L. Clayton, H. L. T. Mobley, Eds. (Humana Press Inc.) pp. 145-152.

17. **Ge, Z. and Taylor, D. E.** 1999. Contributions of genome sequencing to understanding the biology of *Helicobacter pylori* . Ann. Rev. Microbiol. **53**: 353-387.

18. **Gibson, J. R., Chart, H. and Owen, R. J.** 1998. Intra-strain variation in expression of lipopolysaccharide by *Helicobacter pylori*. Lett. Appl. Microbiol. **26**: 399-403.

19. **Graham, D. Y.** 1991. *Helicobacter pylori:* Its epidemiology and its role in duodenal ulcer disease. J. Gastroenterol. Hepatol. **6**: 1359-1362.

20. **Hardy, E., Pupo, E., Castellanos-Serra, L., Reyes, J. and Fernandez-Patron, C.** 1997. Sensitive reverse staining of bacterial lipopolysaccharides on polyacrylamide gels by using zinc and imidazole salts. Anal. Biochem. **244**: 28-32.

21. **Hitchcock, P. J. and Brown, T. M.** 1983. Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver stained polyacylamide gels. J. Bacteriol. **154**: 269-277.

22. International Agency for Research on Cancer. 1994. Shistosomes, liver flukes and *Helicobacter pylori*. WHO Monograph 61. Lyon, France:IARC, pp. 177-240.

23. **Inzana, T. J., Hensley, J., McQuiston, J., Lesse, A. J., Campagnari, A. A., Boyle, S. M. and Apicella, M. A.** 1997. Phase variation and conservation of lipopolysaccharide epitopes in *Haemophilus somnus*. Infect. Immun. **65**: 4675-4681.

24. **Janvier, B., Grignon, B., Audibert, C., Pezennec, L. and Fauchere, J. L.** 1999. Phenotypic changes of *Helicobacter pylori* components during an experimental infection in mice. FEMS Immunol. Med. Microbiol. **24**: 27-33.

25. **Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. and Bird, M. I.** 1997. Lewis X biosynthesis in *Helicobacter pylori*. molecular cloning of an α(1,3) fucosyltransferase gene. J. Biol. Chem. **272**: 21349-21356.

26. **Maskell, D. J., Szabo, M. J., Butler, P. D., Williams, A. E. and Moxon, E. R.** 1991. Phase variation of lipopolysaccharide in *Haemophilus influenzae*. Res. Microbiol. **142**: 719-724.

27. **Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P. and Perry, M. B.** 1998. Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem. **273**: 11533-11543.

28. **Moran, A. P., Appelmelk, B. J. and Aspinall, G. O.** 1996. Molecular mimicry of host structures by lipopolysaccharides of *Campylobacter* and *Helicobacter* Spp: implications in pathogenesis. J. Endotox. Res. **3**: 521-531.

29. **Moran, A. P., Prendergast, M. M. and Appelmelk, B. J.** 1996. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. FEMS Immunol. Med. Microbiol. **16**: 105-115.

30. **Nikaido, H. and Vaara, M.,** 1987. Outer Membrane in *Escherichia coli and Salmonella typhimurium : Cellular and Molecular Biology* F. C. Niedhardt, Ed. (American Society for Microbiology, Washington D.C., 1987), vol. 1, pp. 7-22.

31. **Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N. and Sibley, R. K.** 1991. *Helicobacter pylori* infection and the risk of gastric cancer. N. Engl. J. Med. **325**: 1127-1131.

32. **Peterson, W. L.** 1991. *Helicobacter pylori* and peptic ulcer disease. N. Engl. J. Med. **324**: 1043-1048.

33. **Sambrook, J., Fritsch, E. F. and Maniatis, T.,** 1989. *Molecular cloning: a laboratory manual.* Second edition. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

34. **Simon, P. M., Goode, P. L., Mobasseri, A. and Zopf, D.** 1997. Inhibition of *Helicobacter pylori* Binding to gastrointestinal epithelial cells by sialic acid containing oligosaccharides. Infect. Immun. **65**: 750-757.

35. **Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B.**

S., Rudnica, W., Savio, A. and De Graaff, J. 1996. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol. **34**: 2196-2200.

36. **Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W. and Jewell, L. D.** 1987. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. Am. J. Clin. Path. **87**: 49-54.

37. **Taylor, D. E., Rasko, D. A. and Sherburne, R.** 1996. Growth phase dependent expression of Lewis X by *Helicobacter pylori*. Gut. **34, Supplemental**: A68.

38. **Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C. and Jewel, L. D.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

39. **Taylor, D. N. and Parsonnet, J.** 1995. in *Infections of the gastrointestinal tract* M. J. Blaser, P. F. Smith, J. Ravdin, H. Greenberg, R. L. Guerrant, Eds. (Raven Press, New York, New York,) pp. 551-564.

40. **Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L. X., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M. and Venter, J. C.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. **388**: 539-547.

41. **Towbin, H., Staehelin, T. and Gordon, J.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. **76**: 4350-4354.

42. **Van Putten, J. P. M. and Robertson, B. D.** 1995. Molecular mechanisms and implications for infection of lipopolysaccharide variation in *Neisseria*. Mol. Microbiol. **16**: 847-853.

43. **Walsh, E. J. and Moran, A. P.** 1997. Influence of medium composition on the growth and antigen expression of *Helicobacter pylori*. J. Appl. Microbiol. **83**: 67-75.

44. **Wang, G., Rasko, D. A., Sherburne, R. and Taylor, D. E.** 1999. Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori* : analysis of the α(1-2) fucosyltransferase gene. Mol. Microbiol. **31**: 1265-1274.

45. **Warren, J. R. and Marshall, B. J.** 1983. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet. **1**: 1273-1275.

46. **Wirth, H.-P., Yang, M., Peek, R. M., Hook-Nikanne, J., Fried, M. and Blaser, M. J.** 1999. Phenotypic diversity in Lewis expression of *Helicobacter pylori* isolates from the same host. J. Lab. Clin. Med. **133**: 488-500.

47. **Wirth, H. P., Yang, M. Q., Karita, M. and Blaser, M. J.** 1996. Expression of the human cell surface glycoconjugates Lewis X and Lewis Y by *Helicobacter pylori* isolates is related to CagA status. Infect. Immun. **64**: 4598-4605.

48. **Wotherspoon, A. C., Doglioni, C., Diss, T., Pan, L., Moschini, A., De Boni, M. and Isaacson, P. G.** 1993. Regression of primary low grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. Lancet. **342**: 575-577.

Chapter 4

Cloning and Characterization of the α 1,3/4 Fucosyltransferase of *Helicobacter pylori*

A version of this work has been previously published:

Rasko, D. A., Wang, G., Palcic, M. M. and Taylor, D. E. 2000. Cloning and characterization of the α1,3/4 fucosyltransferase of *Helicobacter pylori*. J. Biol. Chem. **276**: 4988-4994.

4. Preface

The identification of an isolate that expressed both Le^a and Le^x simultaneously in Chapter 2 allowed the investigation of the fucosyltransferase enzymes that are responsible for the synthesis of these antigens. Previous cloning and enzymatic characterization of *H. pylori* FucT enzymes indicated that those enzymes had the capabilities of producing only the Le^x and Le^y antigens but not Le^a or Le^b. Using the knowledge of the antigens present on the bacterial surface, the enzymes responsible were cloned and enzymatically characterized using methods developed for the analysis of mammalian enzymes. Mutagenesis allowed the determination that only a single FucT was active in this isolate and deletion of it abolished Lewis antigen expression. Comparison of DNA and amino acid sequence has identified multiple features which are characteristic of the *H. pylori* FucTs.

4.1. Introduction

Lipopolysaccharide (LPS) is an essential structural and functional component of the gram negative bacterial cell envelope. LPS is composed of lipid A, oligosaccharide core and the antigenic O-polysaccharide chain. *Helicobacter pylori* express a range of Lewis antigens in their LPS O-chain. Most *H. pylori* strains express the type II glycoconjugate antigens, Lewis X (Le^x) and Lewis Y (Le^y), while a small proportion have the ability to express the type I glycoconjugates, Lewis A (Le^a) and Lewis B (Le^b) ((26, 27)). The expression of the Lewis antigens by *H. pylori* is thought to provide a way by which the organism can avoid detection by the immune system as these antigens are also expressed by the human gastric epithelium (4, 37). It has been suggested that the expression of Lewis antigens by *H. pylori* may be the cause for an autoimmune response leading to chronic type B gastritis and gastric as well as duodenal ulcers (28), but the role of Lewis antigens in the disease process has recently been questioned by two separate groups (2, 9). Presently, it is unclear what role, if any, these antigens play in the disease process because *H. pylori* isolates that do not express Lewis antigens may still colonize and cause infection (32, 34).

In the human body the expression and distribution of Lewis antigens is tightly regulated by a series of glycosyltransferases which add monosaccharides to precursor structures (12, 21). The synthesis of Lewis antigens which contain two fucose moieties can occur in two ways, a terminal fucosylation, by an α(1,2) fucosyltransferase (FucT), followed by subterminal fucosylation, by an α(1,3/4) FucT, or subterminal followed by terminal fucosylation (Figure 4.1). Inactivation or lack of expression of one of these FucTs leads to the expression of monofucosylated Lewis antigens. The Le^a structure is synthesized by addition of fucose by an α(1,3/4) FucT encoded by the *FUT-3* gene to a type I acceptor (lacto-*N*-biose (Galβ1-3GlcNAc, Figure 4.1A)(22). Six separate human FucT enzymes have been identified which have the capacity to catalyse the transfer of fucose to the type II precursor, LacNAc, to produce Le^x; Fuc-TIII-VII and Fuc-TIX

Figure 4.1. Lewis antigens used and/or detected in this study. Panel A demonstrates how the mono- and difucosylated Lewis antigens can be produced from the hypothetical starting Type I carbohydrate chain of Lewis C. Panel B shows how Type II carbohydrates can be synthesized from the starting carbohydrate backbone, LacNAc. The only difference between Type I and Type II carbohydrate chains is the linkage of the sugars in the backbone being β1-3 and β1-4 respectively.

A

B

(14, 30) (Figure 4.1B). Among them, Fuc-TIII and Fuc-TV, have demonstrated both α(1,3) and α(1,4) FucT activity, and thus have the potential to produce Le^a and Le^x (22, 42), although the α(1,3) activity of Fuc-TIII has been disputed (10, 13, 17). It has been demonstrated in Fuc-TIII that a single amino acid change resulting from a single nucleotide change in the gene is responsible for enzyme inactivation (15, 16, 25, 31). Whereas, other single amino acid changes are found to be responsible for alteration of enzyme substrate specificity (differential fucose transfer to type I or type II carbohydrate acceptors) (14, 23, 44). A consensus amino acid sequence for the α (1,3) FucT enzymes (6, 7, 30) has also been identified.

Two highly homologous copies of the α(1,3) *fucT* have been identified in both of the *H. pylori* genomes sequenced to date (1, 38). It is thought that the *fucT* genes are controlled by a slip-strand repair mechanism at tracts of cytosines and adenosines in the 5` end of the gene (3). In previous studies a *fucTa* gene from NCTC11637 and a *fucT* gene from NCTC11639 have been cloned and *in vitro* characterization of the FucT enzymes did not demonstrate α(1,4) FucT activity (18, 24). This is consistent with the observation that the lipopolysaccharide of these strains does not contain any type I Lewis antigens ((5), D.A. Rasko, unpublished data). We have previously identified an *H. pylori* strain, UA948, which expresses both type I (Le^a) and type II (Le^x) carbohydrate structures simultaneously (27). For *H. pylori* to produce the Lewis structures (Le^a and Le^x) in the LPS O-chain like the production of Lewis antigens by human cells, there is a requirement for the addition of fucose in both α(1,3) and α(1,4) linkages. Thus it is expected that UA948 contains both α(1,3) and α(1,4) FucT activities (Figure 4.1).

We have examined the FucTs from the *H. pylori* strain UA948 in an attempt to isolate the α(1,4) FucT activity. In this study, we demonstrated that a single *H. pylori* FucT enzyme from UA948 contains both α(1,3) and α(1,4) FucT activity responsible for the production of both Le^a and Le^x in the LPS O-side chain, while the other copy of the *fucT* gene does not encode a functional FucT enzyme. Comparisons of the nucleotide and

amino acid sequences of the newly identified *H. pylori* α(1,3/4) FucT with the previously identified *H. pylori* α(1,3) FucTs has allowed us to predict some of the domains of the enzyme that may be potentially responsible for broadening the range of acceptors used by this enzyme.

4.2 Experimental Procedures

4.2.1. Bacterial Strains and Media. *H. pylori* strains identified from the UA culture collection were cultured by standard methods described by Taylor *et al.* (36). Isolates from a frozen stock were thawed and plated out on BHI-YE agar plates (3.7% brain heart infusion, 0.5% yeast extract, 15 μg/ml of both vancomycin and amphoterocin B, 5% of fetal bovine serum, 1.2% agar). These plates were incubated at 37°C under microaerobic conditions for 2-4 days. Positive *H. pylori* cultures were confirmed by urease test, and microscopy. Transformants containing the chloramphenicol acetyltransferase gene (41) inserted into the *fucT* genes were isolated as described previously (18) and cultured on BHI-YEA plates as described above containing 50 μg/ml chloramphenicol (Sigma-Aldrich, Canada). *Escherichia coli* DH10β and *E. coli* K38(pGP1-2) were grown on Luria broth agar plates containing 100 μg/ml ampicillin, and/or 50 μg/ml chloramphenicol or 50 μg/ml kanamycin depending on the resistance markers present on the plasmids within the cells.

4.2.2. DNA manipulation techniques. Standard DNA manipulation techniques including the isolation, transformation and restriction enzyme digestion analysis of plasmid DNA were described by Sambrook *et al.* (33). Both strands of the appropriate PCR fragments were sequenced using the Thermosequenase sequencing kit according to the manufacturer's instruction. Sequence analyses were performed with the BLAST program from the National Center of Biotechnology Information (Bethesda, MD, USA). The Wisconsin Package (version 9.0) of the Genetics Computer Group (GCG; Madison, WI, USA) was used for the editing and alignment of sequences.

4.2.3. Cloning and Overexpression of the *H. pylori fucT* genes. The primers used for amplification and cloning of the *fucTs* are as follows: To clone *fucTa* DAVE55 5`-cgggatcccgGCGTGAATTACTACCTTTCTG -3' (positions 389088 - 389108) and DAVE56 5'- cggaattccgCAAAACCCTCCTTTCTACTAATG (positions 390887 - 390868) were used. To clone *fucTb* DAVE53 5`-cgggatcccgAGCGACCAATCATTACAG-3` (position 698868 - 698852) and DAVE54 5`-cggaattccgACCTGGCAATTAGACAAC-3` (position 696838 - 696855) were used. The uppercase letters denote sequence derived directly from the published sequence from the strain 26695 (38) while, the lowercase letters of the primers denote the restriction endonuclease sites used to facilitate cloning. PCR was performed as previously described (18) producing fragments of 1769 and 1774 nucleotides for the UA948*fucTa* and UA948*fucTb* fragments respectively. Restriction with *Eco*RI and *Bam*HI allowed cloning into a similarly digested pBluescript II KS+. The respective clones containing the *H. pylori fucTa* or *fucTb* were screened using the primers described above. The proposed coding region of the UA948*fucTa* and UA948*fucTb* were placed under the control of the T7 promoter.

Recombinant plasmids pB948fucTa and pB948fucTb were introduced by electroporation into *E. coli* K38 containing the plasmid pGP1-2 (which encodes a heat inducible T7 RNA polymerase) (35) . The proteins encoded by the recombinant plasmids were expressed as follows. *E. coli* K38(pGP1-2) harbouring the plasmids containing the recombinant UA948*fucTa* or UA948*fucTb* plasmids were grown in 20 ml liquid LB medium with appropriate antibiotics (kanamycin and ampicillin) at 30°C to an optical density of 0.5-0.7 at 600nm. After collection by centrifugation, the cells were washed once in M9 medium, resuspended in 5 ml supplemented M9 medium, and further incubated for 1 hour at 30°C. To induce the expression of the recombinant gene, the culture was shifted to 42°C by adding 5 ml of 55°C supplemented M9 medium and incubated for a further 15 minutes at 42°C. Rifampicin was added to a final concentration

of 200μg/ml and incubation continued for 30 minutes. An aliquot was removed and incubated a further 30 minutes with {^{35}S}-methionine (50 nCi, Mandel Scientific Company Ltd. Guelph, ON.), after which the cells were harvested by centrifugation, resuspended in sample buffer, and subjected to SDS-PAGE as previously described (18). The remaining culture was prepared for enzyme assay by methods previously described (18).

4.2.4. Fucosyltransferase Assay. The FucT assays were performed as previously described (8) with some modification. Reactions were conducted at 37°C for 20 minutes in a 20 μl volume containing 1.8 mM acceptor, 50μM GDP-fucose, 60000 dpm GDP-{^{3}H}fucose (American Radiolabeled Chemicals Inc., St. Louis, MO), 20 mM Hepes buffer (pH 7.0), 20 mM $MnCl_2$, 0.1 M NaCl, 35 mM $MgCl_2$, 1 mM ATP, 5 mg/ml bovine serum albumin, and 9.0 μl of the enzyme preparation. Acceptors used in this study were: type I {β Gal 1-3 β GlcNAc-O-$(CH_2)_8CO_2CH_3$}, H Type I{αFuc1-2β Gal 1-3 β GlcNAc-O-$(CH_2)_8CO_2CH_3$}, LacNAc {βGal 1-4 βGlcNAc-O-$(CH_2)_8CO_2CH_3$}, H Type II {αFuc 1-2 β Gal 1-4 β GlcNAc-O-$(CH_2)_8CO_2CH_3$}. These acceptors were kindly provided by Dr. O. Hindsgaul. For calculation of the specific activity of the enzyme [milli-units (the amount of enzyme needed to convert 1 nmol of acceptor to product within the defined time period) per milligram of protein], protein concentrations of the cell extracts were determined with a BCA protein assay kit (Pierce, Rockford, IL) using bovine serum albumin as a standard according to the supplier's instructions.

4.2.5. Functional Inactivation of the UA948*fucTa*. A chloramphenicol cassette inserted at a unique *XmnI* site in the *fucT* gene and the resultant construct (18) was used for transformation. Natural transformation of UA948 was accomplished by the method of Ge and Taylor (19). Briefly, a 48 hour culture from frozen stock was restreaked and grown for 5 hours on a BHI-YEA plate, 5 μg of DNA containing the *fucT*::CAT was added to the growth. After a further incubation for 20 hours at 37°C the culture was plated onto BHI-YEA plate containing 50μg/ml of chloramphenicol. Cultures were grown from

single colonies and the genomic DNA was extracted according to Ge and Taylor (19). Insertion of the CAT cassette in the *fucT* gene on the chromosome was confirmed by PCR with the previously described primers which were specific to each copy of the *fucT* gene. PCR products were subjected to electrophoresis on a 1% agarose gel and photographed under UV light.

4.2.6. ELISA for Lewis Antigens

The conditions for the ELISA were previously described (27, 43). The primary antibodies used were anti-Lewis A (MAb BG-5, clone T174), anti-Lewis B (MAb BG-6, clone T218), anti-Lewis X (MAb BG-7, clone P12), anti-Lewis Y (MAb BG-8, clone F3) from Signet Laboratories Inc. (Dedham, MA, USA). These primary antibodies were diluted 1:100, while the secondary antibody, goat anti-mouse IgG + IgM conjugated to horseradish peroxidase (HRP) (Biocan #115 035 068, Mississauga, ON.), was diluted 1:2000. Absorbance values were recorded at 405 nm using a Titretek Multiscan MC microtitre plate reader. Absorbance values are an average of triplicate wells with blanks subtracted. Values below 0.1 absorbance units were considered negative.

4.2.7. Analysis of LPS by Acrylamide Gel Electrophoresis and Immunoblot. Whole cell extracts of the *H. pylori* strains were treated with proteinase K, processed and subjected to electrophoresis as described previously (27). These gels were stained with either zinc imidazole, according to the method of Hardy *et al.* (20), or transferred to nitrocellulose membrane (Micron Separations Inc. Westboro MA, USA poresize 0.22um) according to the method described by Towbin *et al.* (39). Nitrocellulose membranes, with transferred LPS, were probed with the primary and secondary antibodies described above [Anti-Lewis structure (Signet Lab. Inc. Dedham, MA, USA) antibodies 1:500 dilution and goat anti-mouse conjugated to horseradish peroxidase diluted 1:2000, respectively]. Blots were developed using an enhanced chemiluminescene kit (Amersham Life Sciences, Oakville, ON.) according to the manufacturer's specifications and images were visualized on BioMax BM film (Kodak, Rochester NY, USA).

4.3. Results

4.3.1. Features of the α (1,3/4) *fucT* gene. PCR and subsequent sequence analysis of the inserts of the pB948fucTa and pB948fucTb demonstrated a significant difference in the size of the UA948*fucTb* gene compared to the predicted size from strain 26695. The insert in pB948fucTb contained 1774 base pairs, whereas the predicted size is 2030 base pairs. The main reason for the difference in size is the absence of a number of repeated sequences in UA948*fucTb* that exist in the 3` region of 26695*fucTb*. On the other hand, the PCR product of UA948*fucTa* is 1769 base pairs long which is similar to the predicted size of 1799 base pairs. The nucleotide sequence of UA948*fucTa* is 85.5% identical to the *fucTa* of 26695, whereas, the UA948*fucTb* is 87.6% identical when compared to the *fucTb* gene in 26695. Like in other *H. pylori fucT* genes identified (1, 18, 24, 38) characteristic cytosine and adenosine tracts exist in the 5' end of the UA948*fucT* genes (Table 4.1).

The protein translation of the sequenced PCR fragments revealed that the UA948*fucTa* gene encodes an open reading frame (ORF) of 475 amino acids with a predicted molecular weight of 55.9 kDa whereas UA948*fucTb* does not encode an ORF containing a full length FucT protein. An arbitrary increase or decrease in the cytosine and/or adenosine tract of the UA948*fucTb* gene by any small number of nucleotides does not provide a full length *fucT* ORF from this gene, eliminating the possibility that the lack of functional protein is caused by slip strand mispairing. A similar modification was discovered to be necessary for the production of full length protein by NCTC11637 (24) and J99FucTa (*JHP1002*) (1). An ORF with homology to the identified FucTs does exist in the UA948*fucTb* insert, but it is truncated.

A comparison of the UA948FucTa amino acid sequence with previously identified *H. pylori* FucTs demonstrates a high level of homology (Figure 4.2). Overall, a greater than 70% amino acid identity was noted between the identified *H. pylori* FucTs.

Table 4.1 : Comparison of Nucleotide and Amino Acid Repeats of Fucosyltransferases

	Nucleotide		Amino Acid	
Strain and *fucT* Copy	Adenosine Repeat	Cytosine Repeat	Number of Repeats	Sequence of Repeat
26695A	6	13	2	DDLRVNY
26695B	6	13	10	DDLRVNY
J99A	9[a]	13	4	DDLRVNY
J99B	3	5	7	DDLRVNY
NCTC111639	6	10	10	DDLR(V/I)NY[b]
NCTC11637	6	9[a]	7	D(D/N)LR(V/I)NY[c]
UA948A	3	5	8	DDLRVNY/DDLRRD(H/R)[d]
UA948B[e]	9	9	NA[e]	NA[e]

[a] Artificially adjusted by 1 nucleotide to produce a full length FucT protein.

[b] Repeats 3,5 and 8 contain a isoleucine instead of a valine at position five of the repeat.

[c] The first two repeat sequences contain the sequence DNLRVNY, the following two repeats contain the consensus repeat sequence DDLRVNY, and the final three repeats contain the sequence DDLRINY.

[d] Repeats 1 and 8 contain the sequence DDLRRDH, while repeat seven has the sequence DDLRRDR.

[e] N/A - not applicable as no protein is produced. Adjustment of either polynucleotide repeat region does not allow the production of full length protein.

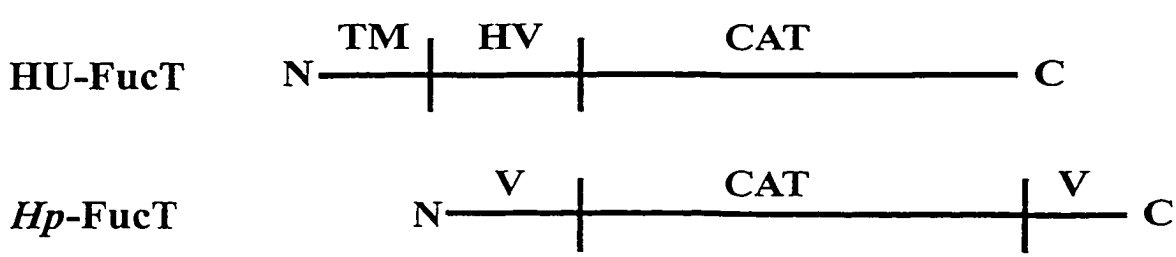
Figure 4.2. **A**: Comparison of the amino acid sequences of the known *H. pylori* FucTs. J99A - JHP1002 (AAD06573), J99B - JHP0596 (AAD06169), 26695A - HP0379 (AAD07447), 26695B - HP0651 (AAD07710), NCTC11639 - the one copy identified (AAB81031), NCTC11637 - the one copy identified (AAB93985), UA948A - FucTa of *H. pylori*UA948 (AF194963). Numbers in the brackets refer to the genbank accession number. * indicate amino acid identity, : indicates a conserved amino acid substitution and a blank indicates a non-conservative amino acid substitution. The conserved catalytic domains are designated by a line overtop of the sequence and the cross species conserved amino acids within this region are bolded. The roman numerals refer to the domain number assigned by Breton et al. (6,7). Amino acid sequences were aligned by CLUSTAL alignment.

B: A diagrammatic alignment of human FucTs with *H. pylori* FucTs. In both cases N denotes the amino terminus and C is the carboxy terminus whereas CAT represents the catalytic domains of the FucT enzymes. HV is the hypervariable region identified in the human FucTs which contain mutations responsible for the alteration of enzyme characteristics. TM is the transmembrane region present only in the human FucTs. V is the variable regions identified in the *H. pylori* FucTs.

A

```
J99A          MFQPLLDAFIESTPIKKKITFKSPPPPLKIAVANWW----GGAEEFKKSTLYFILSQRYTITLHQNPNEPSDLVLGSPIGSARKILSYQNTKRVFYTGEN
J99B          MFQPLLDAYTDST----RLDETDYKPPLNIALANWWPLDKRESKGFRRFILYFILSQRYTITLHQNPNEPSDLVFGSPIGSARKILSYQNTKRVFYTGEN
26695A        MFQPLLDAFIESASI-EKMASKSPPPPLKIAVANWW--GDEEIKEFKKSVLYFILSQRYAITLHQNPNEFSDLVFSNPLGAARKILSYQNTKRVFYTGEN
26695B        MFQPLLDAFIESASI-EKMVSKSPPPPLKIAVANWW--GDEEIKEFKKSVLYFILSQRYAITLHQNPNESSDLVFSNPLGAARKILSYQNTKRVFYTGEN
NCTC11639     MFQPLLDAYVESASI-EKMASKS-PPPLKIAVANWW--GDEEIKEFKNSVLYFILSQRYTITLHQNPNEFSDLVFGNPLGSARKILSYQNAKRVFYTGEN
NCTC11637     MFQPLLDAFIESASI-EKMASKS-PPPLKIAVANWW--GDEEIKEFKKSTLYFILSQHYTITLHRNPDKPADIVFGNPLGSARKILSYQNTKRIFYTGEN
UA948A        MFQPLLDAFIDSTHLDE----TTHKPPFKCSLANWWPLKNSEKKGFRDFILTLLLKQRYKILLQHNPNEPSDLVFGNPLEQARKILSYQNTKRVFYTGEN
              ********: :*:            **:: ::****       : *:   * ::* *:* * *::**:: :*:*:   *:  *********:**:******
                                                                                                  I

J99A          EVPNFNLFDYAIGFDELDFRDRYLRMPLYYASLHYKAESVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESDPLKRGFASFVASNPNAPIRN
J99B          EVPNFNLFDYAIGFDELDFRDRYLRMPLYYASLHYKAESVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESDPLKRGFASFVASNPNAPIRN
26695A        ESPNFNLFDYAIGFDELDFNDRYLRMPLYYAHLHYKAELVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESDLLKRGFASFVASNANAPMRN
26695B        ESPNFNLFDYAIGFDELDFNDRYLRMPLYYAHLHYEAELVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESDLLKRGFASFVASNANAPMRN
NCTC11639     ESPNFNLFDYAIGFDELDFNDRYLRMPLYYDRLHHKAESVNDTTAPYKLKDNSLYALKKPSHCFKEKHPNLCAVVNDESDPLKRGFASFVASNPNAPIRN
NCTC11637     ESPNFNLFDYAIGFDELDFRDRYLRMPLYYDRLHHKAESVNDTTAPYKIKGNSLYTLKKPSHCFKENHPNLCALINNESDPLKRGFASFVASNANAPMRN
UA948A        EVPNFNLFDYAIGFDELDFNDRYLRMPLYYAYLHYKAMLVNDTTSPYKLK--ALYTLKKPSHKFKENHPNLCALIHNESDPWKRGFASFVASNPNAPIRN
              * ********************* **********  **::*  *****:***:*  :**:******* ***:*******::::***  *********** ***:**
                                                          II

J99A          AFYDALNSIEPVTGGGSVKNTLGYNVKNKSEFLSQYKFNLCFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVCDFKNFDEAIDYVRY
J99B          AFYDALNSIEPVTGGGSVKNTLGYNVKNKSEFLSQYKFNLCFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVCDFKNFDEAIDYVRY
26695A        AFYDALNSIEPVTGGGSVRNTLGYKVGNKSEFLSQYKFNLCFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIKY
26695B        AFYDALNSIEPVTGGGSVRNTLGYKVGNKSEFLSQYKFNLCFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIKY
NCTC11639     AFYDALNSIEPVTGGGSVRNTLGYNVKNKNEFLSQYKFNLCFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFKNFDEAIDYIKY
NCTC11637     AFYDALNSIEPVTGGGAVKNTLGYKVGNKSEFLSQYKFNLCFENSQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYVRY
UA948A        AFYDALNAIEPVASGGSVKNTLGYKVKNKNEFLSQYKFNLCFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIRY
              *******:****: **:*:******:* ** ***************:**********:**************************** **:*********::*

J99A          LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYH--DNPF-IFYRDLNEPLVAI-----------------------------
J99B          LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYH--DNPF-IFYRDLNEPLVAIDDLRVNYDDLRV----------------
26695A        LHTHPNAYLDMLYENPLNTLDGKAYFYQDLSFKKILDFFKTILENDTIYHKFSTSF-MWEYDLHKPLVSI-----------------------------
26695B        LHTHPNAYLDMLYENPLNTLDGKAYFYQDLSFKKILDFFKTILENDTIYH--NNPF-IFYRDLHEPLISIDDLRVNYDDLRVNYDDLRVNYDDLRVNYDD
NCTC11639     LHTHKNAYLDMLYENPLNTLDGKAYFYQNLSFKKILAFFKTILENDTIYH--DNPF-IFCRDLNEPLVTIDDLRVNYDDLRVNYDDLRINYDDLRVNYDD
NCTC11637     LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYH--NNPF-IFYRDLNEPLVSIDNLRINYDNLRV----------------
UA948A        LHAHQNAYLDMLYENPLNTIDGKAGFYQDLSFEKILDFFKNILENDTIYHCNDAHYSALHRDLNEPLVSVDDLRRDHDDLRV----------------
              **:* ***************:**** ***:***:*** *** **********      :     **::**:::

J99A          ------------DDLRVNYDDLRVNYDDLRVNYDDLRVN-------YDRLLQNASPLLELSQNTTFKIYRKAYQKSLPLLRAIRRWVKKLGL--------
J99B          ---NYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVN-------YDRLLQNASPLLELSQNTTFKIYRKAYQKSLPLLRTIRRWVKK-----------
26695A        -------------------------DDLRVNYDDLRVN-------YDRLLQNASPLLELSQNTTFKIYRKAYQKSLPLLRAVRKLVKKLGL--------
26695B        LRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVN-------YDRLLQNASPLLELSQNTTFKIYRKAYQKSLPLLRTIRRWVKK-----------
NCTC11639     LRINYDDLRVNYDDLRVNYDDLRINYDDLRVNYDDLRVN-------YERLLSKATPLLELSQNTTSKIYRKAYQKSLPLLRAIRRWVKKLGL--------
NCTC11637     ---NYDDLRVNYDDLRVNYDDLRINYDDLRINYDDLRIN-------YERLLQNASPLLELSQNTSFKIYRKIYQKSLPLLRVIRRWVKK-----------
UA948A        ---NYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRRDRDDLRRDHERLLSKATPKTPL-----LKSIRKAYQKSLPLLRAIRRWLENRFVKPIKPLAL
                                  ****:****** :       ::*** :*:*  *      *  ** ********* :*: :::

J99A          -------
J99B          -------
26695A        -------
26695B        -------
NCTC11639     -------
NCTC11637     -------
UA948A        SSQTLLS
```

B



However, this homology is largely confined to an internal region of approximately 270 amino acids which demonstrates a 82.2% identity among all *H. pylori* FucTs. The highly conserved internal 270 amino acids of *H. pylori* FucTs contains the hypothetical α(1,3) catalytic domains identified in eukaryotic FucTs by sequence alignments (6, 7)(I and II, Figure 4.2A). All *H. pylori* FucTs contain a conserved domain I with a low level identity with only 2 of 19 amino acids being conserved, while a much greater level of identity is present in domain II, with 12 of 23 amino acids being conserved (Figure 4.2A). The human FucTs (HU-FucT) have a conserved carboxy terminus containing the catalytic domain and an amino terminus containing the transmembrane and variable regions, whereas the *H. pylori* FucT (Hp-FucT) have an internally conserved region, containing the catalytic domain, and both the amino and carboxy termini are variable (Figure 4.2B).

Among *H. pylori* FucTs the first 82 amino acids exhibit only 32.0% identity. It is difficult to calculate the degree of identity over the carboxy terminal portion of these proteins (final approximately 100 amino acids) because all identified *H. pylori* FucTs (1, 18, 24, 38) have a variable number and sequence of a seven amino acid repeat (heptad repeat)(Table 4.1 and Figure 4.2). These heptad repeats are thought to function as a leucine zipper in dimerization which may be essential for function (18, 24). In most other *H. pylori* FucTs identified, the heptad repeat consists of the amino acids DDLRVNY. UA948FucTa contains five internal repeats which are of this consensus heptad sequence whereas the remaining three repeats show divergence. The two heptads which border the repeat region contain the amino acid sequence DDLRRDH, while the second last heptad contains the amino acid sequence DDLRRDR (Table 4.1 and Fig. 4.2A). Following the heptad repeats there is also a 15 amino acid addition at the carboxy terminus of the UA948FucTa protein (Figure 4.2), which does not show homology with any protein or motif presently in the databases.

4.3.2. Protein Expression. Both the UA948*fucTa* and UA948*fucTb* were directionally cloned into pBluscript KS+ under the control of the T7 promoter. Expression

of the protein was accomplished by utilization of the strain K38 containing a heat inducible T7 polymerase on the plasmid pGP1-2 (35). Under expression conditions pBUA948fucTa-containing cells expressed a protein of approximately 52 kDa which is in close agreement with the molecular weight of 55.9 kDa predicted from the sequence analysis (Figure 4.3, lane 2). We infer that the 52 kDa is the active species as previous attempts to truncate full length FucT proteins have resulted in inactivation (18). As predicted from the sequence analysis the clone containing pBUA948fucTb did not show expression of full length protein, but did express some lower molecular weight proteins, which may have arisen from alternate start sites within the gene (Figure 4.3, Lane 1).

4.3.3. FucT Enzyme Activity. By using a panel of acceptor carbohydrate molecules the specificity and activity of the cloned UA948FucT enzymes were determined. Extracts from the clone containing the pBUA948fucTb did not have any measurable FucT enzyme activity on the carbohydrate acceptors studied (data not shown), which is in agreement with both the sequence analysis and protein expression data. Significant FucT enzyme activity was detected in the extract from the clone containing the pBUA948fucTa (Table 4.2). The UA948FucTa enzyme adds fucose to type II carbohydrate acceptors (LacNAc and H type II) demonstrating α(1,3) FucT activity. Transfer of fucose to type I carbohydrate acceptors (type I chain and H Type I) was also observed. This demonstrated α(1,4) FucT activity, although it was 5-20 fold lower than the α(1,3) FucT activity (0.22 and 0.066 mu/mg versus 1.26 and 1.47 mu/mg , Table 4.2).

The addition of the fucose to type II carbohydrate chains occured irrespective of the fucosylation state of the terminal galactose. There was no significant difference in the enzyme activity between the transfer of fucose to the unfucosylated LacNAc as compared to the transfer of fucose to the already terminally fucosylated H Type II carbohydrate (Table 4.2, 1.26 versus 1.47 mu/mg). In contrast, the addition of fucose to type I chains is significantly reduced (~70%) when the terminal galactose moiety is fucosylated (type I (0.22 mu/mg)vs. H Type I(0.066 mu/mg)).

Figure 4.3. Heterologous protein expression in *E. coli* K38(pGP1-2) grown under induction conditions described in experimental procedures. Lane 1 contains extract of K38(pGP1-2) containing plasmid pBUA948fucTb, Lane 2 contains cell extract from the host containing plasmid pBUA948fucTa, Lane 3 contains sample from the host strain with pBluscript KS+. The arrow indicates an 52 kDa protein which is expressed by the clone containing the full length active FucT. The clone containing the pBUA948fucTb does produce protein under these conditions, but none would code for a full length FucT as is demonstrated by the lack of FucT activity (see text).

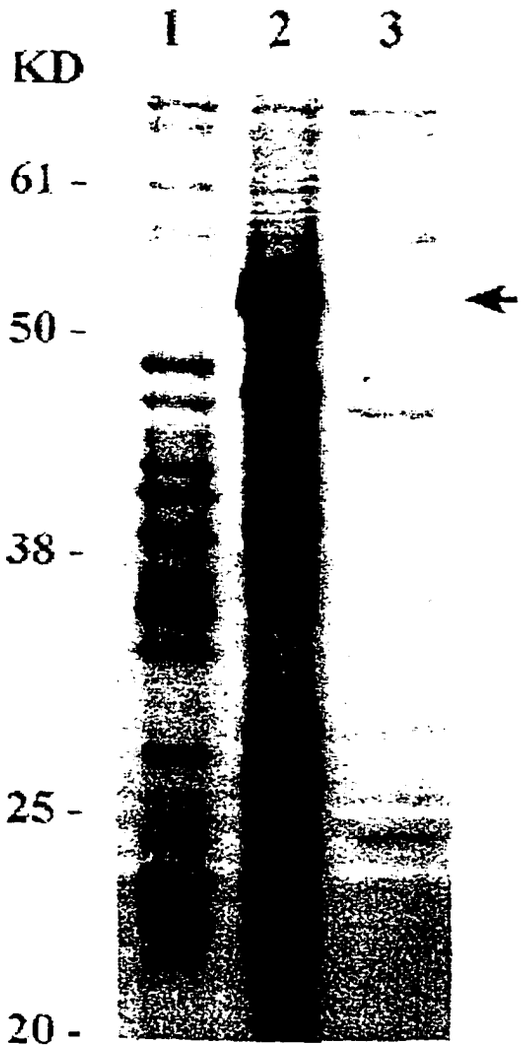


Table 4.2: Transfer of fucose by the heterologously expressed UA948fucTa enzyme.

Acceptor[a]	Final product[a]	Activity[b]	Relative Activity[c]
Type I	Lewis A	0.22 ± 0.02	100
H type I	Lewis B	0.066 ± 0.013	30
LacNAc	Lewis X	1.26 ± 0.2	570
H type II	Lewis Y	1.47 ± 0.2	667

[a] Refer to Figure 4.1 for the carbohydrate structures.

[b]The enzyme activity expressed (munit/mg of protein) is an average from three determinations with standard deviations

[c] The relative activity is measured with respect to transfer of fucose to type I chain

4.3.4. Effects of UA948*fucTa* mutation. The mutation of the UA948*fucT* gene was accomplished by homologous recombination of the *fucT* gene containing a chloramphenicol acetyltransferase gene (experimental procedures). Many chloramphenicol resistant colonies were selected and screened. Each contained an increase in size of the *fucT* PCR fragment, using genomic DNA from transformants as the PCR template, but all expressed the same genotype. Using primers specific for each copy of the *fucT* gene, the PCR fragments for UA948*fucTa* and UA948*fucTb* were 1769 and 1774 base pairs respectively. Using the same primers, it was demonstrated that the UA948*fucT*⁻ transformants all contained CAT insertions into UA948*fucTa,* but not into UA948*fucTb* (Figure 4.4). Since the nucleotide sequences of the *fucT* genes are highly homologous, it was expected that the *fucT*::CAT fragment would have a similar chance to recombine into both genes.

It has been previously demonstrated that UA948 expressed both Lewis A and X simultaneously on the same LPS (27). The mutation of UA948*fucTa* has a dramatic effect on the mobility of the LPS and the expression of Lewis antigens in the LPS (Figure 4.5 and Table 4.3). The LPS from UA948*fucTa*⁻ migrates faster through the SDS-PAGE gel, as indicated by the arrow, when compared to the wild type LPS (Figure 4.5 panel A). The band is more diffuse and thus less intense than the wild type LPS. The expression of the Lewis antigens have also been eliminated in UA948*fucTa* ⁻ (Figure 4.5 panel B and C). The results were confirmed by quantitative determination of the Lewis antigens expression by ELISA (Table 4.3). This indicates that the fucose cannot be transferred to the growing LPS O side chain to produce the Lewis antigenic structures in UA948*fucTa*⁻, indicating that the only functional FucT in *H. pylori* UA948 is FucTa.

4.4. Discussion

The single functional *H. pylori* FucT enzyme identified in this study is a novel enzyme in both activity and specificity when compared to the previously characterized *H. pylori* FucTs (18, 24). Both previously identified FucTs, almost exclusively, transferred

Figure 4.4. Gel of PCR products after *fucT* inactivation. Lanes 1 and 2 contain the PCR products for the *fucTb* (1774 bp) and *fucTa* (1769 bp) respectively from the wild type UA948. Lanes 3 and 4 are the PCR products from the UA948*fucT*⁻ using the *fucTb* and *fucTa* specific primers respectively, described in experimental procedures. The UA948*fucTa* is the only gene that appears to have the chloramphenicol acetyltransferase gene insertion as is demonstrated by the increase in size of the PCR product (approximately 700 bp).

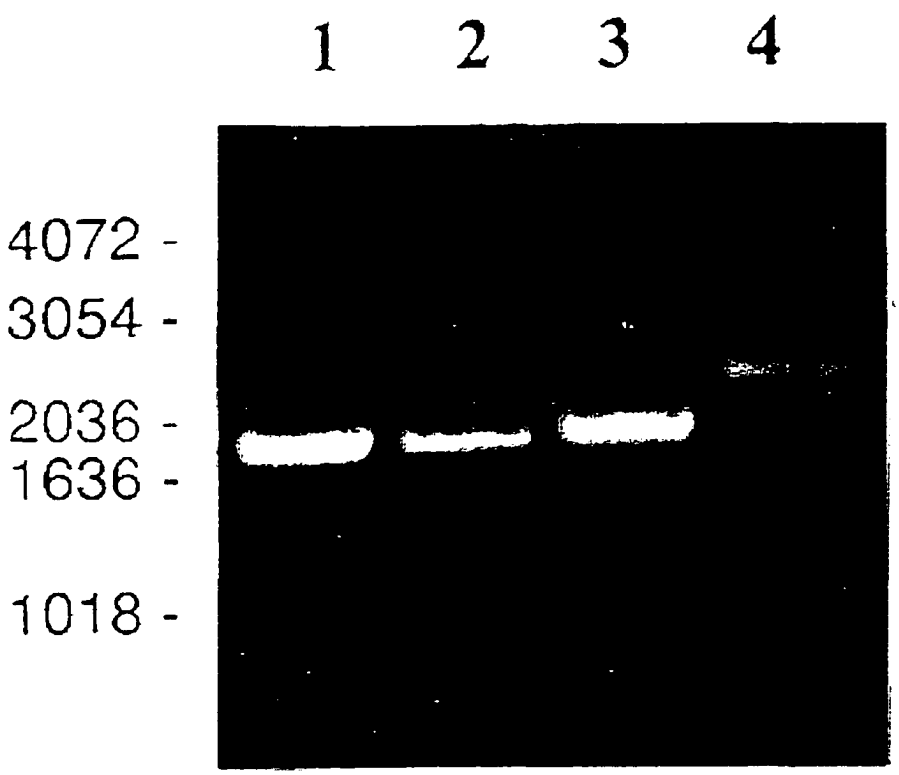


Figure 4.5. LPS and Immunoblot analysis of the UA948 and UA948*fucTa*$^-$. In all panels sample 1 is a proteinase K treated sample of UA948 and sample 2 is a similarly treated sample of UA948*fucTa*$^-$. Panel A is a zinc-imidazole stained polyacrylamide gel showing the change in mobility of the LPS from the UA948*fucTa*$^-$ isolate as is indicated by the arrow. Panels B and C are immunoblots probed with anti-Lewis A and anti-Lewis X respectively. Both panels demonstrate that the UA948*fucTa*$^-$ isolate no longer expresses Lewis antigens.

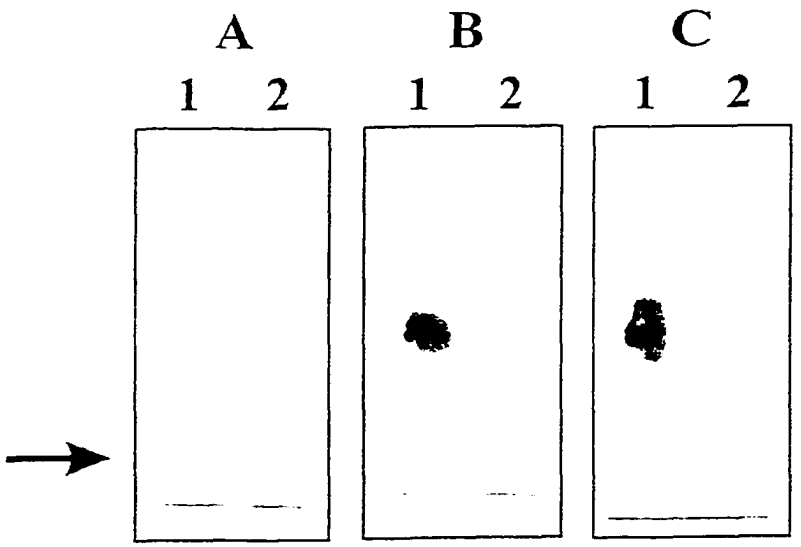


Table 4.3 : ELISA Values of UA948Wild Type Versus UA948fucTa-

Lewis Antigen	UA948	UA948fucTa-
Lewis A[a]	3.34 ± 0.04	0.032 ± 0.002
Lewis X[a]	0.78 ± 0.06	0.003 ± 0.001

[a] Antigens screened for by using method previously described in Monteiro *et al.* (27)

fucose to type II carbohydrate acceptors (18, 24). Martin *et al.* (24) did note some enzyme activity with an elongated type I carbohydrate acceptor, but no activity was noted with the minimal type 1 disaccharide lacto-*N*-biose (type I chain) used in this study. The UA948FucTa enzyme identified in this work can add fucose to both type I and II carbohydrate acceptors (Table 4.2), representing the first α(1,3/4) FucT to be identified in *H. pylori*. There is a greater than five fold preference for the type II carbohydrate acceptors over type I carbohydrate acceptors, which is more similar to the enzyme characteristics exhibited by the human Fuc-TV than any other enzyme (11). The human Fuc-TV enzyme shows a slight preference for type II acceptors, but still retains significant activity on type I carbohydrate receptors.

It was also noted in the study by Martin *et al.* (24) that the α(1,3)FucT isolated could not use the type II carbohydrate, H Type II, as an acceptor to produce Lewis Y, thus leading the authors to believe that the production of the difucosylated Lewis antigens, Le^b and Le^y, may be routed through a subterminal monofucosylation by an α(1,3) or α(1,3/4) FucT followed by the terminal fucosylation by an α(1,2)FucT (40) [Figure 4.1 panels A and B right-hand pathway]. It is apparent from the data presented in this study that this is not true for all *H. pylori* α(1,3)FucTs, as UA948FucTa has the ability to add fucose in a subterminal position on the GlcNAc of H Type II very efficiently, as well as to H Type I, with reduced efficiency, to producing difucosylated antigen [Figure 4.1 panels A and B left-hand pathway]. Our recent observations (unpublished data) show that the α(1,2) FucT of some *H. pylori* isolates can use both the subterminally fucosylated (Lewis A or Lewis X) as well as the unfucosylated carbohydrate chains (type I and LacNAc) as acceptors. More work with both the α(1,2) FucT and the α(1,3/4) FucT is necessary to definitively identify the pathway for the synthesis of difucosylated antigens by *H. pylori*.

Although two homologous copies of the *fucT* gene exist within the *H. pylori* genome of most *H. pylori* strains, only one appears to be active in *H. pylori* UA948. Interestingly, even though the UA948*fucTb* gene is inactive with respect to FucT activity,

no insertions of the CAT cassette into this gene could be obtained. We have also observed this phenomenon in other *H. pylori* isolates while attempting to make a double mutant in which both of the *fucT* genes are insertionally inactivated (D.A. Rasko unpublished data). This suggests that UA948*fucTb* may encode an unknown but essential gene product or that the genes flanking UA948*fucTb* are essential and may be transcriptionally or translationally linked. Upstream of UA948*fucTb* is the gene encoding a cytochrome C biogenesis protein, which terminates only 13 nucleotides upstream of the proposed start codon of the *fucTb* ORF in both 26695 (38) and J99 (1). It is possible that these genes are coordinately transcribed and insertion of the CAT cassette into the *fucTb* gene affects the synthesis of the cytochrome C biogenesis protein which would be deleterious to the cell. Whereas, there are amino acid biosynthetic genes in both the upstream and downstream positions of UA948*fucTa*. Considering the richness of the media used for culture the inactivation of these genes do not pose a strong selective pressure on the *H. pylori*.

The LPS of UA948*fucTa*$^-$ no longer contains any of the complex, fucose-containing, Lewis antigens that are present in the wild type UA948 strain even though only a single *fucT* gene has been inactivated (Figure 4.5 and Table 4.3). This further supports the conclusion that only one copy of the *fucT* gene is active in this *H. pylori* isolate. The inactivation of the only *fucT* gene in this *H. pylori* isolate provides an opportunity to determine what role, if any, the formation of complexed fucose containing carbohydrates in the LPS plays in the infectious process of *H. pylori* .

It has been noted in the identification of the key amino acids involved in the specificity of the human FucTs that single amino acid changes can change or eliminate the expression of the FucT activity (14-16, 23, 25, 31, 44). Due to the low level homology between the prokaryotic and eukaryotic α(1,3) FucTs it is difficult to use the amino acid sequence of the human FucTs to predict which amino acid changes may be responsible for the alteration of acceptor specificity of *H. pylori* FucTs. However, the human system may provide clues as to the identification of the region responsible for this activity in the *H.*

pylori FucTs. Breton *et al.* (6, 7) identified two conserved motifs within the catalytic domain that are identical in all of the human α(1,3) FucT enzymes regardless of the acceptor specificity. These domains are also present in the α(1,3) FucT of other species (30). By sequence analysis, these two motifs have been localized in the *H. pylori* α(1,3) FucTs (Figure 4.2A domain I and II) and they are also present in the α(1,3/4) FucT of *H. pylori*. The *H. pylori* FucTs have a high level of amino acid identity (~82%) in the internal 270 amino acids of the FucT proteins corresponding to the proposed catalytic domain. In the human FucTs, mutations in this highly conserved region generally correspond to the inactivation of the FucT activity of the protein (15, 16, 29, 31).

Obvious differences were observed when the UA948FucTa is compared to the previously identified *H. pylori* FucTs. Firstly, there is a low level of homology (~30%) in the amino terminal 80 amino acids of *H. pylori* FucTs, which in the human FucTs is the location of the transmembrane and hypervariable region (Figure 4.2B). The hypervariable region is the location of essential amino acids responsible for the determination of the enzyme acceptor specificity (14, 30). All of the *H. pylori* FucTs examined by our group (unpublished data) appear to have differing rates for transfer of fucose to identical acceptors, which may be a direct reflection of the variability exhibited in the primary sequence in the amino terminus of the proteins (Figure 4.2A).

Significant differences are also observed in the carboxy terminal portions among the different *H. pylori* FucTs. This region contains the heptad repeats, that form the proposed leucine zipper region and UA948FucTa exhibits significant amino acid sequence variability here when compared to previously identified *H. pylori* α(1,3) FucTs (Figure 4.2). The first four amino acids of the UA948FucTa heptad repeat are conserved, DDLR, but the final three amino acids are divergent. The UA948FucTa heptad repeats still contain the leucine moieties in the appropriate spacing to continue acting as a leucine zipper. It is clear that although variable, there is some conservation of physical attributes of the variable amino acids in the repeat region as the net charge of these three amino acids is relatively

conserved. It is unclear at this time if the non-homologous flanking heptad repeats are involved in the broadening of the acceptor range to include the type I carbohydrate acceptors, or if these regions are responsible only for the hypothesized function of dimerization (18, 24). Finally, there is also a carboxy terminal 15 amino acid addition in UA948FucTa, which is not present in the other *H. pylori* α(1,3) FucTs and does not share any homology with any identified sequence. It was noted in Ge *et al.* (18) that a carboxy terminal 115 amino acid truncation eliminated all FucT enzyme activity, proving that this region is essential for enzyme activity.

We have demonstrated the existence of an α(1,3/4)FucT from *H. pylori* which is responsible for the production of both Le^a and Le^x (Figure 4.1). This enzyme exhibits significant sequence divergence at both the nucleotide and amino acid level from previously identified *H. pylori* FucTs. The regions of variability will need further investigation to determine their role, if any, in the activity and expanded acceptor range of UA948FucTa. It will require a careful molecular study, identification of more *H. pylori* α1,3/4 FucTs, and domain swapping experiments similar to those performed with the human FucTs, to determine exactly which of the changes are responsible for the broader acceptor range of UA948FucTa.

4.5. Bibliography.

1. **Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F. and Trust, T. J.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. **397**: 176-180.

2. **Amano, K., Hayashi, S., Kubota, T., Fijii, N. and Yokata, S.** 1997. Reactivities of Lewis monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. Clin. Diag. Lab. Immunol. **4**: 540-544.

3. **Appelmelk, B. J., Shiberu, B., Trinks, C., Tapsi, N., Zheng, P. Y., Verboom, T., Maaskant, J., Hokke, C. H., Schiphorst, W., Blanchard, D., Simoons-Smit, I. M., van den Eijnden, D. H. and Vandenbroucke-Grauls, C.** 1998. Phase variation in *Helicobacter pylori* lipopolysaccharide. Infect. Immun. **66**: 70-76.

4. **Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A. and Degraaff, J.** 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun. **64**: 2031-2040.

5. **Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1996. Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. Biochem. **35**: 2489-2497.

6. **Breton, C., Oriol, R. and Imberty, A.** 1996. Sequence alignment and fold recognition of fucosyltransferases. Glycobiology. **6**: vii-xii.

7. **Breton, C., Oriol, R. and Imberty, A.** 1998. Conserved structural features in eukaryotic and prokaryotic fucosyltransferases. Glycobiology. **8**: 87-94.

8. **Chan, N. W. C., Stangier, K., Sherburne, R., Taylor, D. E., Zhang, Y. N., Dovichi, N. J. and Palcic, M. M.** 1995. The biosynthesis of Lewis X in *Helicobacter pylori*. Glycobiology. **5**: 683-688.

9. **Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R. and Kirchner, T.** 1998. The gastric H+, K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. Gastroenterology. **115**: 340-347.

10. **Costa, J., Grabenhorst, E., Nimtz, M. and Conrad, H. S.** 1997. Stable expression of the golgi form and secretory variants of human fucosyltransferase III from BHK-21 cells. J. Biol. Chem. **272**: 11613-11621.

11. **Costache, M., Apoil, P.-A., Cailleau, A., Elmgren, A., Larson, G., Henry, S., Blancher, A., Iordachescu, D., Oriol, R. and Mollicone, R.** 1997. Evolution of fucosyltransferase genes in vertebrates. J. Biol. Chem. **272**: 29721-29728.

12. **Costache, M., Cailleau, A., Fernandez-Mateos, P., Oriol, R. and Mollicone, R.** 1997. Advances in molecular genetics of α-2 and α-3/4-fucosyltransferases. Transfus. Clin. Biol. **4**: 367-382.

13. **de Vries, T., Srnka, C. A., Palcic, M. M., Swiedler, S. J., van den Eijnden, D. and Macher, B. A.** 1995. Acceptor specificity of different length constructs of human recombinant α1,3/4 fucosyltransferases. J. Biol. Chem. **270**: 8712-8722.

14. **Dupuy, F., Petit, F.-M., Mollicone, R., Oriol, R., Julien, R. and Maftah, A.** 1999. A single amino acid in the hypervariable stem domain of vertebrate α1,3/1,4-fucosyltransferases determines the type 1/type 2 transfer: characterization of acceptor substrate specificity of the Lewis enzyme by site-directed mutagenesis. J. Biol. Chem. **274**: 12257-12262.

15. **Elmgren, A., Borjeson, C., Svensson, L., Rydberg, L. and Larson, G.** 1996. DNA sequencing and screening for point mutations in the human Lewis (*FUT3*) gene enables molecular genotyping of the human Lewis blood group system. Vox Sang. **70**: 97-103.

16. **Elmgren, A., Mollicone, R., Costache, M., Borjeson, C., Oriol, R., Harrington, J. and Larson, G.** 1997. Significance of individual point mutations, T202C and C314T, in the human Lewis (*FUT3*) gene for expression of Lewis antigens by the human α (1,3/1,4)-fucosyltransferase, Fuc-TIII. J. Biol. Chem. **272**: 21994-21998.

17. **Gallet, P. F., Vaujour, H., Petit, J.-M., Maftah, A., Oulmouden, A., Oriol, R., Le Narvor, C., Guiloton, M. and Julien, R.** 1998. Heterologous expression of an engineered truncated form of human Lewis fucosyltransferase (FucT-III) by the methylotrophic yeast *Pichia pastoris*. Glycobiology. **8**: 919-925.

18. **Ge, Z., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1997. Cloning and heterologous expression of an α1,3 fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. J. Biol. Chem. **272**: 21357-21363.

19. **Ge, Z. and Taylor, D. E.** 1992. *H. pylori* DNA transformation by natural competence and electroporation in *Helicobacter pylori Protocols* C. L. Clayton, H. L. T. Mobley, Eds. (Humana Press Inc.) pp. 145-152.

20. **Hardy, E., Pupo, E., Castellanos-Serra, L., Reyes, J. and Fernandez-Patron, C.** 1997. Sensitive reverse staining of bacterial lipopolysaccharides on polyacrylamide gels by using zinc and imidazole salts. Anal. Biochem. **244**: 28-32.

21. **Henry, S., Oriol, R. and Samuelsson, B.** 1995. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang. **69**: 166-182.

22. **Kukowska-Latallo, J., Larsen, R. D., Nair, R. P. and Lowe, J. B.** 1990. A cloned human cDNA determines expression of a mouse stage-specific embryonic antigen and the Lewis blood group α(1,3/1,4) fucosyltransferase. Genes Dev. **4**: 1288-1303.

23. **Legault, D. J., Kelly, R. J., Natsuka, Y. and Lowe, J. B.** 1995. Human α(1,3/1,4)-fucosyltransferases discriminate between different oligosaccharide acceptor substrates through a discrete peptide fragment. J. Biol. Chem. **270**: 20987-20996.

24. **Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. and Bird, M. I.** 1997. Lewis X biosynthesis in *Helicobacter pylori*. molecular cloning of an α(1,3) fucosyltransferase gene. J. Biol. Chem. **272**: 21349-21356.

25. **Mollicone, R., Reguigne, I., Kelly, R. J., Fletcher, A., Watt, J., Chatfield, S., Aziz, A., Cameron, H. S., Weston, B. W., Lowe, J. B. and Oriol, R.** 1994. Molecular basis for Lewis α(1,3/1,4)-fucosyltransferase gene deficiency (*FUT3*) found in Lewis-negative Indonesian pedigrees. J. Biol. Chem. **269**: 20987-20994.

26. **Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Moran, A. P., Hynes, S. O., McLean, L. L., Chan, K. H., Michael, F. S., Logan, S. M., O'Rourke, J., Lee, A., Taylor, D. E. and Perry, M. B.** 2000. Lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, mouse model *H. pylori* Sydney strain, *H. pylori* P466 carrying sialyl Lewis X, and *H. pylori* UA915 expressing Lewis B: Classification of *H. pylori* lipopolysaccharides into glycotype families. European Journal of Biochemistry. **276**: 305-320

27. **Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P. and Perry, M. B.** 1998. Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem. **273**: 11533-11543.

28. **Negrini, R., Savio, A., Poiesi, C., Appelmelk, B. J., Buffoli, F., Paterlini, A., Cesari, P., Graffeo, M., Vaira, D. and Franzin, G.** 1996. Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. Gastroenterology. **111**: 655-665.

29. **Nishihara, S., Yazawa, S., Iwasaki, H., Nakazato, M., Kudo, T., Ando, T. and Narimatsu, H.** 1993. α (1,3/1,4) Fucosyltransferase (FucT-III) gene is inactivated by a single amino acid substitution in Lewis histo-blood type negative individuals. Biochem. Biophy. Res. Commun. **196**: 624-631.

30. **Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L. and Breton, C.** 1999. Divergent evolution of fucosyltransferase genes from vertebrates, invertebrates, and bacteria. Glycobiology. **9**: 323-334.

31. **Pang, H., Koda, Y., Soejima, M. and Kimura, H.** 1998. Significance of each of three missense mutations G484A, G667A, and G808A, present in an inactive allele of the human Lewis gene (*FUT3*) for α(1,3/1,4) fucosyltransferase inactivation. Glycoconj. J. **15**: 961-967.

32. **Rasko, D. A., Wilson, T. J. M., Zopf, D. and Taylor, D. E.** 2000. Lewis antigen expression and stability in *Helicobacter pylori* isolated from serial gastric biopsies. J. Infect. Dis. : In press.

33. **Sambrook, J., Fritsch, E. F. and Maniatis, T.**, 1989. *Molecular cloning: a laboratory manual.* Second edition. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

34. **Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B. S., Rudnica, W., Savio, A. and De Graaff, J.** 1996. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol. **34**: 2196-2200.

35. **Tabor, S. and Richardson, C. C.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA. **82**: 1074-1078.

36. **Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W. and Jewell, L. D.** 1987. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. Am. J. Clin. Path. **87**: 49-54.

37. **Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C. and Jewel, L. D.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

38. **Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L. X., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M. and Venter, J. C.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. **388**: 539-547.

39. **Towbin, H., Staehelin, T. and Gordon, J.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA. **76**: 4350-4354.

40. **Wang, G., Rasko, D. A., Sherburne, R. and Taylor, D. E.** 1999. Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori* : analysis of the α(1-2) fucosyltransferase gene. Mol. Microbiol. **31**: 1265-1274.

41. **Wang, Y. and Taylor, D. E.** 1990. Chloramphenicol resistance in *Campylobacter coli:* nucleotide sequence, expression and cloning vector construction. Gene. **94**: 23-28.

42. **Weston, B. W., Nair, R. P., Larsen, R. D. and Lowe, J. B.** 1992. Isolation of a novel human α(1,3) fucosyltransferase gene and molecular comparison to the human Lewis blood group α (1,3/1,4) fucosyltransferase gene. J. Biol. Chem. **267**: 4152-4160.

43. **Wirth, H. P., Yang, M. Q., Karita, M. and Blaser, M. J.** 1996. Expression of the human cell surface glycoconjugates Lewis X and Lewis Y by *Helicobacter pylori* isolates is related to CagA status. Infect. Immun. **64**: 4598-4605.

44. **Xu, Z., Vo, L. and Macher, B. A.** 1996. Structure-function analysis of human α1,3-fucosyltransferase: amino acids involved in acceptor substrate specificity. J. Biol. Chem. **271**: 8818-8823.

Chapter 5

Synthesis of mono and di-fucosylated type I Lewis blood group antigens by *Helicobacter pylori*

A version of this work has been previously submitted:

Rasko, D.A., Wang, G., Montiero, M.A., Palcic, M.M. and D.E. Taylor. Synthesis of mono and di-fucosylated type I Lewis blood group antigens by *Helicobacter pylori*. European Journal of Biochemistry.

5. Preface

The identification of *Helicobacter pylori* isolates that exclusively express type I Lewis antigens is necessary to determine the biosynthetic pathway of these antigens. FAB-MS provides evidence that the *H. pylori* isolate UA1111 expresses predominantly Le^b, with H type I and Le^a in lesser amounts. (Please note- The FAB-MS data presented in this Chapter was not gathered by the author but by Dr. M.A. Monteiro and is included in this Chapter for completeness). The enzymes responsible for the production of these antigens are similar to the ones described in Chapter 4 and their identification will allow for comparison to other *H. pylori* FucT enzymes to identify the region that enables these enzymes to synthesize the type I antigens. The identification of all three *H. pylori* FucT enzymes from this isolate allowed identification of the novel pathway involved in the synthesis of the type I antigens by *H. pylori*.

5.1. Introduction. It has been conclusively demonstrated that the gastric pathogen, *Helicobacter pylori,* can express a range of human histo-blood group antigens as a component of their lipopolysaccharide (LPS) (5, 17). Greater than 80% of *H. pylori* isolates express the type II carbohydrate antigens, Le^x and Le^y (21, 23, 34), whereas the expression of the type I Lewis antigens, Le^a and Le^b, by *H. pylori* is rare (23, 34). To date, less than 5% of *H. pylori* isolates screened express the type I Lewis antigens (23, 34). The true proportion of *H. pylori* isolates that express the Le^b antigen is unknown as it has been demonstrated by detailed chemical analysis that the monoclonal antibodies used in routine serological testing of *H. pylori* likely cross reacts with Le^b precursor structures (18). The first definitive chemical characterization of a Le^b expressing *H. pylori* has recently been reported (17).

The role of the Lewis antigens expressed by *H. pylori* originally was proposed to be molecular mimicry (4), but recent work has demonstrated that carbohydrate reactive antibodies elicited in colonized individuals reacted with an undefined region of the LPS core oligosaccharide rather than with the Lewis antigens (2, 8, 36). The identification of *H. pylori* isolates that do not express the Lewis antigens demonstrates that these antigens are not essential for colonization (21, 23). It has also been suggested that the Lewis antigens, especially Le^x, may play some role in adhesion (9, 26), and the LPS molecule as a whole has also been previously implicated as an adhesin (28). Therefore, the role of the Lewis antigens in the *H. pylori* infectious process is unknown.

The type I carbohydrates, Le^a and Le^b, are part of the human ABO blood typing system (13). In human cells the synthesis of Lewis antigens is regulated by a series of glycosyltransferases that act sequentially upon a precursor molecule. The fucosyltransferase (FucT) enzymes are responsible for the final steps in this process. The biosynthetic pathway of Le^a and Le^b in humans has been investigated extensively. Synthesis of Le^b has been shown to be accomplished by a terminal $\alpha(1,2)$ fucosylation, by Fuc-TI or Fuc-TII (19), to produce the H Type I antigen followed by internal fucosylation

by an α(1,3/4) FucT, Fuc-TIII (15) or Fuc-TV (32) (Figure 5.1). The α(1,3/4) FucTs are also responsible for the production of Le^a by fucosylation of the type I chain, but the Le^a antigen cannot be further fucosylated to produce the Le^b antigen (13).

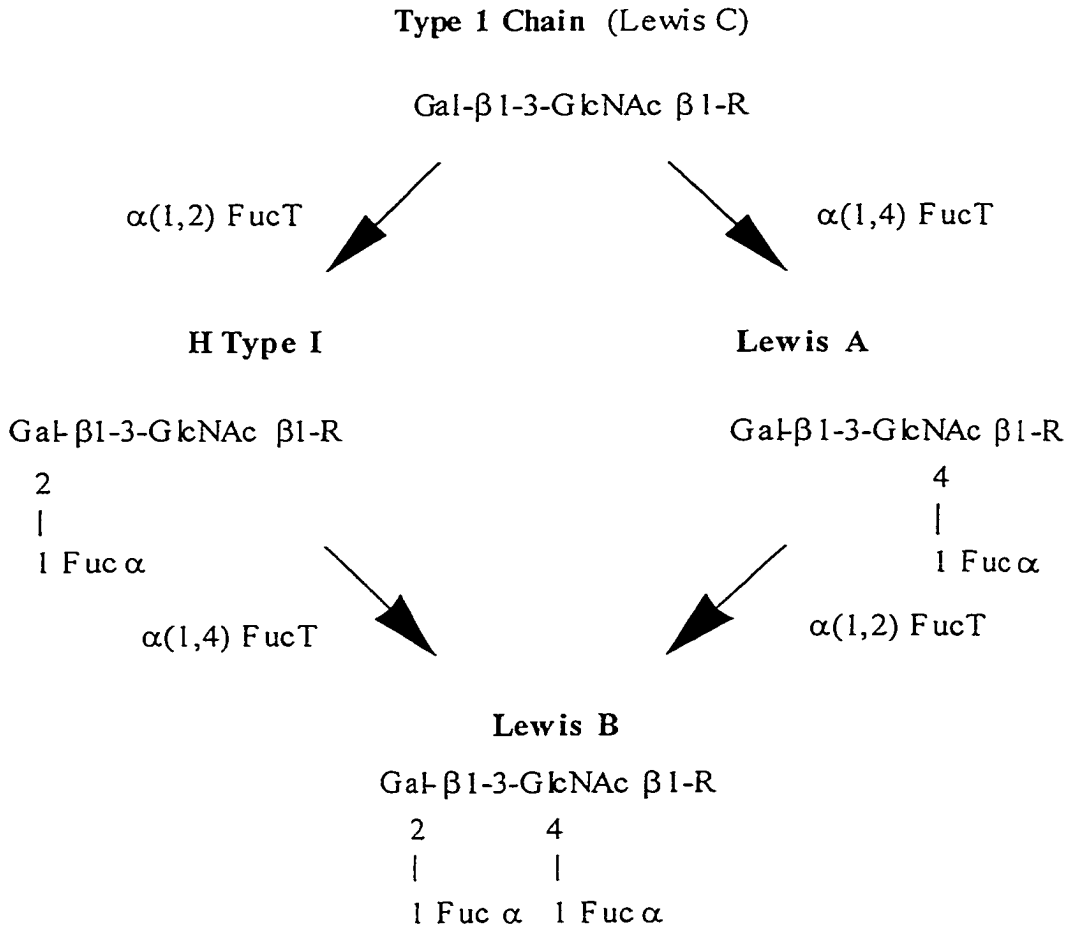
Three *fucT* genes, two α(1,3)*fucTs* {or α(1,3/4)*fucTs*} and one α(1,2)*fucT* (1, 12, 27, 30), are present in each *H. pylori* isolate investigated to date. All of the *H. pylori* α(1,3)*fucT* and α(1,3/4)*fucT* genes contain characteristic polynucleotide tracts which have been identified as the cause of slipped strand mispairing leading to phase variation of Lewis antigens (3). The *H. pylori* α(1,2)*fucT* contains similar polynucleotide tracts, which may be responsible for phase variation. The polynucleotide tracts are also present preceding and within a translational frameshifting cassette, in the *fucT2* genes of some *H. pylori* strains, which is responsible for overcoming the effects of slipped strand mispairing (30). The *H. pylori* α(1,3)FucT and α(1,3/4)FucT enzymes also contain a series of carboxy terminal heptad repeats which may play a role in the dimerization of the FucT proteins (12).

Synthesis of Le^y by *H. pylori* was distinct from the human biosynthetic pathway because fucose is added first by the α(1,3) FucT, followed by the α(1,2) FucT, reverse order to that observed in the human system (30) . The identification of an *H. pylori* isolate exclusively expressing type I antigens provides an opportunity to investigate the enzymes and biosynthetic pathway involved in type I carbohydrate antigen synthesis in *H. pylori.*

5.2. Experimental Procedures

5.2.1. *H. pylori* Isolation. Strains were cultured by standard methods described by Taylor *et al.* (25). Biopsy samples were plated out on brain heart infusion yeast extract agar (BHI-YE) plates (3.7% brain heart infusion broth [Difco], 0.5% yeast extract, 15 μg/ml of both vancomycin and amphoterocin B, 5.0% fetal bovine serum, and 1.2% agar). These plates were incubated at 37°C under microaerobic conditions for 2-4 days.

Figure 5.1. Possible biosynthetic pathways of Type I Lewis antigens. The left-hand side of the diagram exemplifies the production of Le^b by humans, while the right-hand side is an alternative method by which *H. pylori* produces Le^b.



Positive *H. pylori* cultures were confirmed by urease test, and microscopy. *H. pylori* containing the chloramphenicol acetyltransferase (CAT) cassette (31) inserted into either the *fucTa* or *fucT2* genes were selected for and cultured on BHI-YE agar plates as described above containing 50 μg/ml chloramphenicol (Sigma-Aldrich, Canada). *Escherichia coli* DH10β was used as the standard cloning host and DT1967 (*E. coli* K38(pGP1-2) {heat inducible T7 polymerase(24)}) was used as the heterologous expression host. *E. coli* containing the appropriate antibiotic resistances were grown on Lauria broth agar containing 100 μg/ml ampicillin, 50 μg/ml kanamycin, or 50 μg/ml chloramphenicol (Sigma-Aldrich, Canada).

5.2.2. DNA manipulation techniques. Standard DNA manipulation techniques including the isolation, transformation and restriction enzyme digestion analysis of plasmid DNA were performed as detailed by Sambrook *et al.* (22). Both strands of the appropriate PCR fragments were sequenced using the Thermosequenase sequencing kit (Amersham) according to the manufacturer's instruction. Sequence analyses were performed with the BLAST program from the National Center of Biotechnology Information (Bethesda, MD, USA). The Wisconsin Package (version 9.0) of the Genetics Computer Group (GCG; Madison, WI, USA) was used for the editing and alignment of sequences.

5.2.3. Cloning and Characterization. Cloning of the fucosyltransferase genes (*fucTs*) of UA1111 was accomplished by using primers previously described in Rasko *et al.* (20) for the α(1,3/4)*fucTs* and Wang *et al.* (30) for the α(1,2)*fucT*. PCR was performed as previously described (11) producing fragments of 1733, 1905 and 1111 nucleotides for UA1111*fucTa*, UA1111*fucTb* and UA1111*fucT2* respectively. Restriction of the fragments with *Eco*RI and *Bam*HI allowed ligation with a similarly digested pBluscript KS+ placing the UA1111*fucTs* under control of the T7 promoter. Screening for positive clones was done using the aforementioned primers. Protein expression for enzyme analysis and autoradiography was performed using the previously described procedure of Rasko *et al.* (20) (Chapter 4).

5.2.4. ELISA for Lewis Antigens. The conditions for the ELISA were previously described (18, 34). The primary antibodies used were anti-H Type I (MAb BG-4) anti-Le^a (MAb BG-5), anti-Le^b (MAb BG-6), from Signet Laboratories Inc. (Dedham, MA, USA). These primary antibodies were diluted 1:100 while the secondary antibody, goat anti-mouse IgG + IgM conjugated to horseradish peroxidase (HRP) (Biocan #115 035 068, Mississauga, ON.), was diluted 1:2000. Absorbance values are an average of triplicate wells with blanks subtracted and values below 0.2 absorbance units were considered negative.

5.2.5. Acrylamide gel electrophoresis and Immunoblots. Whole cell extracts of the *H. pylori* strains were treated with Proteinase K and processed as described by Monteiro *et al.* (18). Anti-Lewis structure (Signet Lab. Inc.) antibodies diluted 1:500 were used as the primary antibody, and goat anti-mouse conjugated to HRP diluted 1:2000 was used as the secondary antibody. Blots were developed using an enhanced chemiluminescence kit (Amersham Life Sciences) according to the manufacturer's specifications. Blots were developed on BioMax BM film (Kodak, Rochester NY, USA).

5.2.6. FAB-MS. All experimental procedures are detailed in Monteiro *et al.* (18).

5.2.7. Fucosyltransferase Assay. The FucT assays were performed as previously described (20). Acceptors used in this study were: type I chain {βGal1-3-β-GlcNAc-O-$(CH_2)_8CO_2CH_3$}, Le^a {βGal1-3β(αFuc1-4)GlcNAc-O-$(CH_2)_8CO_2CH_3$}, H Type I {αFuc1-2β Gal-1-3 β GlcNAc-O-$(CH_2)_8CO_2CH_3$}, LacNAc {βGal-1-4-βGlcNAc-O-$(CH_2)_8CO_2CH_3$}, Le^x {βGal1-4β(αFuc1-3)GlcNAc-O-$(CH_2)_8CO_2CH_3$}, H Type II {αFuc 1-2 β Gal 1-4 β GlcNAc-O-$(CH_2)_8CO_2CH_3$}. These acceptors were kindly provided by Dr. O. Hindsgaul. For calculation of the specific activity of the enzyme (micro-units {the amount of enzyme per millilitre needed to convert 1 pmol of acceptor to product within the defined time period} per milligram of protein), protein concentrations of the cell extracts were determined with a BCA protein assay kit (Pierce, Rockford, IL) using bovine serum albumin as a standard according to supplier's instructions.

5.3. Results

5.3.1. Antigen Identification. *H. pylori* UA1111 was identified during screening of the culture collection maintained at the University of Alberta. This strain exhibited a strong positive reaction with the anti-Le^b antibody in both ELISA and immunoblot. Further detailed chemical analysis of the UA1111 was necessary due to the crossreactivity of Le^b monoclonal antibodies (13), as well as previous experience with isolates that appeared to be Le^b positive by ELISA, but do not contain the complete Le^b antigen (18). The LPS of *H. pylori* UA1111 was analyzed by the chemical methods employed in the studies of the previously investigated *H. pylori* LPS (18). The sugar analysis of UA1111 intact LPS disclosed the presence of (approximate molar ratios in brackets) L-Fuc (2), D-Glc (3), D-Gal (2), D-GlcNAc (1), DD-Hep (2) and LD-Hep (2). The Le^b antigen is identified by the observation of *m/z* 812 [Fuc_2, Gal, GlcNAc] and the secondary ion *m/z* 402 from the loss, through β-elimination, of Fuc-1-2-Gal-OH from the *O*-3 position of GlcNAc of Le^b {α-L-Fuc-(1-2)-β-D-Gal-[Fuc-(1-4)]-β-D-GlcNAc-(1-} in the FAB-MS spectrum of the methylated UA1111 LPS (Figure 5.2 and Table 5.1). The FAB-MS spectrum of UA1111 LPS also showed, albeit in smaller intensity, *m/z* 638 [Fuc, Gal, GlcNAc] which carried two secondary ions, *m/z* 228 and trace amounts of *m/z* 402 (Figure 5.2). The presence of the secondary ion at *m/z* 228 is due to the loss of Fuc-Gal-OH from *O*-3 of GlcNAc indicating the presence of the H type 1 (Le^d) antigen {α-L-Fuc-(1-2)-β-D-Gal-(1-3)-β-D-GlcNAc-(1-} (Table 5.1 and Figure 5.2), whereas, the secondary ion at *m/z* 402 identifies the presence of the Le^a structure {β-D-Gal-[Fuc-(1-4)]-β-D-GlcNAc-(1-} due to liberation of the Gal-OH from the *O*-3 of GlcNAc (Table 5.1 and Figure 5.2).

H. pylori strain UA1111 was shown to express the type 1 Le^b antigen, the H-type 1 antigen with decreased intensity and trace amounts of Le^a in its O chain. The analyses point towards a low-M_r semi rough-form LPS with a short O chain composed of a single type 1 determinant (Le^b, H Type I or Le^a) attached to the core oligosaccharide. Thus the

Figure 5.2. The complete FAB-MS spectrum of methylated intact LPS of *H. pylori* UA1111. The primary ion *m/z* 812 and the secondary ion *m/z* 402 indicates the presence of Le^b. The other primary ion *m/z* 638 produces the secondary ions *m/z* 228 and *m/z* 402, indicative of the presence of H Type I and Le^a respectively.

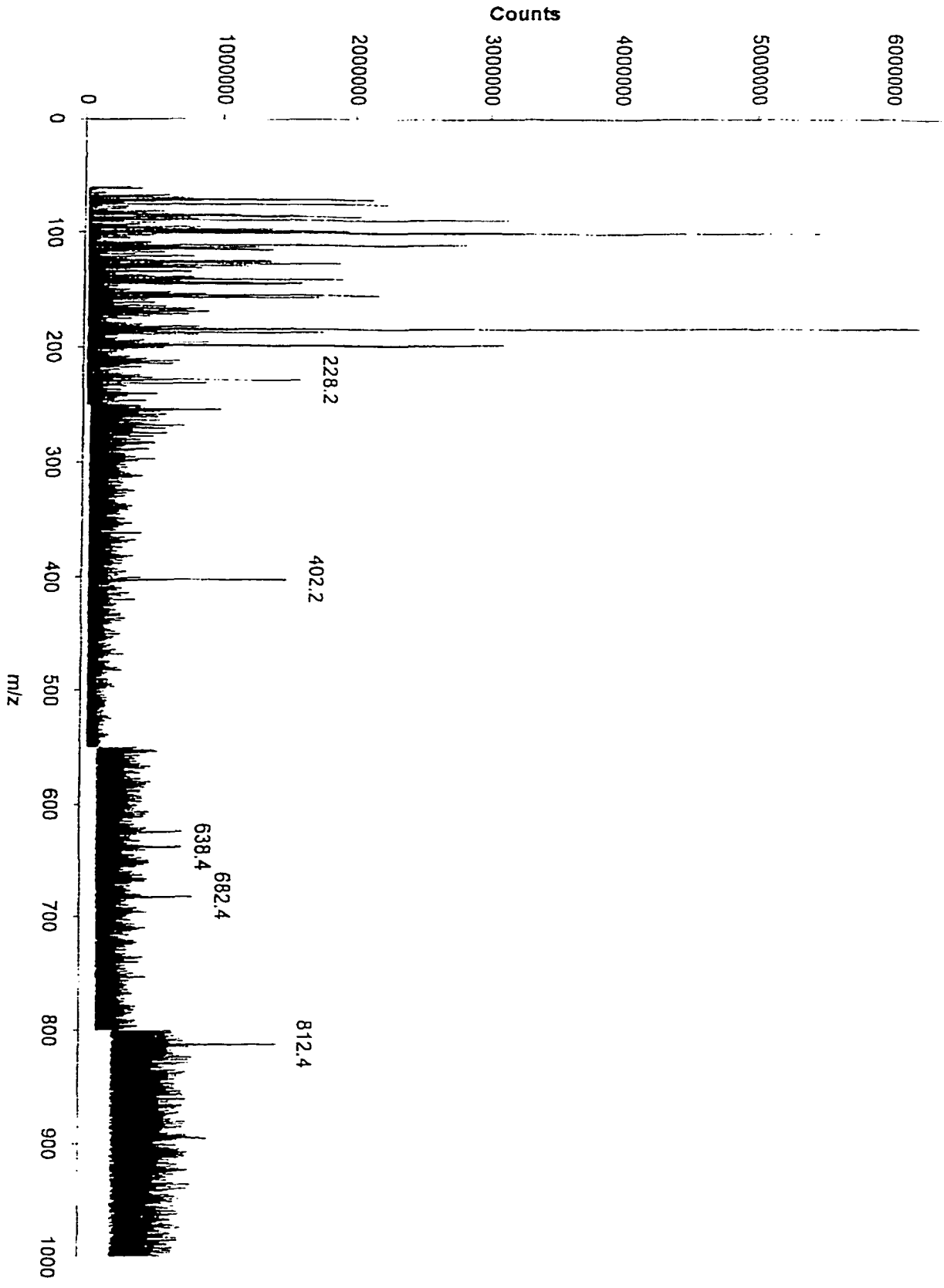


Table 5.1. Interpretation of the ions from the FAB-MS spectrum (Figure 5.2) of methylated intact LPS of H. pylori UA1111

Primary ions	Secondary ions from β -elimination	Structure	
812	402	Fuc-1-2-Gal-1-3-GlcNAc$^+$ 4 | Fuc-1	Lewis B
638	228	Fuc-1-2-Gal-1-3-GlcNAc$^+$	H Type I
638	402	Gal-1-3-GlcNAc$^+$ 4 | Fuc-1	Lewis A

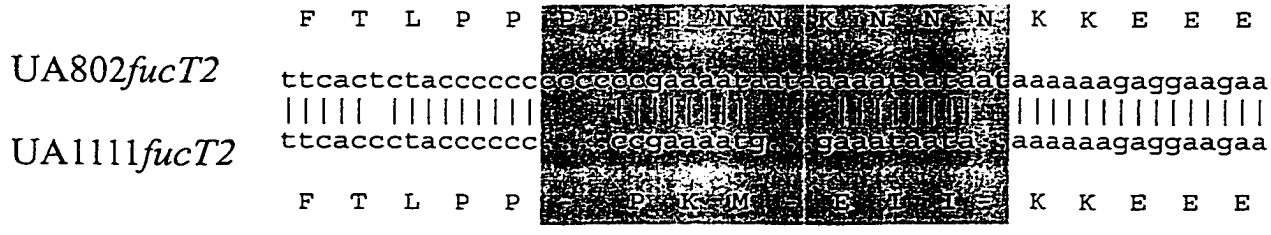
LPS of UA1111 belongs to the glycotype H family containing exclusively type I antigens (17). The core oligosaccharide had the same sugar linkage types as the cores from other *H. pylori* strains and all the typical *H. pylori* core derivatives were also accounted for in the methylation analysis of UA1111 LPS (data not shown).

5.3.2. Features of the UA1111 Fucosyltransferases. Characteristic features of the *H. pylori fucT*s were identified in the DNA of all three *fucTs* investigated. The α(1,2)*fucT* gene, UA1111*fucT2*, is greater than 95% identical to the previously identified *H. pylori fucT2* genes (30). One interesting feature of UA1111*fucT2* is the presence of 2 frameshift mutations and nucleotide deletions in the polynucleotide repeat regions. This resulted in a deletion of three amino acids as well as producing a distinct 5 amino acid cluster, compared to the previously characterized *H. pylori* UA802FucT2 (Figure 5.3A). Since the two compensatory frameshifts are relatively close together the translation of the protein shifts out of frame and then back in again while maintaining the integrity of the open reading frame. Translation of the UA1111*fucT2* insert indicates that a protein of 297 amino acids with a predicted molecular weight of 34.9 kDa may be produced from this insert.

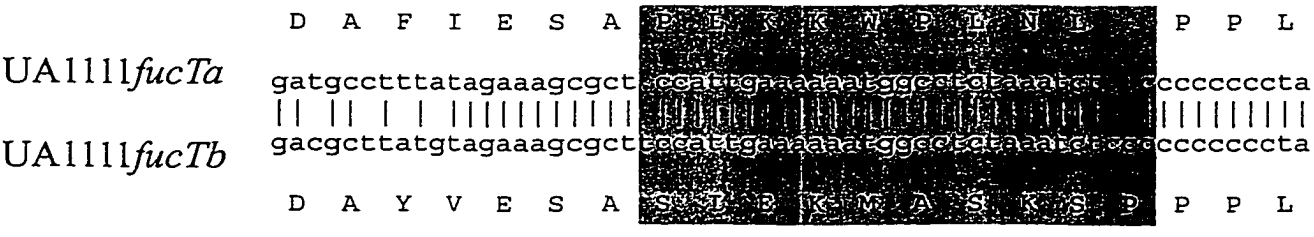
Two homologues of the α(1,3) *fucT* gene, designated *fucTa* and *fucTb*, exist in UA1111 as in most *H. pylori* isolates (1, 27). The polynucleotide tracts responsible for phase variation are present in both of these genes; UA1111*fucTa* contains 8 cytosines and 6 adenines, whereas UA1111*fucTb* contains 10 cytosines and 6 adenines. The UA1111*fucTa* gene, compared to UA1111*fucTb*, contains two frameshift mutations (Figure 5.3B) within and around the poly-A-poly-C tracts. In this case the mutations cause the deletion of only a single amino acid, but produce an eight amino acid block of divergence not homologous to any other *H. pylori* FucT, whereas the predicted amino acid sequence in this region of UA1111FucTb is identical to other identified *H. pylori* FucTs (data not shown). Translation of the sequenced inserts containing UA1111*fucTa* and

Figure 5.3. The effect of the frameshift mutations on the UA1111*fucT* genes. All UA1111*fucT* genes encode open reading frames which correspond to complete full length FucT proteins. Both the frameshift mutations and the nucleotide deletions are responsible for the peptide sequence variation observed in the shaded areas. A - Comparison of nucleotide and peptide sequences of UA1111*fucT2* and UA802*fucT2*. B - Comparison of nucleotide and peptide sequences of UA1111*fucTa* and UA1111*fucTb*.

A



B



UA1111*fucTb* indicates that both inserts can produce full length *H. pylori* FucTs. UA1111*fucTa* encodes an ORF of 435 amino acids with a predicted molecular mass of 50.9 kDa, whereas UA1111*fucTb* encodes a ORF of 453 amino acids with a predicted molecular mass of 52.9 kDa.

Comparison of the proteins encoded by UA1111*fucTa* and UA1111*fucTb* with the other known *H. pylori* α(1,3) FucTs (12) and the *H. pylori* α(1,3/4) FucT (20) demonstrates an overall amino acid identity of approximately 80%. Much of the identity is restricted to the internal, hypothetical catalytic domain while both the amino and carboxy termini of the proteins demonstrate divergence (data not shown), as is the case with previously identified *H. pylori* α(1,3) FucTs (20). The size of the *H. pylori* FucT enzymes varies mainly due to the carboxy terminal heptad repeat region which is variable in both number and sequence of the repeats between the homologous FucT proteins even within the same isolate (12). A consensus heptad repeat sequence, DDLRVNY, has been identified in all *H. pylori* α(1,3) FucTs and in the α(1,3/4) FucT examined and is thought to play a role in dimerization (12). UA1111FucTa contains three repeats of the consensus sequence preceded by one heptad of the alternative sequence, DGLRVNY, whereas UA1111FucTb has 6 repeats of the consensus sequence.

5.3.3. Fucosyltransferase Activities of the Isolated FucT enzymes of UA1111. The activities of the heterologously expressed UA1111FucTa, UA1111FucTb and UA1111FucT2 enzymes were examined to determine if they demonstrate any differences in specificity when compared to the previously identified *H. pylori* FucT enzymes. Autoradiography demonstrated that all three enzymes were expressed in similar amounts and had approximately the predicted molecular weights (Figure 5.4). Both UA1111α(1,3/4) FucTs exhibited a level of FucT activity *in vitro* that is ten-100 fold lower than previously characterized *H. pylori* FucTs. UA1111FucTa had an extremely low level of activity *in vitro* for all of the acceptors tested which is close to the sensitivity

Figure 5.4. Autoradiograph of protein over expression from UA1111*fucT* inserts. Lane 1 - vector alone (pBluscript KS+), Lane 2 - UA1111*fucTa*, Lane 3 - UA1111*fucTb*, Lane 4 - UA1111*fucT2*. Arrows designate the protein product from the appropriate insert. All proteins are expressed in similar amounts and protein products are similar to the predicted molecular weights indicated in the text.

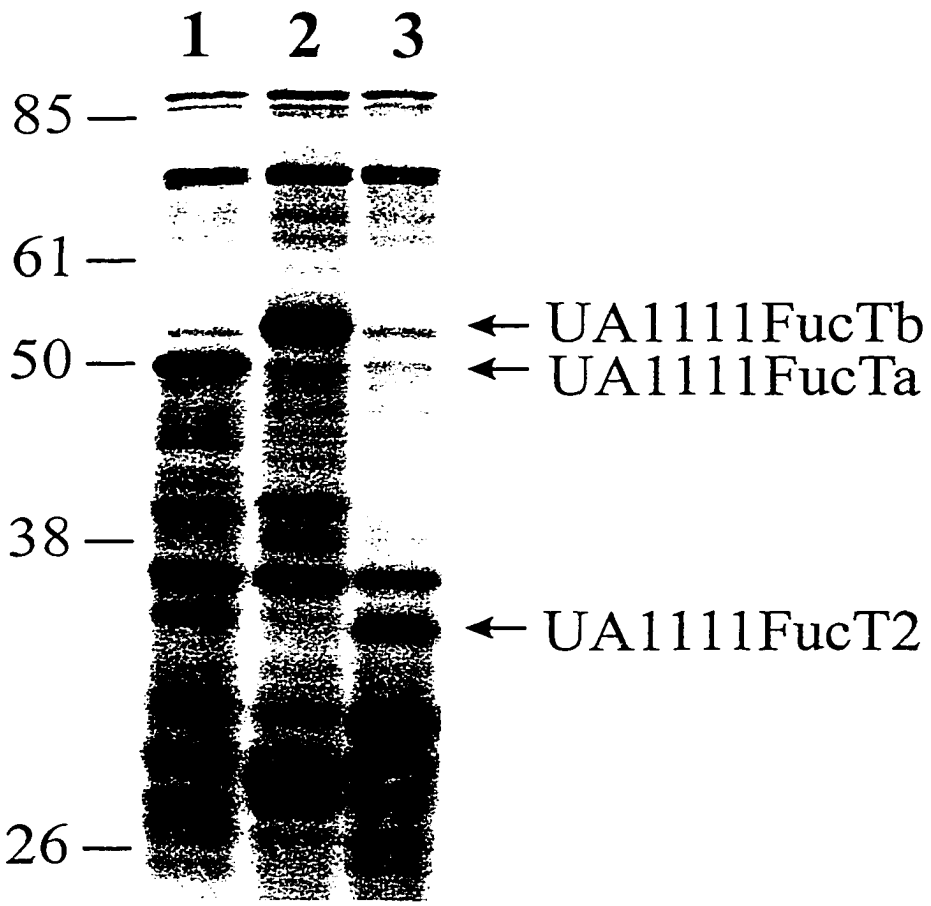


Table 5.2 : Specific Activities[a] of Heterologously Expressed UA1111 FucTs

α(1,3/4) Activity			α(1,2) Activity	
Acceptor[b]	UA1111FucTa	UA1111FucTb	Acceptor[b]	UA1111FucT2
LacNAc	0.65 ± .056	14.5 ± 0.65	LacNAc	22.7 ± 0.7
H Type II	2.75 ± 0.45	28.4 ± 1.0	Lewis X	1.8 ± 0.1
type I Chain	1.0 ± 1.0	10.7 ± 0.6	type I Chain	8.85 ± 0.15
H Type I	0.9 ± 0.9	1.5 ± 0.3	Lewis A	3.1 ± 0.1

[a]Specific activities are measured in μU/mg of protein (μU is measured as the amount of enzyme needed per milligram of protein to catalyze the transfer of 1pmol of fucose to the acceptor). mU were observed by UA948FucTa in Chapter 4.

[b] Carbohydrate structures can be found in Figure 5.1

limit of the assay. The activity of UA1111FucTb was also low but measurable. The UA1111FucTb enzyme demonstrated a preference for the addition of fucose to type II carbohydrate acceptors (Table 5.2), even though type II antigens are not produced by this isolate. There was approximately a 2 fold preference for the type II acceptor, H Type II, containing fucose on the terminal galactose over the unfucosylated chain (LacNAc) (Table 5.2). With the type I carbohydrate acceptors a lower level of enzyme activity is measured for UA1111FucTb and the enzyme preference is reversed, as the unfucosylated type I chain acceptor was preferred greater than 7 fold over the terminally fucosylated H Type I acceptor (Table 5.2). The activity measured for UA1111FucTb indicates that the synthesis of the Le^a antigen from the type I chain would be favored over the production of Le^b from the H Type I antigen.

The UA1111FucT2 also displays a similar preference for type II carbohydrate acceptors (Table 5.2). In contrast to the α(1,3/4)FucTs described above, UA1111FucT2 prefers the non-fucosylated carbohydrate acceptors (LacNAc and type I chain) over the internally fucosylated acceptors (Le^x and Le^a) regardless of type I or II linkage within the carbohydrate acceptors (Table 5.2). With the type I acceptors UA1111FucT2 prefers the unfucosylated type I chain with an activity almost 3 fold greater than that measured for the Le^a acceptor. From the *in vitro* enzyme activities measured, the synthesis of the H Type I antigen from the type I acceptor would be produced predominantly by UA1111FucT2 over the production of Le^b from the Le^a antigen.

5.3.4. Effect of *fucT* and *fucT2* knock out mutations on the expression of Le^b and Le^a in *H. pylori*. To demonstrate the requirement of the FucTs in the biosynthesis of Le^b by UA1111 the *fucT* genes (*fucTa* and *fucT2*) were inactivated by inserting a CAT cassette (11). The CAT cassette was inserted into either the UA1111*fucTa* or UA1111*fucT2* gene by homologous recombination as previously described (11, 30). The insertions of the CAT cassette into the UA1111 genome was verified by PCR amplification of the fragment and the observation of an increase in PCR

fragment size (data not shown), no CAT insertions into UA1111*fucTb* could be identified, as was previously noted (20). The Le^b expression of these *H. pylori* UA1111 mutants was examined by ELISA and immunoblot.

The ELISA and immunoblot data demonstrated that UA1111 strongly expresses Le^b, H type 1 to a lesser extent, while not expressing Le^a (Table 5.3 and Figure 5.5). These results are in agreement, to the limits of the ELISA, with the FAB-MS data which demonstrated the presence of all three antigens (Table 5.1 and Figure 5.2). The Le^a identified by FAB-MS is only in trace amounts which is below the detection limits of ELISA or immunoblot. The UA1111*fucT2*$^-$ mutant, as predicted, was negative for the expression of Le^b and H Type I (Table 5.3 and Figure 5.5, lane 3). This strain can express only Le^a (Table 5.3 and Figure 5.5) and this eliminates the possibility that the α(1,3/4)FucTs contain any α(1,2) FucT activity. The phenotype of the UA1111*fucTa*$^-$ mutant is similar to the wild type but with decreased Le^b expression (Table 5.3), indicating that both α(1,3/4)FucT enzymes are active in this *H. pylori* isolate, and both play a role in the *in vivo* fucosylation of the LPS. The amount of H Type I produced by UA1111*fucTa*$^-$ is not significantly altered from the wild type UA1111 (Table 5.3). From these data we can conclude that all three UA1111 FucT enzymes are active *in vivo* in the production of the Le^b antigen.

5.4. Discussion

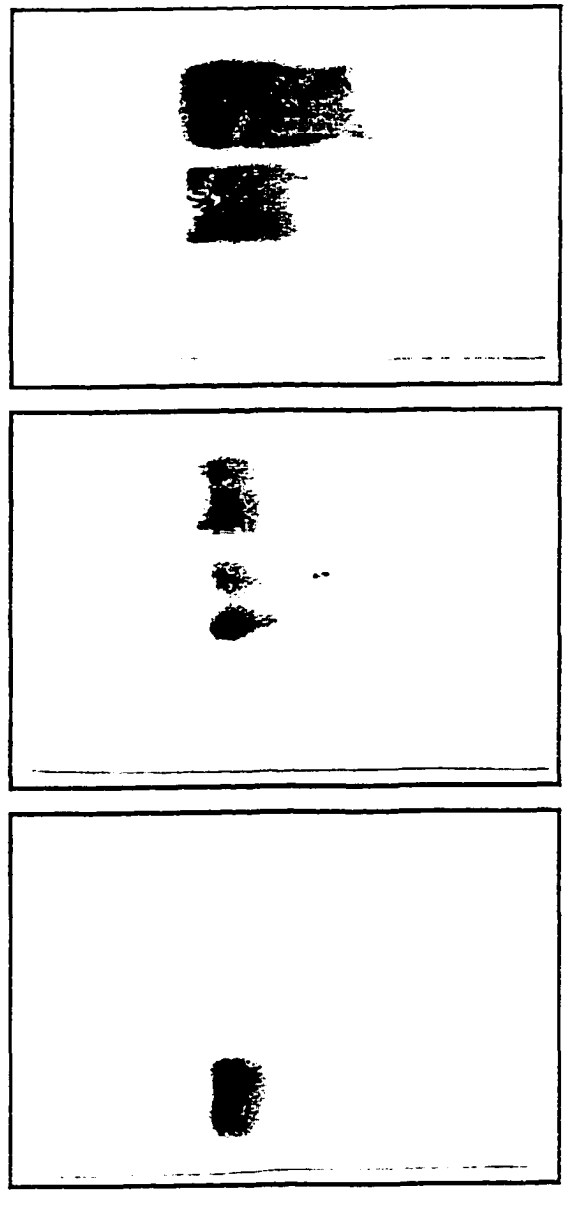
Although there have been positive ELISA reports of *H. pylori* isolates that express Le^b (18, 34, 35), detailed chemical analysis has revealed them to be false positive results, most likely due to the cross reactivity of the Le^b monoclonal antibody with other surface exposed carbohydrates (18). This report definitively demonstrates the expression of Le^b from an *H. pylori* isolate and examines the fucosyltransferase enzymes involved in its production. The expression of type I antigens exclusively by *H. pylori* enables us to determine if the type I carbohydrates are synthesized in a manner that is similar to the

Table 5.3 : Expression of Lewis antigens By *H. pylori* UA1111 and its *fucT* mutants as measured by ELISA*

Strain	Lewis B	Lewis A	H Type I
UA1111	2.415 ± 0.015	0.004 ± 0.002	0.305 ± 0.013
UA1111*fucTa*⁻	0.567 ± 0.020	0.009 ± 0.005	0.237 ± 0.005
UA1111*fucT2*⁻	0.125 ± 0.012	0.397 ± 0.001	0.018 ± 0.004

* ELISA values are an average of three individual trials with triplicates per trial and the control values have been subtracted. Values under 0.2 are considered negative.

Figure 5.5. Immunoblots demonstrating the difference in the Lewis antigen expression in the *fucT* mutants created of UA1111. The MAb used as the primary antibody is indicated above each blot. In all cases the lanes contain proteinase K treated whole cell lysates as follows; Lane 1 - UA1111, Lane 2 - UA1111*fucTa*$^-$, Lane 3 - UA1111*fucT2*$^-$.



expression of type II carbohydrates by *H. pylori* (30) or alternatively if the synthesis is more similar to the human pathway of synthesis for these antigens (13).

From the structures identified by FAB-MS it appeared as though *H. pylori* UA1111 may have produced Le^b in a manner similar to that of the human system with a terminal fucosylation of the Type I chain followed by a subterminal fucosylation (Figure 5.1, left hand pathway), based on the presence of the H Type I antigen. Since structural studies cannot conclusively identify the biosynthetic pathway of the type I Lewis antigens in *H. pylori* UA1111, we investigated the FucT enzymes involved. All of the UA1111*fucT* genes have characteristic features of the *H. pylori fucTs*. The UA1111 α(1,3/4) FucTs contain heptad repeats of the established consensus sequence in their carboxy termini (12). Polynucleotide tracts in both the α(1,2)*fucT* and α(1,3/4)*fucTs* allow for phase variation by a slipped strand mispairing mechanism (3). Frameshift mutations are present in two of the three *fucT* genes which would have created a truncated protein, but a second frameshift mutation in each gene coupled with nucleotide deletions allow for the production of full length FucT proteins with regions of diversity (Figure 5.3A and 5.3B). These regions of diversity may be responsible for the altered enzyme activities measured. UA1111FucT2 demonstrated altered enzyme characteristics; both lower activity and altered specificity were observed in comparison to the previously characterized UA802FucT2 (29). A lower level of enzyme activity is also exhibited by the UA1111 α(1,3/4) FucTs when compared to the previously characterized *H. pylori* α(1,3) and α(1,3/4) FucTs (11, 16, 20). The effects of the mutations in these regions on enzyme activity and acceptor specificity is under further investigation.

The activities of the UA1111 FucTs measured from the heterologous host were low in comparison to previously analyzed *H. pylori* FucT enzymes (11, 16, 20, 29). The clone containing UA1111FucTa appears to express almost no α(1,3/4) FucT activity, but mutational analysis indicated that FucTa contains 76% of the α(1,3/4) FucT activity produced *in vivo* (Table 5.3). The low activities were not due to lack of protein

expression (Figure 5.4), or predicted protein instability (http://www.expasy.ch/tools/protparam.html). The lack of *H. pylori* FucT activity *in vitro* may be due to the carbohydrate acceptors utilized in this study. The acceptors used for *in vitro* measurement may not be identical to the acceptors utilized within the *H. pylori* cells and thus the *H. pylori* FucT enzymes did not function at peak efficiency under the reaction conditions employed. Interestingly all UA1111FucTs expressed a preference for the type II carbohydrate acceptors *in vitro* even though the type II antigens are not synthesized *in vivo* by this isolate. This suggests that UA1111 may have produced type II carbohydrate chains containing Le^x and Le^y, like most other *H. pylori* (11, 16). The ability to fucosylate both type I and type II carbohydrate acceptors is a trait demonstrated by only the human Fuc-TV enzyme (19).

The activities of the fucosyltransferase enzymes, carbohydrate acceptors and donors and their relative amounts determine which structures will be present on the bacterial surface (12). The α(1,2) FucT preferentially produces the H type I structure but will also, at a lower level, fucosylate Le^a to produce the Le^b antigen (Table 5.2). The mutational analysis indicated that a significant amount of Le^a is produced in the absence of any α(1,2) FucT (Table 5.3 and Figure 5.5). It would seem likely that the same level of this antigen would be made in the presence of the α(1,2) FucT and all but trace amounts of the Le^a antigen produced are converted to Le^b. The expression pattern obtained from UA1111*fucT2*⁻ also demonstrated that the α(1,3/4) FucTs did not contain any α(1,2) FucT activity. UA1111FucTa has very low *in vitro* activity, whereas UA1111FucTb demonstrated a greater than 7 fold preference for the unfucosylated type I chain over the H type I antigen. This indicated that the Le^a antigen will be primarily produced by this enzyme rather than the production of Le^b from the H Type I antigen. Mutational analysis confirmed this pathway, as the inactivation of UA1111*fucTa* exhibited a decrease in the expression of Le^b, although no corresponding increase in the expression of H Type I was observed (Table 5.3). UA1111FucTb is still functional, which leads to the production of a

lower level of Le^b, but an increase in the H Type I antigen should have been observed if the pathway truly proceeded via the H Type I antigen.

Combining the enzymatic and mutational data it appears that difucosylated type I antigen synthesis proceeds primarily via an intermediate antigen containing an internal fucose, Le^a, followed by terminal fucosylation to produce Le^b. In contrast, the production of the H Type I antigen in UA1111 appears to be a result of the activity of α(1,2)FucT on the type I chain.

The *H. pylori* difucosylated antigens, Le^b and Le^y, are produced via the internally fucosylated antigen intermediates, Le^a and Le^x, regardless of the carbohydrate backbone linkage. The difference between the type I and type II Lewis antigens is the linkage of the galactose (Gal) to the N-acetlyglucosamine (GlcNAc) being β1,3 or β1,4 respectively. An *H. pylori* enzyme capable of transferring galactose to GlcNAc in either linkage has not yet been identified, but is thought to be one of the *waaJ* (*rfaJ*) homologues previously identified (6, 27). Conclusive identification of this gene and comparison of its product to the galactosyltransferases involved in human and other *H. pylori* Lewis antigen synthesis pathways will be of great importance in determining the biosynthetic pathway of the Lewis antigen backbone in *H. pylori*.

Considering that the Le^b antigen can act as a receptor for the *H. pylori* BabA protein (section 1.5.5.6), the one hypothetical advantage of the expression of Le^b by UA1111 is apparent (7, 10, 14). *H. pylori* expressing BabA could adhere to the Le^b expressed by the gastric epithelium or a Le^b expressing *H. pylori* that has already bound, forming a microcolony. Phase variants expressing Le^a or H Type I may result in loss of microcolony architecture and thus these *H. pylori* could be released and would be able to colonize secondary niches throughout the gastric environment. Although the mechanism appears to be advantageous for *H. pylori* colonization, only one other isolate has been identified to date that could benefit from this mechanism (17). One could conclude that other factors, including the immunological response toward these *H. pylori*, as well as

physical forces within the gastric environment, may be preventing this mechanism from becoming widespread among *H. pylori*.

The expression of Le^b in this case, as well as the intermediate steps in synthesis, Le^a and H Type I, support the hypothesis that *H. pylori* LPS O specific chain is produced in a sugar by sugar synthesis and not the block by block synthesis that is most common for gram negative bacteria (33). Other gram negative bacteria assemble blocks of the O side chain which are then added to the growing O side chain; an incomplete O side chain block can not be added to the growing chain (33). It appears as though *H. pylori* has the ability to synthesize the LPS O side chain with incomplete O side chain units. This is also consistent with the results from the phase variation mutants that have been characterized by Appelmelk *et al.* (3), indicating that the LPS O side chain production is not dependent on complete O side chain fucosylation. This is a novel mechanism of LPS biosynthesis among gram negative bacteria. The LPS O side chain assembly mechanism, sugar by sugar, in *H. pylori* is in accordance with the distribution of LPS biosynthetic genes throughout the genome (1, 27) rather than the block by block synthesis which accompanies the operon arrangement of genes observed in other gram negative bacteria.

Overall, *H. pylori* produces the difucosylated Lewis antigen, Le^b, via an internally fucosylated antigen, Le^a, by an LPS biosynthetic pathway that is unique among gram negative bacteria.

5.5 Bibliography

1. **Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F. and Trust, T. J.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. **397**: 176-180.

2. **Amano, K., Hayashi, S., Kubota, T., Fijii, N. and Yokata, S.** 1997. Reactivities of Lewis monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. Clin. Diag. Lab. Immunol. **4**: 540-544.

3. **Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M. J. E. and Kusters, J. G.** 1999. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α3-fucosyltransferase genes. Infect. Immun. **67**: 5361-5366.

4. **Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A. and Degraaff, J.** 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun. **64**: 2031-2040.

5. **Aspinall, G., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1994. O antigen chains in the lipopolysaccharide of *Helicobacter pylori* NCTC11637. Carbohydr. Lett. **1**: 151-156.

6. **Berg, D. E., Hoffman, P. S., Appelmelk, B. J. and Kusters, J. G.** 1997. The *Helicobacter pylori* genome:genetic factors for a long life in the gastric mucosa. Trend. Microbiol. **5**: 468-474.

7. **Boren, T., Falk, P., Kevin, A. R., Larson, G. and Normark, S.** 1993. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science. **262**: 1892-1895.

8. **Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R. and Kirchner, T.** 1998. The gastric H+, K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. Gastroenterology. **115**: 340-347.

9. **Edwards, N. J., Montiero, M., Walsh, E., Moran, A. P., Roberts, I. S. and High, N. J.** 1999. The role of lipopolysaccharide in adhesion of *Helicobacter pylori* to gastric mucosa. 10th International Workshop on *Campylobacter*, *Helicobacter* and related organisms. Abstract HP23.

10. **Falk, P., Roth, K. A., Boren, T., Westblom, T. U., Gordon, J. I. and Normark, S.** 1993. An *in vitro* adherence assay reveals that *Helicobacter pylori* exhibits cell-lineage specific tropism in human gastric epithelium. Proc. Natl. Acad. Sci. USA. **90**: 2035-2039.

11. **Ge, Z., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1997. Cloning and heterologous expression of an α1,3 fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. J. Biol. Chem. **272**: 21357-21363.

12. **Ge, Z. and Taylor, D. E.** 1992. *H. pylori* DNA transformation by natural competence and electroporation in *Helicobacter pylori Protocols* C. L. Clayton, H. L. T. Mobley, Eds. (Humana Press Inc.) pp. 145-152.

13. **Henry, S., Oriol, R. and Samuelsson, B.** 1995. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang. **69**: 166-182.

14. **Ilver, D., Arnqvist, A., Ogren, J., Frick, I. M., Kersulyte, D., Incecik, E. T., Berg, D. E., Covacci, A., Engstrand, L. and Boren, T.** 1998. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science. **279 (5349)**: 373-377.

15. **Kukowska-Latallo, J., Larsen, R. D., Nair, R. P. and Lowe, J. B.** 1990. A cloned human cDNA determines expression of a mouse stage-specific embryonic antigen and the Lewis blood group α(1,3/1,4) fucosyltransferase. Genes Dev. **4**: 1288-1303.

16. **Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. and Bird, M. I.** 1997. Lewis X biosynthesis in *Helicobacter pylori*. molecular cloning of an α(1,3) fucosyltransferase gene. J. Biol. Chem. **272**: 21349-21356.

17. **Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Moran, A. P., Hynes, S. O., McLean, L. L., Chan, K. H., Michael, F. S., Logan, S. M., O'Rourke, J., Lee, A., Taylor, D. E. and Perry, M. B.** 2000. Lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, mouse model *H. pylori* Sydney strain, *H. pylori* P466 carrying sialyl Lewis X, and *H. pylori* UA915 expressing Lewis B: Classification of *H. pylori* lipopolysaccharides into glycotype families. European Journal of Biochemistry. **276**: 305-320.

18. **Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P. and Perry, M. B.** 1998. Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem. **273**: 11533-11543.

19. **Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L. and Breton, C.** 1999. Divergent evolution of fucosyltransferase genes from vertebrates, invertebrates, and bacteria. Glycobiology. **9**: 323-334.

20. **Rasko, D. A., Wang, G., Palcic, M. M. and Taylor, D. E.** 2000. Cloning and characterization of the α1,3/4 fucosyltransferase of *Helicobacter pylori*. J. Biol. Chem. **275**: 4988-94.

21. **Rasko, D. A., Wilson, T. J. M., Zopf, D. and Taylor, D. E.** 2000. Lewis antigen expression and stability in *Helicobacter pylori* isolated from serial gastric biopsies. J. Infect. Dis. : In press.

22. **Sambrook, J., Fritsch, E. F. and Maniatis, T.**, 1989. *Molecular cloning: a laboratory manual.* Second edition. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

23. **Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B. S., Rudnica, W., Savio, A. and De Graaff, J.** 1996. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol. **34**: 2196-2200.

24. **Tabor, S. and Richardson, C. C.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA. **82**: 1074-1078.

25. **Taylor, D. E., Hargreaves, J. A., Ng, L. K., Sherbaniuk, R. W. and Jewell, L. D.** 1987. Isolation and characterization of *Campylobacter pyloridis* from gastric biopsies. Am. J. Clin. Path. **87**: 49-54.

26. **Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C. and Jewel, L. D.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

27. **Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L. X., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M. and Venter, J. C.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. **388**: 539-547.

28. **Valkonen, K. H., Wadstrom, T. and Moran, A. P.** 1997. Identification of the N-acetylneuraminyllactose specific laminin binding protein of *Helicobacter pylori*. Infect. Immun. **65**: 916-923.

29. **Wang, G., Boulton, P. G., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1999. Novel *Helicobacter pylori* a1,2-fucosyltransferase, a key enzyme in the synthesis of Lewis antigens. Microbiology. **145**: 3245-3253.

30. **Wang, G., Rasko, D. A., Sherburne, R. and Taylor, D. E.** 1999. Molecular genetic basis for the variable expression of Lewis Y antigen in

Helicobacter pylori : analysis of the α(1-2) fucosyltransferase gene. Mol. Microbiol. **31**: 1265-1274.

31. **Wang, Y. and Taylor, D. E.** 1990. Chloramphenicol resistance in *Campylobacter coli:* nucleotide sequence, expression and cloning vector construction. Gene. **94**: 23-28.

32. **Weston, B. W., Nair, R. P., Larsen, R. D. and Lowe, J. B.** 1992. Isolation of a novel human α(1,3) fucosyltransferase gene and molecular comparison to the human Lewis blood group α (1,3/1,4) fucosyltransferase gene. J. Biol. Chem. **267**: 4152-4160.

33. **Whitfield, C.** 1995. Biosynthesis of lipopolysaccharide O antigens. Trend Microbiol. **3**: 178-185.

34. **Wirth, H. P., Yang, M. Q., Karita, M. and Blaser, M. J.** 1996. Expression of the human cell surface glycoconjugates Lewis X and Lewis Y by *Helicobacter pylori* isolates is related to CagA status. Infect. Immun. **64**: 4598-4605.

35. **Wirth, H. P., Yang, M. Q., Peek, R. M., Tham, K. T. and Blaser, M. J.** 1997. *Helicobacter pylori* Lewis expression is related to the host Lewis phenotype. Gastroenterology. **113**: 1091-1098.

36. **Yokota, S., Amano, K., Shibata, Y., Nakajima, M., Suzuki, M., Hayashi, S., Fujii, N. and Yokochi, T.** 2000. Two distinct antigenic types of polysaccharide chains of *Helicobacter pylori* lipopolysacchairdes characterized by reactivity with sera from humans with natural infection. Infect. Immun. **68**: 151-159.

Chapter 6

Discussion and Conclusions

6.1 Introduction

Helicobacter pylori expression of Lewis antigenic structures has been demonstrated by several groups and yet the clinical significance of these antigens remains a mystery. Previous research into the distribution and synthesis of these antigens was limited. The studies contained in this thesis focused on the distribution of the Lewis antigens expressed by *H. pylori* as well as the fucosyltransferase enzymes that produce these antigens. In the following discussion the results of these studies and their significance are summarized. In addition, areas that require additional investigation are identified.

6.2 Lewis Antigen Distribution

The distribution of the Lewis antigens on *H. pylori* within a population may be able to provide clues as to the role of these antigens in the infectious process and pathogenicity, if indeed one exists. The expression of these antigens by *H. pylori* has been assumed to be for molecular mimicry and immune avoidance (5, 28). It has been suggested that Le^x is correlated with increased inflammation (18), but other factors such as *H. pylori* colonization density also influence this response (23). The distribution of the Lewis antigens among *H. pylori* strains has been correlated with CagA expression (47) as well as the host Lewis phenotype (36, 48). Recent work on the Lewis antigens and their role in infection has focused on the antigenic variation and mechanisms that explain that variation (3, 4). Although there has been extensive research into the role these antigens play during infection, none have been identified. Comparison of the Lewis antigen expression profiles of *H. pylori* isolates from symptomatic and asymptomatic individuals provides interesting clues to the possible role of these antigens during symptomatic and asymptomatic infection.

The most important observation about the study groups surveyed in Chapters 2 and 3 is that a significant proportion of the *H. pylori* isolates were obtained from subjects who were asymptomatic. The Lewis antigen expression of *H. pylori* isolates from this group of individuals had not been previously investigated. The Lewis antigen expression profile

of the *H. pylori* isolates of asymptomatic individuals enrolled in the Neose trial exclusively comprises the study group in Chapter 3. This group plus *H. pylori* isolates obtained from asymptomatic subjects at the University of Alberta Hospital provided a larger pool of data to analyze in Chapter 2. The *H. pylori* isolates obtained from the symptomatic patients were all collected at the University of Alberta Hospital. It is clear from the data that the Lewis antigenic profile differs between *H. pylori* isolates of symptomatic and asymptomatic individuals in three significant ways. The first difference is that the proportion of Le^x-expressing *H. pylori* is significantly decreased in the isolates from the asymptomatic subjects. It has been suggested that the presence of the Le^x antigen on an *H. pylori* isolate increases the potential for immune cell attraction and inflammation (18, 23). In light of these data, it is understandable that in *H. pylori* isolates from asymptomatic subjects, a population who exhibit little inflammation and no overt disease, there would be a concomitant reduction of an antigen that is correlated with that pathology. The data presented here further supports the evidence that *H. pylori* Le^x expression may be correlated to the progression to a severe pathology.

The second significant difference between the *H. pylori* obtained from symptomatic and asymptomatic individuals is the identification of a significantly higher proportion of non-typable *H. pylori* (NTHp) among the isolates from asymptomatic individuals when compared to the NTHp prevalence from symptomatic individuals. This once again suggests that there is a role for the Lewis antigens in the disease pathology caused by *H. pylori*. The exact structures of the LPS antigens expressed by this groups of isolates have not been determined but they are being pursued and it appears as though the LPS architecture may be different among these isolates. Whether this is directly related to the development of an asymptomatic infection will require further investigation. Manipulation of the genes involved with LPS biosynthesis will allow testing the infectious capabilities of the NTHp with altered LPS to determine what role these molecules play in asymptomatic infection.

The third major difference between *H. pylori* obtained from the different study groups is the lack of identification of Le^a or Le^b among the isolates from asymptomatic subjects. There is only a small proportion of *H. pylori* that express these antigens among the *H. pylori* from the symptomatic individuals. It is possible that the expression of these antigens may be so low that they were not detected in this study, but that in itself is also interesting. It may be that the Le^a and Le^b antigens, or other factors associated with this antigenic type cause the pathology and disease most of the time. There are other differences with respect to the LPS O side chain length among the type I antigens that may be important in disease progression and pathology (see section 6.4).

Identification of differences between the groups of *H. pylori* isolates from symptomatic and asymptomatic individuals may be indicate a generalized trend toward decreased *H. pylori* pathogenesis. The decrease in the pathology would benefit both the *H. pylori* as well as the human host. It would not be beneficial for the *H. pylori* to be detected, therefore the carbohydrate antigens may be synthesized to mimic the host gastric epithelium (5, 28). This process in turn would allow the organism to persist without the pressure of the host immune response. It is possible that there is an evolution of infection in the asymptomatic individuals in that the *H. pylori* either do not provoke an immune response, or the host has a decreased response to the bacterial antigens over time. Overall, this is evidence of a trend toward decreased pathogenesis, similar to a commensal relationship between a non-pathogen and its host. One point that needs to be addressed is the designation of an asymptomatic infection. There could be two types of asymptomatic individuals, those who truly do not get any symptoms and then there are individuals who choose not to seek medical advice even if symptoms occur and their infection remains undiagnosed. The asymptomatic individuals included in this work did not present with any disease symptoms and were assumed to be free of disease caused by *H. pylori* .

It must also be noted that the role of Lewis antigens is not clearly defined in these situations. There are still a number of *H. pylori* isolates that expressed Le^x and yet caused

no apparent disease. In addition, there are NTHp isolates that cause disease (Chapter 2)(35). It is evident that other *H. pylori* factors must be involved in this pathogenic process. Identification of antigens that contribute to pathogenesis will be essential in understanding the interaction between *H. pylori* and the gastric epithelium and could be exploited to turn all *H. pylori* infections into asymptomatic infections. It would be interesting to investigate whether individuals who have an asymptomatic infection continue to have an increased gastric cancer risk over time, or if only symptomatic infections progress to these advanced pathologies.

Recent work by several groups has shown that the Lewis antigens are not the target of the human immune response (2, 10, 49, 50). It is not clear why *H. pylori* would continue to produce these complex antigens if they did not serve some purpose. It has been demonstrated in this work that the distributions of the Lewis antigens among the *H. pylori* isolates can be correlated to individuals in different infectious states, but also indicates that there are other factors. Both *H. pylori* and host, appear to be involved in the pathogenic process. Further study into the expression of these antigens as well as other associated antigens may determine which *H. pylori* factors are necessary for progression to symptomatic or asymptomatic infection. Examination of the Lewis antigen expression from expanded numbers of asymptomatics as well as *in vitro* and *in vivo* assessment of the pathogenic potential of the *H. pylori* from this group will most likely identify factors that affect pathogenesis.

Recently, it was demonstrated that the primary culture plate from any biopsy may contain more than one genetically identical *H. pylori* which exhibited a variable LPS profile (17, 46). In Chapter 3, the isolation of one primary colony from each biopsy was also employed, but the study guidelines involved removal and culture of multiple biopsy samples over a brief period of time (86 days). This allowed the isolation of more than one phenotypically distinct but genetically identical *H. pylori* isolate from each subject. This is similar to what was observed in other studies that investigated multiple primary colonies

from a single agar plate (17, 46). It is now clear that the *in vivo* variability of the Lewis antigens contained within the *H. pylori* LPS is higher than the phase variation rate calculated by Appelmelk *et al.* (4) for the *in vitro* growth of *H. pylori*. The *in vitro* phase variation has been demonstrated to be a direct result of slipped strand mispairing mechanism of the polynucleotide tracts in the fucosyltransferase genes (3) {discussed later in this Chapter}. This mechanism of phase variation can explain some of the observed variation among the isolates on a single primary culture plate, but the levels observed on the primary culture plate are much higher than the 0.2-0.5% phase variation rate previously reported.

The variation of the Lewis antigens by apparently genetically identical isolates from one single biopsy sample provided possible clues to the infectious process of *H. pylori*. It is possible that the phase variation is the natural mechanism of survival for *H. pylori*. An increased mutation rate, leading to increased variation of one of the most important antigens on the bacterial surface, may produce progeny that are possibly better adapted to the gastric environment. The continual variation would allow the possibility of producing the optimal phenotype for survival. Some of the phenotypic variability can be attributed to the slipped strand mispairing mechanism that has been identified by Appelmelk *et al.* (3), but there also appears to be other, possibly environmental, signal(s) that *H. pylori* can detect and respond to. It has been noted that the passage of the mouse model strain, SS1, *in vivo* induces changes in the LPS profile of that isolate (21). This may be the same process or signal that causes multiple LPS profiles to be obtained from genetically identical isolates from primary biopsy plates (17, 46). Identification of the signal(s) responsible for the increased variability or induction of certain genes responsible for synthesis of Lewis antigens under different circumstances has already begun. McGowan *et al.* have identified an LPS associated gene that is upregulated in response to acidic conditions (25).

The inherent variability of the Lewis antigens must also be considered when attempting to make correlations between the Lewis antigenic phenotype and other bacterial

factors such as CagA status (44), host Lewis antigen status (36, 48), or host inflammation (18, 19, 23). During *H. pylori* culture and antigen determination, one must be aware of these facts as the conditions for culture or isolate choice may provide a Lewis antigen profile that is not representative of the *H. pylori* population causing the infection. Identification of other mechanisms of Lewis antigenic variation is necessary before correlations can be conclusively determined.

It appears likely, from the discussion of the antigen distribution above, that the Lewis antigens do play an unidentified role in the pathology caused by *H. pylori*, but further work is necessary to identify what that role is. In addition, further investigations are required to identify other mechanisms of Lewis antigenic variation. Understanding the antigenic switching mechanisms will allow confident assignment of correlations between the Lewis antigens and other host as well as bacterial factors.

6.3 *H. pylori* Fucosyltransferases

The identification of the enzymes which are responsible for the production of the *H. pylori* Lewis antigens are essential in understanding the role that these antigens play in the infectious process. By creating isogenic mutants, such as the ones described in Chapters 4 and 5, which have a defined Lewis antigen expression, the tools are at hand to delineate the contribution of these antigens. The creation of isogenic mutants of the *H. pylori* urease and flagellar proteins demonstrated conclusively the essential role of these proteins in colonization and pathogenesis. It will be interesting to see if the loss of Lewis antigen expression inhibits the colonization and disease causing capabilities of *H. pylori* in both *in vitro* and *in vivo* model systems.

In this work we have identified novel *H. pylori* fucosyltransferases (Chapter 4 and 5). The identification of type I Lewis antigens expressed by *H. pylori* isolates indicated that there was the requirement for enzymes with increased acceptor specificities responsible for the synthesis of these alternative structures. UA948 expressed both Le^a and Le^x (Chapter 4), whereas UA1111 expressed exclusively the type I antigens, Le^a, Le^b

and H Type I (Chapter 5). The production of the type I structures, Le^a and Le^b, indicates that these enzymes are different from those previously identified and shown to be responsible for the more common production of the type II antigens, Le^x and Le^y (16, 24). Upon cloning and characterization of these genes it was discovered that there were significant differences between the newly identified FucTs and previously identified enzymes.

Overall, there was a high level of identity between all identified α(1,3/4)*fucT* genes of *H. pylori*. The existence of a cross species conserved FucT catalytic domain was noted previously by Oriol *et al.* (30). The hypothetical catalytic domain is contained within a highly conserved region of the *fucT* genes (Appendix 1A). It has been shown among the human enzymes that this region is responsible for the transfer of fucose from the GDP linked donor to the acceptor, but not for acceptor or donor recognition (34). One report on human FucT enzymes indicated that conserved domain II was responsible for enzyme activity (34) but further work will be necessary to determine if this is the same for the *H. pylori* enzymes. It must be noted that among *H. pylori* α(1,3(4))FucTs investigated the catalytic domains are highly conserved regardless of enzyme specificity, with only conserved amino acid substitutions being observed.

Another conserved feature of the α(1,3(4))*fucTs* is the presence of polynucleotide tracts in the 5' regions of the ORFs. Each gene homologue contains a characteristic number of adenines and cytosines in this region which allows the production of a functional FucT enzyme (Appendix 1A). The polynucleotide regions have an increased mutation frequency due to slipped stand mispairing that has been implicated in the phase variation of the Lewis antigen expression (3). The lack of an SOS response and repair system in *H. pylori* may allow an increased number of mutations to occur at these sites (43). In one case, UA1111*fucTb*, the presence of a potentially deleterious frameshift in the polynucleotide region is compensated for by another frameshift mutation which

precedes the polynucleotide region, creating a localized region of diversity (Figure 5.3) but maintains the integrity of the ORF.

Upon translation to amino acid sequence there is greater than 80% identity among the *H. pylori* α(1,3(4))FucT proteins, although over the first approximately 80 amino acids, corresponding to the polynucleotide regions there is a low level of amino acid identity (~30%). In fact, there has been the identification of 2 groups of *H. pylori* FucTs based upon nucleotide diversity in this region (42). Interestingly, the enzyme specificity of the human FucTs is determined by differences in the amino terminal portions of the protein (29). In the human system, a single amino acid change has been implicated as being responsible for this change in acceptor specificity (14), but there does not appear to be an obvious conserved change in any one amino acid in the amino terminus of the enzymes which contain α(1,4) FucT activity (UA948FucTa, UA1111FucTa, UA1111FucTb). This region may contain amino acid changes that are responsible for the enzyme acceptor specificity but the changes that are noted in this work and the assignment of *fucT* groups do not correlate to changes in the enzyme specificity. Further work on this region is necessary.

A final characteristic feature of the *H. pylori* α(1,3(4)) FucTs is the presence of a carboxyl terminal heptad repeat (Table 1.5). This region has no homologous structure in any of the other identified FucTs from any species (11, 12, 30). It is thought that the heptad repeat may form a leucine zipper which in other proteins containing this motif is responsible for protein dimerization (22, 38). There is a significant amount of diversity in this region of the FucTs (Table 1.5), even within the two homologues from the same isolate, but the locations of the leucine moieties in all cases are located appropriately to function as a leucine zipper. It is not possible to group the *H. pylori* α(1,3(4))FucTs based upon the sequence of the heptad repeats. The exact function of these repeats remains unclear, but a carboxyl terminal 115 amino acid deletion which removed the

heptad repeat region from the protein also abrogated the enzyme function, indicating that this region is essential for protein function (16).

A detailed molecular study of the domains of these *H. pylori* α(1,3(4))FucTs should be undertaken to determine which regions of these proteins are essential for FucT activity and specificity. A series of domain switching experiments, including both the amino terminal variable region and the heptad repeat regions, will be essential in the identification of the region responsible for the enzyme specificity and work of this nature should be undertaken in future studies.

In this work, and others significant assumptions are made about the *H. pylori* FucT enzymes which may not all be valid and only further work can determine if these interpretations are correct. The biggest assumption about the *H. pylori* FucT enzymes is that they fundamentally act in a similar manner as the human FucT enzymes. The carbohydrates used as acceptors in all of the studies, to date, are the same acceptors used to test human fucosyltransferases. These acceptors may not be ideal for the *H. pylori* enzymes as the LPS biosynthetic pathway is unknown. Although these *H. pylori* enzymes are able to act on synthetic acceptors *in vitro,* it is not clear if these same acceptors are present within the *H. pylori* cell. Work described n Chapter 5 demonstrated that the UA1111FucTa and UA1111FucTb proteins were being produced from *E.coli*, but very little or no enzyme activity was ever detected. A reason for this lack of activity may be that the acceptors used were not appropriate acceptors for these particular *H. pylori* enzymes. In the case of UA1111, the Le^b, Le^a and H Type I antigens are produced in variable quantities but it is clear that, in all cases, there is only ever a single unit of O side chain produced by this isolate. When the enzyme activity was tested the acceptor used was the type I backbone conjugated to a carrier molecule. The true acceptor may be the carbohydrate backbone conjugated to the LPS core and lipid A. The UA1111 enzymes may be specific for this complex acceptor and the lack of acceptor recognition results in little or no enzyme activity. One other possibility to explain the lack of enzyme activity is

that the reaction conditions employed are not saturated and thus the maximal enzyme activity is not being measured. Further work is necessary to identify the optimal carbohydrate acceptors that are utilized by *H. pylori* enzymes.

One essential feature of the *H. pylori* FucT enzymes that has not been addressed in any study to date is the control and regulation of these enzymes. It is unclear whether these genes are constitutively expressed or if they are induced in response to environmental signal(s). Identification of a possible control mechanism for these genes will be important in the understanding of the processes which may contribute to the variation of the Lewis antigens during *in vivo* growth.

6.4 Synthesis of Lewis Antigens by *H. pylori*

The identification of the biosynthetic pathway of the Lewis antigens in *H. pylori* LPS is essential in the understanding of potential control mechanisms of these antigens. The sequential addition of the sugar molecules to create the Lewis antigens allows for the expression of multiple antigens without altering the biosynthetic pathways. The relative levels of the enzymes involved, as well as donor and acceptor carbohydrate concentrations, will all influence the ability of *H. pylori* to produce certain antigens. From an energetics point of view the synthesis of Lewis antigens by *H. pylori* is more economical than the human system as each antigen in the human system is an endpoint for synthesis (20). In contrast to the human system, the *H. pylori* pathway can use the systematic addition of carbohydrates to produce each of the Lewis antigens because the enzymes have a broader specificity. Each sequential addition of the carbohydrates in *H. pylori* synthesizes a new antigen as well as another substrate for other biosynthetic enzymes (Chapter 5). This is accomplished in large part by the ability of the *H. pylori* FucTs to utilize more acceptor molecules than do their human counterparts.

The synthesis of the Lewis antigens by *H. pylori* appears to be different from the human system with respect to the order of addition of the fucose. The order of addition of other carbohydrates cannot be commented on as the enzymes responsible have not been

identified (1, 32, 37). As was described in the Introduction (Chapter 1) humans produce the Lewis antigens by way of an addition of fucose to the terminal galactose of the type I or II backbone by the α(1,2) FucT to produce H Type I and H Type II, respectively. Further synthesis is completed by the α(1,3(4)) FucT which adds the subterminal fucose to complete the Lewis antigenic structures, Le^b and Le^y. From work that has been presented here, as well as other work from the Taylor laboratory, it has become clear that *H. pylori* enzymes are more promiscuous than their human homologues (16, 31, 41, 44). The pathway for the synthesis of Le^x and Le^y was the first to be identified in *H. pylori*. It was demonstrated that inactivation of the α(1,2)*fucT* gene lead to the production of only the Le^x antigen from an isolate that expressed only Le^y previously (44). This alone did not prove that the biosynthetic pathway of Le^y was routed through Le^x, but additional enzymatic characterization demonstrated that the *H. pylori* α(1,2) FucT had the ability to add fucose to the Le^x antigen as well as the type II backbone (44). This is an activity not displayed by the human enzymes (11, 12, 20, 30) and it proved that the synthesis of Le^y from the Le^x antigen was a possible pathway in *H. pylori*. Also, the continued lack of identification of the H Type II antigen indicated that this antigen is most likely not produced.

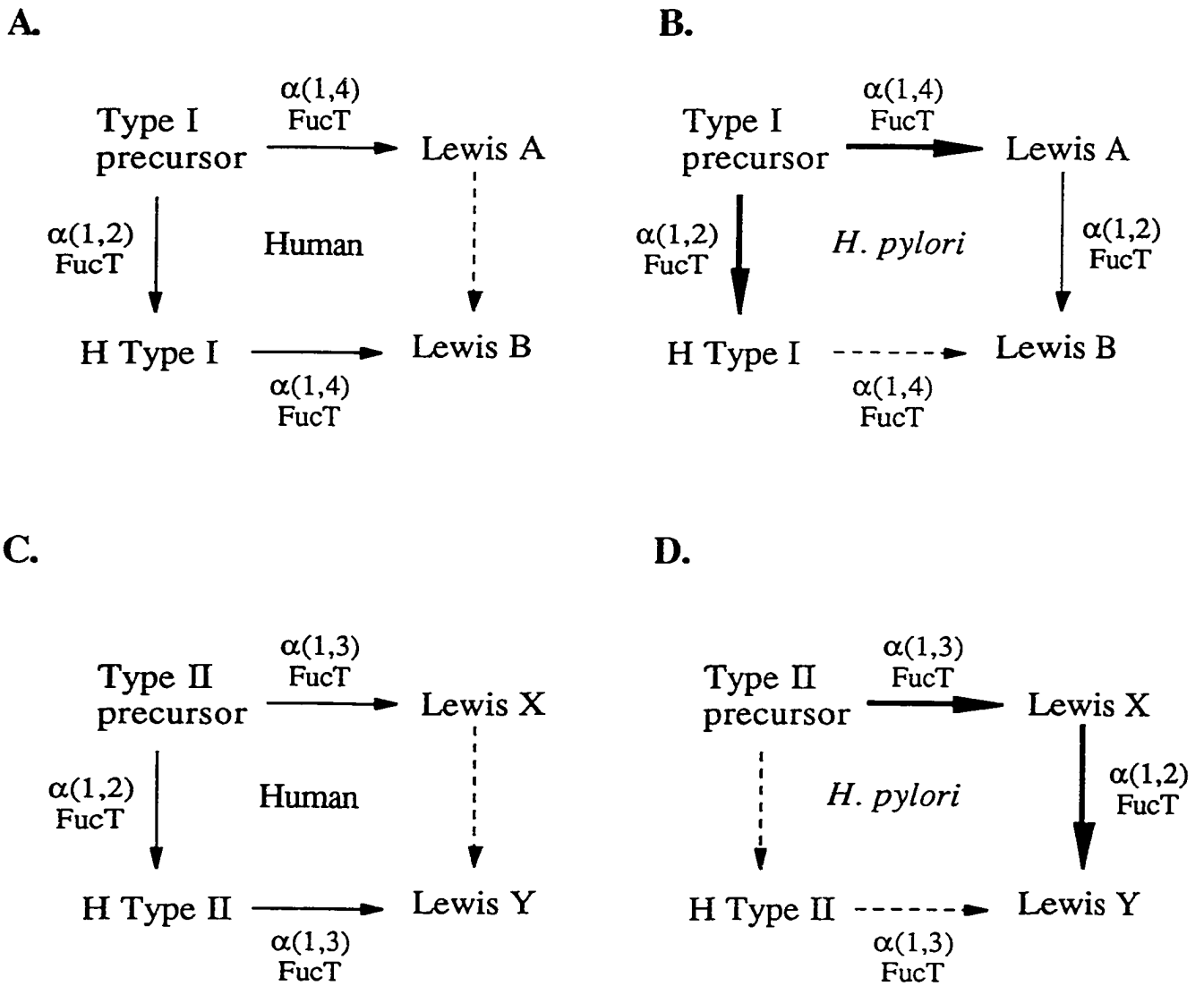
The expression of type I Lewis antigens exclusively allowed examination of a biosynthetic pathway which was not previously examined in *H. pylori*. Evidence presented in Chapter 5 demonstrated that the type I carbohydrates, Le^a and Le^b, are also produced in a similar manner to the type II carbohydrates, Le^x and Le^y. The terminal fucosylation of the type I precursor in UA1111 can occur leading to the production of the H Type I antigen due to the relatively high level of the α(1,2) FucT enzyme activity in this isolate. This antigen is not thought to be further fucosylated to produce the Le^b antigen as is the case with the human system. To produce the Le^b antigen one of the two active α(1,3(4)) FucT enzymes fucosylates the type I precursor to produce the Le^a antigen which is then further fucosylated by the α(1,2) FucT. The α(1,2) FucT enzyme from this isolate

fucosylates all but trace amounts of the Le^a antigen to produce Le^b. It is clear that no matter which type of Lewis antigens are produced, type I or type II, *H. pylori* utilizes a common biosynthetic pathway which uses one antigen as the substrate for synthesis of another (Figure 6.1). This promiscuity of the enzymes allows for the synthesis of a complex carbohydrate O antigen even if not all of the enzymes are present to completely fucosylate the carbohydrate backbone.

The relative amounts of the enzymes, acceptors and donors present at the time of synthesis will determine which antigenic structures are presented on the bacterial surface. An isolate which has a low activity or low production of the α(1,2) FucT will express an increased amount of the monofucosylated Le^a or Le^x antigens, whereas if an isolate has an increased level of α(1,2) FucT virtually all of the chains will become the difucosylated antigens, Le^y or Le^b. A high relative α(1,2) FucT level may also lead to the production of the H Type I or H Type II antigens, theoretically, although no H Type II has even been observed. The activity and expression of the FucT enzymes are not the only factors that can affect the surface expression of the antigens. Availability of the substrate and donor will also play a role. All of these factors lead to the expression of variable LPS isoforms. Until we identify the processes and control mechanisms which are responsible for the synthesis and transport of these antigens to the bacterial surface, we will not fully grasp why the antigens are present as a myriad of structures at different degrees of completion (see section 6.5).

It has been determined definitively that in the *H. pylori* system their FucTs use GDP-fucose as the fucose donor. A conserved operon of three genes is responsible for the conversion of mannose-1-phosphate to GDP-fucose (HP0043-HP0045) (37). Inactivation of any of these genes by insertion of an antibiotic cassette leads to *H. pylori* that no longer express fucosylated antigens {(Hp0043 - (15)), (HP0044 - Rasko unpublished), (Hp0045 - (25))}. The phenotype of these mutants is characterized as being the same as an isolate lacking all functional fucosyltransferases. One of the genes in this

Figure 6.1. Proposed Biosynthetic pathways of Lewis antigens of *H. pylori* and Humans. The solid arrows indicate the demonstrated fucosyltransferase activities. The dotted arrows indicate which enzyme activities have not been demonstrated or are not commonly utilized. **A.** Human synthesis of type I antigens proceeds to Le^a or to H Type I and then further to Le^b. Le^a is rarely further fucosylated to produce Le^b. **B.** *H. pylori* type I antigen synthesis. Le^b is produced via further fucosylation of Le^a. The H Type I structure is also produced but is not further fucosylated. **C.** Human type II antigen synthesis follows a similar path to type I antigen synthesis with Le^x being produced as a terminal antigen and Le^y being synthesized via the H Type II antigen. **D.** *H. pylori* type II antigen synthesis utilizes Le^x as a precursor structure for the synthesis of Le^y. The H Type II antigen has not been demonstrated to be synthesized by *H. pylori* and is not thought to be a substrate for Le^y synthesis although the enzymes produced by *H. pylori* do contain activity on this substrate.



operon HP0045 has been demonstrated to be acid inducible (25). The expression of this gene product allowed the demonstration of acid tolerance but the mechanism responsible was not elucidated. Examination of the control of other LPS associated genes may identify if the acid inducability is a GDP-fucose synthesis-specific phenomenon or if more genes are involved. Once a better understanding of the biosynthetic pathway of *H. pylori* LPS is obtained it may be easier to answer some of the questions of control and regulation of LPS biosynthesis.

There are many questions still to be answered concerning the synthesis of the Lewis antigens by *H. pylori*. The first and most intriguing is why is there the absence of many genes necessary for the synthesis of the LPS in *H. pylori*? The galactosyl transferase as well as the *N*-acetylglucosamine transferase were not identified from the genome projects (1, 37), and have not yet been identified functionally by any other research groups. Yet these enzymes are essential for the synthesis of Lewis antigens (Figure 6.1). One attractive hypothesis is that these genes are present but because of the low level of genetic similarity among glycosyltransferases these enzymes are actually mis-identified as one of the 3 or 4 homologues of the *waaJ* (*rfaJ*) genes (8). Functional analysis of these genes and their encoded products is essential for determining the differences between the production of type I or type II antigens. The only difference between these antigens is the linkages of the backbone carbohydrates. Type I carbohydrates have a 1-3 linkage of the galactose (Gal) to the *N*-acetyglucosamine (GlcNAc), that subsequently requires a fucosyltransferase with $\alpha(1,4)$ specificity to produce the Le^a and Le^b antigens. In the type II antigens, the Gal is linked 1-4 to the GlcNAc and so an $\alpha(1,3)$ FucT enzyme is needed to internally fucosylate this chain to produce the Le^x and Le^y antigens. It will be interesting to see if, as in the case of the UA948FucTa, which expresses both core types, if a single gene contains multiple functions or if a unique gene will be required for each of the unidentified transferase functions.

6.5 LPS Assembly in *H. pylori*

It is becoming clear from the evidence presented in this thesis, as well as other studies, that the LPS assembly of *H. pylori* may be unique among bacteria. Along with the unique structures that are presented in the LPS O side chain there appears to be a novel system to assemble these antigens. The ability of *H. pylori* to produce O side chains in various states of completion is unique among the LPS gram-negative pathogens which express complex O side chains (45). *Neisseria*species appear to produce an LPS that is assembled in a similar manner to *H. pylori* in that various glycoforms can be produced which are uncompleted derivatives of the same antigen (39). This indicates that the transport mechanism will export even uncompleted LPS, which may be similar but not identical to the *H. pylori* situation. The *Neisseria* species example contains only limited polymerized carbohydrates and does not assemble them into units like the LPS O side chains of *H. pylori* (39). In other Gram-negative organisms, if the O side chain unit is not complete, it is not added to the growing LPS chain and the chain elongation is terminated, even if a portion is not essential to the structure (45). How *H. pylori* overcomes this barrier remains unclear.

One interesting note, with respect to the synthesis of the O side chain type is the length of the O side chain. Investigations to date have revealed that the O side chains containing type I antigens are always short. The longest type I containing chain is produced by UA948 with three O side chain units, but this LPS also contains type II units (27). The LPS of UA1111 and UA915, which both express exclusively type I carbohydrates, contain only a single unit of O side chain ((26), Chapter 5). In contrast, O side chains expressing exclusively type II chains have been shown to be capable of containing greater than 10 O side chain units in various states of fucosylation (6, 7, 26). It is possible that the enzymes which are responsible for the production of the type I chain are incapable of catalyzing the addition of further units of either the galactose or the *N*-acetyglucosamine due to lack of enzyme specificity or activity upon the newly created

substrate. Identification of the enzymes responsible in the production of these antigens will provide insight into the reasons for this difference in length of the O side chains. One hypothesis previously described in this work may link the short O side chains with an increase in pathology. The lack of type I antigen expression among the *H. pylori* from asymptomatic subjects suggested that there may be a correlation between the Lewis antigens and the disease state. This may not be due to the Lewis antigens as much as to the O side chain length. It was noted previously that longer O side chains could inhibit the access of virulence factors to the epithelial cell surface (9, 40). If this is the case it may be that the expression of shorter O side chains will lead to a symptomatic disease and the *H. pylori* with longer chain LPS, possibly containing heptan polymers, may mask the bacterial virulence factors leading to an asymptomatic infection regardless of Lewis antigen expression. Further investigation of this hypothesis is necessary to determine if length of the LPS O side chain or Lewis antigens are the critical factors in the pathogenesis of *H. pylori*. One interesting report recently established that the CagA protein is directly inserted into the epithelial cell membrane (33) and thus intimate bacterial outer membrane to epithelial cell membrane contact is required, a process in which LPS O side chain length may be factor.

Since so few LPS biosynthetic genes have been identified by homology in the genome sequencing projects, much of the LPS biosynthetic pathway remains to be identified. Examples of genes that are required for LPS synthesis, but not identified, are an O side chain ligase, polymerase, or determinant of chain length (*rol* gene). It is clear that the *H. pylori* LPS has a modal distribution that is unique to the isolate and yet no gene or protein with homology has been identified that could control chain length. It almost seems as if the *H. pylori* biosynthetic pathway is not really a pathway at all, but rather a random chance encounter of the enzymes that are responsible for the production of the LPS. When the α(1,2) FucT acts on the growing chain, effectively terminating elongation, the LPS O side chain is transported to the bacterial surface. It appears as

though there cannot be further elongation of the carbohydrate backbone chain once the terminal α(1,2) fucose has been added. The reason for this may be due to either steric hindrance of the carbohydrate acceptor or inaccessibility of the enzymatic site by the acceptor. Nevertheless, there are *H. pylori* isolates that do not contain a functional α(1,2) FucT enzyme, yet maintain the modal distribution where all O side chains are of similar length (i.e. UA948, see Figure 4.5 and (44)). The identification of the genes responsible for LPS biosynthesis in *H. pylori* may represent a novel mechanism of LPS assembly. There are many questions that remain unanswered about the biosynthetic process of LPS in *H. pylori*, including but not limited to:

a) What type of assembly system is employed?

b) What controls chain length? If α(1,2) FucT activity does control chain length what occurs in isolates that lack a functional α(1,2) FucT?

c) How is the LPS transported to the surface?

d) Why does the LPS continue to be made even though the O side chain units are not completed? What is the specificity for the ligase/polymerase/transport enzymes?

Further research on *H. pylori* will yield the answers to these questions. We have just begun to identify the processes of *H. pylori* LPS biosynthesis and have already identified significant differences with respect to the synthesis of the O side chain units and their assembly when compared to other Gram-negative organisms. Many of the identified genes in *H. pylori* LPS biosynthesis are core assembly homologues, but very few genes were identified which would be involved in the assembly and processing of this product (1, 13, 37). It will be difficult to identify these genes as *H. pylori* does not contain an LPS biosynthetic operon but rather has these genes distributed throughout the genome. The further identification of the genes and controls responsible for *H. pylori* LPS biosynthesis is essential in the understanding the pathogenesis of this organism.

6.6. Bibliography

1. **Alm, R. A., Ling, L. L., Moir, D. T., King, B. L., Brown, E. D., Doig, P. C., Smith, D. R., Noonan, B., Guild, B. C., deJonge, B. L., Carmel, G., Tummino, P. J., Caruso, A., Uria-Nickelsen, M., Mills, D. M., Ives, C., Gibson, R., Merberg, D., Mills, S. D., Jiang, Q., Taylor, D. E., Vovis, G. F. and Trust, T. J.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature. **397**: 176-180.

2. **Amano, K., Hayashi, S., Kubota, T., Fijii, N. and Yokata, S.** 1997. Reactivities of Lewis monoclonal antibodies with the lipopolysaccharides of *Helicobacter pylori* strains isolated from patients with gastroduodenal diseases in Japan. Clin. Diag. Lab. Immunol. **4**: 540-544.

3. **Appelmelk, B. J., Martin, S. L., Monteiro, M. A., Clayton, C. A., McColm, A. A., Zheng, P., Verboom, T., Maaskant, J. J., van den Eijnden, D. H., Hokke, C. H., Perry, M. B., Vandenbroucke-Grauls, C. M. J. E. and Kusters, J. G.** 1999. Phase variation in *Helicobacter pylori* lipopolysaccharide due to changes in the lengths of poly(C) tracts in α3-fucosyltransferase genes. Infect. Immun. **67**: 5361-5366.

4. **Appelmelk, B. J., Shiberu, B., Trinks, C., Tapsi, N., Zheng, P. Y., Verboom, T., Maaskant, J., Hokke, C. H., Schiphorst, W., Blanchard, D., Simoons-Smit, I. M., van den Eijnden, D. H. and VandenbrouckeGrauls, C.** 1998. Phase variation in *Helicobacter pylori* lipopolysaccharide. Infect. Immun. **66**: 70-76.

5. **Appelmelk, B. J., Simoons-Smit, I., Negrini, R., Moran, A. P., Aspinall, G. O., Forte, J. G., Devries, T., Quan, H., Verboom, T., Maaskant, J. J., Ghiara, P., Kuipers, E. J., Bloemena, E., Tadema, T. M., Townsend, R. R., Tyagarajan, K., Crothers, J. M., Monteiro, M. A., Savio, A. and Degraaff, J.** 1996. Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect. Immun. **64**: 2031-2040.

6. **Aspinall, G. O. and Monteiro, M. A.** 1996. Lipopolysaccharides of *Helicobacter pylori* strains P466 and MO19: structures of the O antigen and core oligosaccharide regions. Biochem. **35**: 2498-2504.

7. **Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P.** 1996. Lipopolysaccharide of the *Helicobacter pylori* type strain NCTC11637 (ATCC 43504): structure of the O antigen chain and core oligosaccharide regions. Biochem. **35**: 2489-2497.

8. **Berg, D. E., Hoffman, P. S., Appelmelk, B. J. and Kusters, J. G.** 1997. The *Helicobacter pylori* genome:genetic factors for a long life in the gastric mucosa. Trend. Microbiol. **5**: 468-474.

9. **Bilge, S. S., Vary, J. C., Dowell, S. F. and Tarr, P. I.** 1996. Role of the *Escherichia coli* O157:H7 O side chain in adherence and analysis of the *rfb* locus. Infect. Immun. **64**: 4795-4801.

10. **Claeys, D., Faller, G., Appelmelk, B. J., Negrini, R. and Kirchner, T.** 1998. The gastric H+, K+-ATPase is a major autoantigen in chronic *Helicobacter pylori* gastritis with body mucosa atrophy. Gastroenterology. **115**: 340-347.

11. **Costache, M., Apoil, P.-A., Cailleau, A., Elmgren, A., Larson, G., Henry, S., Blancher, A., Iordachescu, D., Oriol, R. and Mollicone, R.** 1997. Evolution of fucosyltransferase genes in vertebrates. J. Biol. Chem. **272**: 29721-29728.

12. **Costache, M., Calleau, A., Fernandez-Mateos, P., Oriol, R. and Mollicone, R.** 1997. Advances in molecular genetics of α-2 and α-3/4-fucosyltransferases. Transfus. Clin. Biol. 4: 367-382.

13. **Doig, P., de Jonge, B. L., Alm, R. A., Brown, E. D., Uria-Nickelsen, M., Noonan, B., Mills, S. D., Tummino, P., Carmel, G., Guild, B. C., Moir, D. T., Vovis, G. F. and Trust, T. J.** 1999. *Helicobacter pylori* physiology predicted from genomic comparison of two strains. Microbiol. Mol. Biol. Rev. **63**: 675-707.

14. **Dupuy, F., Petit, F.-M., Mollicone, R., Oriol, R., Julien, R. and Maftah, A.** 1999. A single amino acid in the hypervariable stem domain of vertebrate α1,3/1,4-fucosyltransferases determines the type 1/type 2 transfer: characterization of acceptor substrate specificity of the Lewis enzyme by site-directed mutagenesis. J. Biol. Chem. **274**: 12257-12262.

15. **Edwards, N. J., Montiero, M., Walsh, E., Moran, A. P., Roberts, I. S. and High, N. J.** 1999. The role of lipopolysaccharide in adhesion of *Helicobacter pylori* to gastric mucosa. 10th International Workshop on *Campylobacter, Helicobacter* and related organisms. Abstract HP23.

16. **Ge, Z., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1997. Cloning and heterologous expression of an α1,3 fucosyltransferase gene from the gastric pathogen *Helicobacter pylori*. J. Biol. Chem. **272**: 21357-21363.

17. **Gibson, J. R., Chart, H. and Owen, R. J.** 1998. Intra-strain variation in expression of lipopolysaccharide by *Helicobacter pylori*. Lett. Appl. Microbiol. **26**: 399-403.

18. **Heneghan, M. A., McCarthy, C. F. and Moran, A. P.** 2000. Relationship of blood group determinants on *Helicobacter pylori* lipopolysaccharide with host Lewis phenotype and inflammatory response. Infect. Immun. **68**: 937-941.

19. **Heneghan, M. A., Moran, A. P., Feeley, K. M., Egan, E. L., Goulding, J., Connolly, C. E. and McCarthy, C. F.** 1998. Effect of host Lewis and ABO blood group antigen expression on *Helicobacter pylori* colonization density and the consequent inflammatory response. FEMS Immunol. Med. Microbiol. **20**: 257-266.

20. **Henry, S., Oriol, R. and Samuelsson, B.** 1995. Lewis histo-blood group system and associated secretory phenotypes. Vox Sang. **69**: 166-182.

21. **Janvier, B., Grignon, B., Audibert, C., Pezennec, L. and Fauchere, J. L.** 1999. Phenotypic changes of *Helicobacter pylori* components during an experimental infection in mice. FEMS Immunol. Med. Microbiol. **24**: 27-33.

22. **Lau, P. C. K., Wang, Y., Patel, A., Labbe, D., Bergeron, H., Brousseau, R., Konishi, Y. and Rawlings, M.** 1997. A bacterial basic region leucine zipper histidine kinase regulating toluene degradation. Proc. Natl. Acad. Sci. U.S.A. **94**: 1453-1458.

23. **Marshall, D. G., Hynes, S. O., Coleman, D. C., O'Morain, C. A., Smyth, C. J. and Moran, A. P.** 1999. Lack of a relationship between Lewis antigen expression and *cagA*, Cag, *vacA*, and VacA status of Irish *Helicobacter pylori* isolates. FEMS Immunol. Med. Microbiol. **24**: 79-90.

24. **Martin, S. L., Edbrooke, M. R., Hodgman, T. C., van den Eijnden, D. H. and Bird, M. I.** 1997. Lewis X biosynthesis in *Helicobacter pylori*. molecular cloning of an α(1,3) fucosyltransferase gene. J. Biol. Chem. **272**: 21349-21356.

25. **McGowan, C. C., Necheva, A., Thompson, S. A., Cover, T. L. and Blaser, M. J.** 1998. Acid-induced expression of an LPS-associated gene in *Helicobacter pylori*. Mol. Microbiol. **30**: 19-31.

26. **Monteiro, M. A., Appelmelk, B. J., Rasko, D. A., Moran, A. P., Hynes, S. O., McLean, L. L., Chan, K. H., Michael, F. S., Logan, S. M., O'Rourke, J., Lee, A., Taylor, D. E. and Perry, M. B.** 2000. Lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, mouse model *H. pylori* Sydney strain, *H. pylori* P466 carrying sialyl Lewis X, and *H. pylori* UA915 expressing Lewis B: Classification of *H. pylori* lipopolysaccharides into Glycotype families. Eur. J. Biochem. **276**: 305-320.

27. **Monteiro, M. A., Chan, K. H. N., Rasko, D. A., Taylor, D. E., Zheng, P. Y., Appelmelk, B. J., Wirth, H.-P., Yang, M., Blaser, M. J., Hynes, S. O., Moran, A. P. and Perry, M. B.** 1998. Simultaneous expression of type 1 and type 2 Lewis blood group antigens by *Helicobacter pylori* lipopolysaccharides. J. Biol. Chem. **273**: 11533-11543.

28. **Moran, A. P., Prendergast, M. M. and Appelmelk, B. J.** 1996. Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. FEMS Immunol. Med. Microbiol. **16**: 105-115.

29. **Nguyen, A. T., Holmes, E. H., Whitaker, J. M., Ho, S., Shetterly, S. and Macher, B. A.** 1998. Human α1,3/4-Fucosyltransferases: I Identification of amino acids involved in acceptor substrate binding by site-directed mutagenesis. J. Biol. Chem. **273**: 25244-25249.

30. **Oriol, R., Mollicone, R., Cailleau, A., Balanzino, L. and Breton, C.** 1999. Divergent evolution of fucosyltransferase genes from vertebrates, invertebrates, and bacteria. Glycobiology. **9**: 323-334.

31. **Rasko, D. A., Wang, G., Palcic, M. M. and Taylor, D. E.** 2000. Cloning and characterization of the α1,3/4 fucosyltransferase of *Helicobacter pylori*. J. Biol. Chem. **276**: 4988-4994.

32. **Saunders, N. J., Peden, J. F., Hood, D. W. and Moxon, E. R.** 1998. Simple sequence repeats in the *Helicobacter pylori* genome. Mol. Microbiol. **27**: 1091-1098.

33. **Segal, E. D., Cha, J., Lo, J., Falkow, S. and Tompkins, L. S.** 1999. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc. Natl. Acad. Sci. USA. **96**: 14559-14564.

34. **Sherwood, A. L., Nguyen, A. T., Whitaker, J. M., Macher, B. A., Stroud, M. R. and Holmes, E. H.** 1998. Human α1,3/4-Fucosyltransferases: III A lys/arg residue located within the 1,3-FucT motif is required for activity but not for substrate binding. J. Biol. Chem. **273**: 25256-25260.

35. **Simoons-Smit, I. M., Appelmelk, B. J., Verboom, T., Negrini, R., Penner, J. L., Aspinall, G. O., Moran, A. P., She, F. F., Shi, B. S., Rudnica, W., Savio, A. and De Graaff, J.** 1996. Typing of *Helicobacter pylori* with monoclonal antibodies against Lewis antigens in lipopolysaccharide. J. Clin. Microbiol. **34**: 2196-2200.

36. **Taylor, D. E., Rasko, D. A., Sherburne, R., Ho, C. and Jewel, L. D.** 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115**: 1113-1122.

37. **Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., Ketchum, K. A., Klenk, H. P., Gill, S., Dougherty, B. A., Nelson, K., Quackenbush, J., Zhou, L. X., Kirkness, E. F., Peterson, S., Loftus, B., Richardson, D., Dodson, R., Khalak, H. G., Glodek, A., McKenney, K., Fitzegerald, L. M., Lee, N., Adams, M. D., Hickey, E. K., Berg, D. E., Gocayne, J. D., Utterback, T. R., Peterson, J. D., Kelley, J. M., Cotton, M. D., Weidman, J. M., Fujii, C., Bowman, C., Watthey, L., Wallin, E., Hayes, W. S., Borodovsky, M., Karp, P. D., Smith, H. O., Fraser, C. M. and Venter, J. C.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature. **388**: 539-547.

38. **Turner, R. and Tjian, R.** 1989. Leucine repeats and an adjacent DNA binding domain mediate the formation of functional cFos-cJun heterodimers. Science. **243**: 1689-1694.

39. **Van Putten, J. P. M. and Robertson, B. D.** 1995. Molecular mechanisms and implications for infection of lipopolysaccharide variation in *Neisseria*. Mol. Microbiol. **16**: 847-853.

40. **Voorhis, D. L., Dillon, S., Formal, S. B. and Isberg, R. R.** 1991. An O antigen can interfere with the function of the *Yersinia pseudotuberculosis* invasin protein. Mol. Microbiol. **5**: 317-325.

41. **Wang, G., Boulton, P. G., Chan, N. W. C., Palcic, M. M. and Taylor, D. E.** 1999. Novel *Helicobacter pylori* α1,2-fucosyltransferase, a key enzyme in the synthesis of Lewis antigens. Microbiology. **145**: 3245-3253.

42. **Wang, G., Ge, Z., Rasko, D. A. and Taylor, D. E.** Submitted. Lewis antigens in *Helicobacter pylori*:biosynthesis and phase variation. Mol. Microbiol.

43. **Wang, G., Humayun, M. Z. and Taylor, D. E.** 1999. Mutation as an origin of genetic variability in *Helicobacter pylori*. Trend. Microbiol. **7**: 488-493.

44. **Wang, G., Rasko, D. A., Sherburne, R. and Taylor, D. E.** 1999. Molecular genetic basis for the variable expression of Lewis Y antigen in *Helicobacter pylori* : analysis of the α(1-2) fucosyltransferase gene. Mol. Microbiol. **31**: 1265-1274.

45. **Whitfield, C.** 1995. Biosynthesis of lipopolysaccharide O antigens. Trend. Microbiol. **3**: 178-185.

46. **Wirth, H.-P., Yang, M., Peek, R. M., Hook-Nikanne, J., Fried, M. and Blaser, M. J.** 1999. Phenotypic diversity in Lewis expression of *Helicobacter pylori* isolates from the same host. J. Lab. Clin. Med. **133**: 488-500.

47. **Wirth, H. P., Yang, M. Q., Karita, M. and Blaser, M. J.** 1996. Expression of the human cell surface glycoconjugates Lewis X and Lewis Y by *Helicobacter pylori* isolates is related to CagA status. Infect. Immun. **64**: 4598-4605.

48. **Wirth, H. P., Yang, M. Q., Peek, R. M., Tham, K. T. and Blaser, M. J.** 1997. *Helicobacter pylori* Lewis expression is related to the host Lewis phenotype. Gastroenterology. **113**: 1091-1098.

49. **Yokota, S., Amano, K., Shibata, Y., Nakajima, M., Suzuki, M., Hayashi, S., Fujii, N. and Yokochi, T.** 2000. Two distinct antigenic types of polysaccharide chains of *Helicobacter pylori* lipopolysacchairdes characterized by reactivity with sera from humans with natural infection. Infect. Immun. **68**: 151-159.

50. **Yokota, S.-I., Amano, K.-I., Hayashi, S., Kubota, T., Fujii, N. and Yokochi, T.** 1998. Human antibody response to *Helicobacter pylori* lipopolysaccharide: presence of an immunodominant epitope in the polysaccharide chain of lipopolysaccharide. Infect. Immun. **66**: 3006-3011.

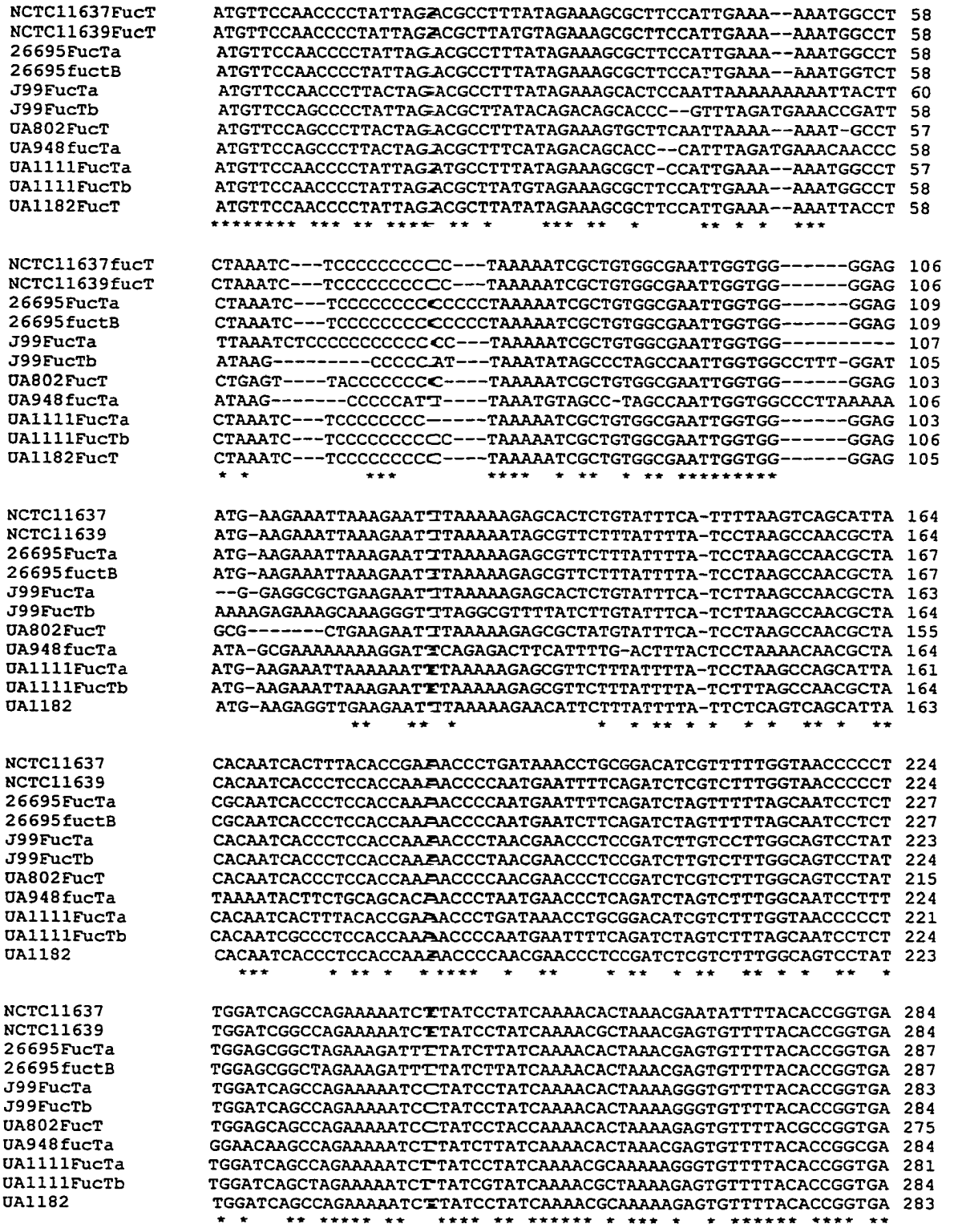
Appendices

Appendix 1. This appendix contains alignments of both nucleotide (A) and amino acid (B) sequences from all known *H. pylori* α(1,3(4)) fucosyltransferases. The accession numbers are as follows:

NCTC11637*	AAB93985
NCTC11639	AAB81031
26695FucTa	AAD07447
26695FucTb	AAD07710
J99FucTa	AAD06573
J99FucTb*	AAD06169
UA802FucT	Z. Ge Unpublished data
UA948FucTa	AF194963
UA1111FucTa	To Be Assigned
UA1111FucTb	To Be Assigned
UA1182FucT	Z. Ge Unpublished data

* denotes that nucleotide sequence has been adjusted to provide full length open reading frame.

A.



```
NCTC11637       AAACGAATCGCCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTAGACTT 344
NCTC11639       AAACGAATCGCCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 344
26695FucTa      AAACGAATCACCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 347
26695fuctB      AAACGAATCACCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 347
J99FucTa        AAATGAAGTCCCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGACTT 343
J99FucTb        AAATGAAGTCCCTAATTTCAATCTCTTTGATTACGCCATAGGCTTTGATGAATTGGACTT 344
UA802FucT       AAATGAAGTCCCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 335
UA948fucTa      AAATGAAGTGCCTAATTTCAATCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 344
UA1111FucTa     AAATGAAGTCCCTAACTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGACTT 341
UA1111FucTb     AAATGAAGTCCCTAACTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 344
UA1182          AAACGAATCGCCTAATTTCAACCTCTTTGATTACGCCATAGGCTTTGATGAATTGGATTT 343
                *** ***    ***** ***** ********************************* ** **

NCTC11637       TAGAGATCGTTATTTGAGAATGCCTTTATATTATGATAGGCTACACCATAAAGCCGAGAG 404
NCTC11639       TAATGATCGTTATTTGAGAATGCCTTTATATTATGATAGGCTACACCATAAAGCCGAGAG 404
26695FucTa      TAATGATCGTTATTTGAGAATGCCTTTGTATTATGCCCATTTGCACTATAAAGCCGAGCT 407
26695fuctB      TAATGATCGTTATTTGAGAATGCCTTTGTATTATGCCCATTTGCACTATGAAGCCGAGCT 407
J99FucTa        TAGAGATCGTTATTTGAGAATGCCTTTATATTACGCTAGCTTGCATTACAAAGCCGAGAG 403
J99FucTb        TAGAGATCGTTATTTGAGAATGCCTTTATATTACGCTAGCTTGCATTATAAAGCCGAGAG 404
UA802FucT       AAGAGATCGTTATTTGAGAATGCCTTTATATTATGATAGACTACACCATAAAGCCGAGAG 395
UA948fucTa      TAACGATCGCTATTTGAGAATGCCTTTGTATTACGCCTATTTGCATTATAAAGCCATGCT 404
UA1111FucTa     TAGAGATCGTTATTTGAGAATGCCTTTGTATTATGCCTATTTGCATTATAAAGCCGAGCT 401
UA1111FucTb     TAGAGATCGTTATTTGAGGATGCCTTTATATTATGATAGGCTACACCATAAAGCCGAGAG 404
UA1182          TAGAGATCGTTATTTAAGAATGCCTTTATATTATGATAGACTACACCATAAAGCCGAGAG 403
                *  ***** ***** ** ******** ***** *       * **  *  *****  *

NCTC11637       CGTGAATGACACCACCGCACCCTACAAGATTAAAGGCAACAGCCTTTATACTTTAAAAAA 464
NCTC11639       CGTGAATGACACCACTGCGCCCTACAAACTCAAAGATAACAGCCTTTATGCTTTAAAAAA 464
26695FucTa      TGTTAATGACACCACTGCGCCCTACAAACTCAAAGACAACAGCCTTTATGCTTTAAAAAA 467
26695fuctB      TGTTAATGACACCACTGCGCCCTACAAACTCAAAGACAACAGCCTTTATGCTTTAAAAAA 467
J99FucTa        CGTGAATGACACCACCGCGCCCTACAAACTCAAAGACAACAGCCTTTATGCTTTAAAAAA 463
J99FucTb        CGTGAATGACACCACCGCGCCCTACAAACTCAAAGACAACAGCCTTTATGCTTTAAAAAA 464
UA802FucT       CGTGAATGACACCACCGCGCCTTACAAGATTAAACCTGACAGCCTTTATACTTTAAAAAA 455
UA948fucTa      TGTTAATGACACCACTTCGCCCTATAAACTCAAAGC------CCTTTATACTTTAAAAAA 458
UA1111FucTa     TGTTAATGACACCACTTCGCCTTATAAACTCCAACCTGACAGCCTTTATGCTTTAAAAAA 461
UA1111FucTb     CGTGAATGACACCACTTCGCCCTACAAACTCAAAGACAACAGCCTTTATACTTTAAAAAA 464
UA1182          CGTGAATGACACCACTTCGCCTTACAAACTCAAACCTGACAGCCTTTATGCTTTAAAAAA 463
                ** ***********  * ** ** **  *  **         ******* **********

NCTC11637       ACCCTCCCATTGTTTTAAAGAAAACCACCCTAATTTGTGCGCGCTCATCAATAATGAGAG 524
NCTC11639       ACCCTCCCATTGTTTTAAAGAAAAACACCCCAATTTATGCGCAGTAGTGAATGATGAGAG 524
26695FucTa      ACCCTCTCATCATTTTAAAGAAAACCACCCTAATTTGTGCGCAGTAGTGAATGATGAGAG 527
26695fuctB      ACCCTCTCATCATTTTAAAGAAAACCACCCTAATTTGTGCGCAGTAGTGAATGATGAGAG 527
J99FucTa        GCCCTCCCATCATTTTAAAGAAAACCACCCTAATTTATGCGCAGTAGTGAATGATGAGAG 523
J99FucTb        GCCCTCCCATCATTTTAAAGAAAACCACCCTAATTTATGCGCAGTAGTGAATGATGAGAG 524
UA802FucT       ACCCTCCCATCATTTTAAAGAAAAACACCCCCATTTATGCGCAGTAGTGAATGATGAGAG 515
UA948fucTa      ACCTTCCCATAAATTTAAAGAAAACCACCCCAATTTATGTGCGCTAATCCATAACGAGAG 518
UA1111FucTa     ACCCTCCCATCATTTTAAAGAAAACCACCCCAATTTGTGCGCAGTAGTGAATAATGAGAG 521
UA1111FucTb     ACCCTCCCATCAATTTAAAGAAAACCACCCTAATTTATGCGCAGTCGTGAATGATGAGAG 524
UA1182          ACCCTCCCATCATTTTAAAGAAAACCACCCCAATTTATGCGCAGTAGTGAACAATGAGAG 523
                ** ** ***    *********** *****  **** ** **  *  *  *  * *****

NCTC11637       CGATCCTTTGAAAAGAGGGTTTGCCAGTTTTGTAGCGAGCAACGCTAACGCTCCTATGAG 584
NCTC11639       CGATCCTTTGAAAAGAGGGTTTGCGAGCTTTGTCGCGAGCAACCCTAACGCCCCTATAAG 584
26695FucTa      CGATCTTTTAAAAAGAGGGTTTGCCAGTTTTGTAGCGAGCAACGCTAACGCTCCTATGAG 587
26695fuctB      CGATCTTTTAAAAAGAGGGTTTGCCAGTTTTGTAGCGAGCAACGCTAACGCTCCTATGAG 587
J99FucTa        CGATCCTTTGAAAAGAGGGTTTGCGAGCTTTGTCGCAAGCAACCCTAACGCTCCTATAAG 583
J99FucTb        CGATCCTTTGAAAAGAGGGTTTGCGAGCTTTGTCGCGAGCAACCCTAACGCTCCTATAAG 584
UA802FucT       CGATCCTTTGAAAAGAGGGTTTGCGAGTTTTGTCGCAAGCAACCCTAACGCTCCTAAAAG 575
UA948fucTa      CGATCCTTGGAAAAGAGGGTTTGCCAGTTTTGTCGCAAGCAATCCTAACGCTCCCATCAG 578
UA1111FucTa     TGATCCTTTGAAAAGAGGGTTTGCGAGCTTTGTCGCAAGCAACCCTAACGCTCCTAGAAG 581
UA1111FucTb     CGATCCTTTGAAAAGAGGGGTTGTGAGCTTTGTAGCGAGCAACGCTAACGCTCCTATGAG 584
UA1182          CGATCCTTTGAAAAGAGGGTTTGCGAGTTTTGTAGCGAGCAACCCTAACGCTCCTAAAAG 583
                **** **   ********* ***   ** ***** ** ******  ******* ** *   **
```

```
NCTC11637       GAACGCTTTCTATGACGCTTTAAATTCTATTGAGCCAGTTACTGGGGGAGGAGCCGTGAA 644
NCTC11639       GAACGCTTTCTATGACGCTCTAAATTCTATTGAACCAGTTACTGGGGGAGGGAGCGTGAG 644
26695FucTa      GAACGCTTTTTATGACGCTCTAAATTCCATAGAGCCAGTTACTGGGGGAGGAAGTGTGAG 647
26695fuctB      GAACGCTTTTTATGACGCTCTAAATTCCATAGAGCCAGTTACTGGGGGAGGAAGTGTGAG 647
J99FucTa        GAACGCTTTCTATGACGCTTTAAATTCTATTGAGCCAGTTACTGGGGGAGGGAGCGTGAA 643
J99FucTb        GAACGCTTTCTATGACGCTTTAAATTCTATTGAGCCAGTTACTGGGGGAGGGAGCGTGAA 644
UA802FucT       GAACGCCTTCTATGACGCTTTAAATTCTATTGAGCCAGTTACTGGGGGAGGGAGCGTGAA 635
UA948fucTa      AAACGCTTTCTATGACGCTTTAAATGCTATTGAGCCAGTGGCTAGTGGAGGGAGTGTGAA 638
UA1111FucTa     GAACGCTTTTTATGAGGCTTTAAACGCTATTGAGCCAGTTGCTGGGGGAGGGAGCGTGAA 641
UA1111FucTb     AAACGCCTTTTATGACGCTTTAAATTCTATTGAGCCAGTTACTGGGGGAGGGAGCGTGAA 644
UA1182          GAATGCTTTCTATGACGTTTTAAATTCTATAGAGCCAGTTATTGGGGGAGGGAGCGTGAA 643
                 ** ** ** ***** * * ****  * ** ** *****   * * *****    ****

NCTC11637       AAACACTTTAGGCTATAAGGTTGGAAACAAAAGCGAGTTTTTAAGCCAATACAAATTCAA 704
NCTC11639       AAACACTTTAGGCTATAACGTCAAAAACAAAAACGAGTTTTTAAGCCAATACAAGTTCAA 704
26695FucTa      AAACACTTTAGGCTATAAGGTTGGAAACAAAAGCGAGTTTTTAAGCCAATACAAGTTCAA 707
26695fuctB      AAACACTTTAGGCTATAAGGTTGGAAACAAAAGCGAGTTTTTAAGCCAATACAAGTTCAA 707
J99FucTa        AAACACTTTAGGCTATAACGTCAAAAACAAGAGCGAGTTTTTAAGCCAATACAAATTCAA 703
J99FucTb        AAACACTTTAGGCTATAACGTCAAAAACAAGAGCGAGTTTTTAAGCCAATACAAATTCAA 704
UA802FucT       AAACACTTTAGGCTATAAAGTTGGAAACAAAAACGAGTTTTTAAGCCAATACAAATTCAA 695
UA948fucTa      AAACACTCTAGGCTATAAGGTCAAAAACAAAAACGAATTTTTAAGCCAATACAAGTTCAA 698
UA1111FucTa     AAACACTTTAGGCTATAATGTCAAAAACAAGAGCGAGTTTTTAAGCCAATACAAATTCAA 701
UA1111FucTb     AAACACTTTAGGCTATAACGTCAAAAACAAGAGCGAGTTTTTAAGCCAATACAAGTTCAA 704
UA1182          AAACACTTTAGGCTATAACATTAAAAACAAGAGCGAGTTTTTAAGCCAATACAAATTCAA 703
                ******* **********  *   ****** * *** ***************** *****

NCTC11637       CCTGTGTTTTGAAAACTCACAAGGCTATGGCTATGTAACCGAAAAAATCATTGACGCTTA 764
NCTC11639       CCTGTGTTTTGAAAACACTCAAGGCTATGGCTATGTAACTGAAAAAATCATTGACGCTTA 764
26695FucTa      TCTCTGTTTTGAAAACTCGCAAGGTTATGGCTATGTAACCGAAAAAATCCTTGATGCGTA 767
26695fuctB      TCTCTGTTTTGAAAACTCGCAAGGTTATGGCTATGTAACCGAAAAAATCCTTGATGCGTA 767
J99FucTa        CCTGTGTTTTGAAAACACTCAAGGCTATGGCTATGTAACTGAAAAAATCATTGACGCTTA 763
J99FucTb        TCTGTGTTTTGAAAACACTCAAGGCTATGGCTATGTAACTGAAAAAATCATTGACGCTTA 764
UA802FucT       TCTGTGTTTTGAAAACTCTCAAGGCTATGGCTATGTAACCGAAAAAATCATTGACGCTTA 755
UA948fucTa      CCTCTGTTTTGAAAACTCACAAGGCTATGGCTATGTAACCGAAAAAATCCTTGATGCGTA 758
UA1111FucTa     TCTGTGTTTTGAAAACACTCAAGGCTATGGCTATGTAACTGAAAAGATCATTGACGCTTA 761
UA1111FucTb     CCTGTGTTTTGAAAACTCACAAGGCTATGGCTATGTAACCGAGAAGATCCTTGACGCTTA 764
UA1182          TCTGTGTTTTGAAAACTCACAAGGCTATGGCTATGTAACTGAAAAAATCATTGACGCTTA 763
                 ** ************ * ***** ************** ** ** *** **** ** **

NCTC11637       CTTTAGCCATACTATTCCCATTTATTGGGGGAGTCCCAGCGTGGCGAAAGATTTTAACCC 824
NCTC11639       CTTTAGCCATACCATTCCTATTTATTGGGGGAGTCCTAGCGTGGCGAAAGATTTTAACCC 824
26695FucTa      TTTTAGCCATACCATTCCTATTTATTGGGGGAGTCCCAGCGTGGCGAAAGATTTTAACCC 827
26695fuctB      TTTTAGCCATACCATTCCTATTTATTGGGGGAGTCCCAGCGTGGCGAAAGATTTTAACCC 827
J99FucTa        TTTCAGCCACACCATTCCCATTTATTGGGGGAGTCCTAGCGTGGCGAAAGACTTTAACCC 823
J99FucTb        TTTCAGCCACACCATTCCCATTTATTGGGGGAGTCCTAGCGTGGCGAAAGACTTTAACCC 824
UA802FucT       CTTTAGCCATACCATTCCTATTTATTGGGGGAGTCCTAGCGTGGCGAAAGATTTTAACCC 815
UA948fucTa      TTTCAGCCACACTATCCCTATTTATTGGGGGAGTCCCAGCGTGGCGAAAGATTTTAACCC 818
UA1111FucTa     TTTCAGCCATACCATTCCTATTTATTGGGGGAGTCCCAGCGTGGCGAAAGATTTTAACCC 821
UA1111FucTb     CTTTAGCCACACCATTCCTATTTATTGGGGGAGTCCTAGCGTGGCGAAAGATTTTAACCC 824
UA1182          CTTTAGCCATACCATTCCTATTTATTGGGGGAGTCCTAGCGTGGCACAAGATTTTAACCC 823
                 ** ***** ** ** ** ***************** ********  **** ********

NCTC11637       TAAGAGTTTTGTGAATGTCCATGATTTCAACAACTTTGATGAAGCGATTGATTACGTGAG 884
NCTC11639       TAAAAGTTTTGTGAATGTGCATGATTTCAAAAACTTTGATGAAGCGATTGACTATATCAA 884
26695FucTa      TAAAAGTTTTGTGAATGTGCATGATTTCAACAACTTTGATGAAGCGATTGATTATATCAA 887
26695fuctB      TAAAAGTTTTGTGAATGTGCATGATTTCAACAACTTTGATGAAGCGATTGATTATATCAA 887
J99FucTa        TAAGAGTTTTGTGAACGTTTGTGATTTTAAAAACTTTGATGAAGCGATTGATTACGTGAG 883
J99FucTb        TAAGAGTTTTGTGAACGTTTGTGATTTTAAAAACTTTGATGAAGCGATTGATTACGTGAG 884
UA802FucT       TAAGAGTTTTGTGAATGTGCATGATTTTAAAAACTTTGATGAAGCGATTGATTACGTGAG 875
UA948fucTa      TAAAAGTTTTGTGAATGTGCATGATTTCAACAACTTTGATGAAGCGATTGATTATATCAG 878
UA1111FucTa     TAAGAGTTTTGTGAATGTCCATGATTTCAACAACTTTGATGAAGCGATTGACTATATCAG 881
UA1111FucTb     TAAAGAGTTTGTGAATGTCCATGATTTCAACAACTTTGATGAAGCGATAGATTATATCAA 884
UA1182          TAAGAGTTTTGTGAATGTTTGTGATTTTAAAGATTTTGATGAAGCGATTGATCATGTGCG 883
                ***    ******** **   ****** **  * ************** **  *  *
```

```
NCTC11637       ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACAC  944
NCTC11639       ATACTTGCACACGCACAAAAACGCTTATTTAGACATGCTTTATGAAAACCCTTTGAACAC  944
26695FucTa      ATACCTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACAC  947
26695fuctB      ATACCTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACAC  947
J99FucTa        ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACAC  943
J99FucTb        ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACAC  944
UA802FucT       ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACAC  935
UA948fucTa      ATATTTACACGCGCACCAAAACGCTTATTTAGACATGCTTTATGAAAACCCCTTAAACAC  938
UA1111FucTa     ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCACTATGAAAACCCTTTAAACAC  941
UA1111FucTb     ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCTCTATGAAAACCCTTTAAACGC  944
UA1182          ATACTTGCACACGCACCCAAACGCTTATTTAGACATGCTTTATGAAAACCCTTTAAACAC  943
                ***   * *** *****  ********************  *********** ** *** *

NCTC11637       CCTTGATGGGAAAGCTTACTTTTACCAAAATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1004
NCTC11639       CCTTGATGGGAAAGCTTACTTTTACCAAAATTTGAGTTTTAAAAAGATCCTAGCTTTTTT 1004
26695FucTa      CCTTGATGGGAAAGCTTACTTTTACCAAGATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1007
26695fuctB      CCTTGATGGGAAAGCTTACTTTTACCAAGATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1007
J99FucTa        CCTTGATGGGAAAGCTTACTTTTACCAAAATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1003
J99FucTb        CCTTGATGGGAAAGCTTACTTTTACCAAAATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1004
UA802FucT       CCTTGATGGGAAAGCTTACTTTTACCAAGATTTGAGTTTTAAAAAAATCCTAGATTTTTT 995
UA948fucTa      CATTGATGGGAAAGCGGGTTTTTACCAAGATTTGAGTTTTGAAAAGATCTTAGATTTTTT 998
UA1111FucTa     TATTGATGGGAAAGCTTACTTTTACCAAAATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1001
UA1111FucTb     CCTTGATGGGAAAGCTTACTTTTACCAGGATTTGAGTTTTAAAAAAATCCTAGCTTTTTT 1004
UA1182          CCTTGATGGGAAAGCTTACTTTTACCAAAATTTGAGTTTTAAAAAAATCCTAGATTTTTT 1003
                  *************     ********  *********** **** *** *** ******

NCTC11637       TAAAACGATTTTAGAAAACGACACGATTTATCA---TAA-----TAAC-CC-----TTTC 1050
NCTC11639       TAAAACGATTTTAGAAAACGATACGATTTATCA---CGA-----TAAC-CC-----TTTC 1050
26695FucTa      TAAAACGATTTTAGAAAACGATACGATTTATCA---CAAATTCTCAACATC-----TTTC 1059
26695fuctB      TAAAACGATTTTAGAAAACGACACGATTTATCA---CAA-----TAAC-CC-----TTTC 1053
J99FucTa        TAAAACGATTTTAGAAAACGACACGATCTATCA---CGA-----TAAC-CC-----TTTC 1049
J99FucTb        TAAAACGATTTTAGAAAACGACACGATCTATCA---CGA-----TAAC-CC-----TTTC 1050
UA802FucT       TAAAACGATTTTAGAAAACGATACGATCTATCA---CAA-----TAAC-CC-----TTTT 1041
UA948fucTa      CAAAAACATTCTTGAAAACGATACGATTTATCATTGCAA-----TGATGCCCATTATTCT 1053
UA1111FucTa     TAAAACGATTTTAGAAAACGACACGATCTATCA---CGA-----TAAC-CC-----TTTC 1047
UA1111FucTb     TAAAACGATTTTAGAAAACGATACGATTTATCA---CAAATCCTCAACATC-----TTTC 1056
UA1182          TAAAACGATTTTAGAAAACGACACGATTTATCA---CGA-----TAAC-CC-----TTTT 1049
                **** *** * ******** ***** *****     *       *   *     **

NCTC11637       ATTTTCTATCGTGATTTGAATGAGCCGTTAGTATCCATTGATAAT--------------- 1095
NCTC11639       ATTTTCTGTCGTGATTTGAATGAGCCTTTAGTAACTATTGATGAT--------------- 1095
26695FucTa      ATGTGGGAGTACGATCTGCATAAGCCGTTAGTATCCATTGATGAT--------------- 1104
26695fuctB      ATTTTCTATCGTGATCTGCATGAGCCTTTAATATCTATTGATGAT--------------- 1098
J99FucTa        ATTTTCTATCGCGATTTGAATGAGCCTTTAGTAGCTATTGATGAT--------------- 1094
J99FucTb        ATTTTCTATCGCGATTTGAATGAGCCTTTAGTAGCTATTGATGAT--------------- 1095
UA802FucT       GTTTTCTATCGTGATTTGAATGAGCCGTTAGTATCTATTGATGAT--------------- 1086
UA948fucTa      GCTCTTCATCGTGATTTGAATGAGCCGTTAGTGTCTGTTGATGAT--------------- 1098
UA1111FucTa     ATTTTCTATCGTGATTTGAATGAGCCTTCAGTATCTATTGATGGT--------------- 1092
UA1111FucTb     ATGTGGGAGTGCGATCTCGATGAGCCGTTAGCGTCTATTGATGAT--------------- 1101
UA1182          ATTTTTTATCGTGATTTGAATGAGCCGTTAATATCTATTGATGATGATTTGAGGGTTAAT 1109
                        *** *  ** **** * *    *  *****  *

NCTC11637       ------------------------------------------------------------
NCTC11639       ------------------------------------------------------------
26695FucTa      ------------------------------------------------------------
26695fuctB      ------------------------------------------------------------
J99FucTa        ------------------------------------------------------------
J99FucTb        ------------------------------------------------------------
UA802FucT       ------------------------------------------------------------
UA948fucTa      ------------------------------------------------------------
UA1111FucTa     ------------------------------------------------------------
UA1111FucTb     ------------------------------------------------------------
UA1182          TATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTT 1169
```

```
NCTC11637       ----------------------------------------------------TTGAGA 1101
NCTC11639       ----------------------------------------------------TTGAGG 1101
26695FucTa      ----------------------------------------------------TTGAGG 1110
26695fuctB      ----------------------------------------------------TTGAGG 1104
J99FucTa        ----------------------------------------------------TTGAGG 1100
J99FucTb        ----------------------------------------------------TTGAGG 1101
UA802FucT       ----------------------------------------------------TTGAGA 1092
UA948fucTa      --------------------------------TTGAGAAGAGATCATGATGATTTGAGG 1125
UA1111FucTa     ----------------------------------------------------TTGAGG 1098
UA1111FucTb     ----------------------------------------------------TTGAGG 1107
UA1182          AATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGG 1229
                                                                    *****

NCTC11637       ATCAATTATGATAATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT--------- 1152
NCTC11639       GTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGAATTAATTAT--------- 1152
26695FucTa      GTTAATTATGATGATTTGAGGGTTAATTATGAC---------------------------- 1143
26695fuctB      GTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT--------- 1155
J99FucTa        GTTAATTATGATGATTTGAGGGTTAATTA---T---------------------------- 1130
J99FucTb        GTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTG 1161
UA802FucT       GCCGATTATAATAATTTGAGAGCCGATTATAATAATTTGAGAGCCGATTAT--------- 1143
UA948fucTa      GTTAATTATGATGATTTGAGAGTTAATTATGATGATTTGAGAGTTAATTAT--------- 1176
UA1111FucTa     GTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT--------- 1149
UA1111FucTb     GTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT--------- 1158
UA1182          GTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT--------- 1280
                    ***** ** ******* *    ****

NCTC11637       --------------------------------GATGATTTGAGGGTTAATTATGATGAT 1179
NCTC11639       --------------------------------GATGATTTGAGGGTTAATTATGATGAT 1179
26695FucTa      ---------------------------------------------------------- 
26695fuctB      --------------------------------GATGATTTGAGGGTTAATTATGATGAT 1182
J99FucTa        --------------------------------GATGATTTGAGGGTTAATTATGATGAT 1157
J99FucTb        AGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGAT 1221
UA802FucT       --------------------------------AATAATTTGAGAGCCGATTATAATAAT 1170
UA948fucTa      --------------------------------GATGATTTGAGAGTTAATTATGATGAT 1203
UA1111FucTa     --------------------------------GATGATTTGAGGGTTAATTA------- 1169
UA1111FucTb     --------------------------------GATGATTTGAGGGTTAATTATGATGAT 1185
UA1182          --------------------------------GATGATTTGAGGGTTAATTATGATGAT 1307

NCTC11637       TTGAGAATCAATTATGATGATTTGAGAATCAATTATGATGATTTGAGAATTAATTATGA- 1238
NCTC11639       TTGAGAATTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGAT 1239
26695FucTa      ---------------------------------------------------------- 
26695fuctB      TTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGAT 1242
J99FucTa        TTGAGGGTTAATTATG---AT---------------------------------------- 1175
J99FucTb        TTGAGGGTTAATTATG---AT---------------------------------------- 1239
UA802FucT       TTGAGAGCCGATTACG---AT---------------------------------------- 1188
UA948fucTa      TTGAGAGTTAATTATGATGATTTGAGAAGAGATCGTGA----------------------- 1241
UA1111FucTa     -------------TG---A------------------------------------------ 1172
UA1111FucTb     TTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGAG---------------------- 1224
UA1182          TTGAGGGTTAATTATG---AG---------------------------------------- 1325

NCTC11637       ----------------------G----------------------------------- 1239
NCTC11639       GATTTGAGAATTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT 1299
26695FucTa      ---------------------------------------------------------- 
26695fuctB      GATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTATGATGATTTGAGGGTTAATTAT 1302
J99FucTa        ---------------------------------------------------------- 
J99FucTb        ---------------------------------------------------------- 
UA802FucT       ---------------------------------------------------------- 
UA948fucTa      ---------------------------------------------------------- 
UA1111FucTa     ----------------------G----------------------------------- 1173
UA1111FucTb     ---------------------------------------------------------- 
UA1182          ---------------------------------------------------------- 
```

```
NCTC11637          ---CGCCTTTTGCAAAACGCTT---CACCT-TTATTGGAA-TT-GTCCCAAAACACCTCT 1290
NCTC11639          GAGCGCCTCTTATCAAAAGCTA---CCCCT-CTTTTGGAA-TT-ATCCCAAAACACCACT 1353
26695FucTa         ---CGGCTTTTACAAAACGCTT---CGCCT-TTATTAGAA-CT-CTCTCAAAACACCACT 1194
26695fuctB         GACCGGCTTTTACAAAACGCTT---CGCCT-TTATTAGAA-CT-CTCTCAAAACACCACT 1356
J99FucTa           ---CGCCTTTTACAAAACGCTT---CGCCT-TTATTAGAA-CT-CTCTCAAAACACCACT 1226
J99FucTb           ---CGCCTTTTACAAAACGCTT---CGCCT-TTATTAGAA-CT-CTCTCAAAACACCACT 1290
UA802FucT          ---CGCCTGTTACAAAACCGTT---CGCCT-TTGTTGGAA-CT-CTCTCAAAACACCACT 1239
UA948fucTa         ---TGATTTGAGAAGAGATCATGAACGCCTCTTATCAAAGGCTACCCCCAAAACACCTCT 1298
UA1111FucTa        ---CGCCTTTTACAAAACGCCT---CGCCT-TTATTAGAA-CT-CTCTCAAAACACCACT 1224
UA1111FucTb        ---CGCCTTTTGCAAAACGCTT---CACCT-TTATTGGAA-TT-ATCCCAAAACACCTCT 1275
UA1182             ---CGGCTCTTACAAAACGCCT---CGCCT-TTATTAGAA-CT-CTCTCAAAACACCACT 1376
                      *  *       *          * ***  * *   *   *   * ********* **

NCTC11637          TTTAAAATCTAT-CGCAAAATTTATCAAAA-----------ATCCTTACCCTTATTGCGT 1338
NCTC11639          TCTAAAATCTAT-CGCAAAGCTTACCAAAA-----------ATCCTTACCTTTGTTGCGC 1401
26695FucTa         TTTAAAATCTAT-CGCAAAGCTTATCAAAA-----------ATCCTTGCCTTTGTTGCGC 1242
26695fuctB         TTTAAAATCTAT-CGCAAAGCTTATCAAAA-----------ATCCTTACCCTTGCTACGC 1404
J99FucTa           TTTAAAATCTAT-CGCAAAGCCTATCAAAA-----------ATCCTTACCTTTGTTGCGC 1274
J99FucTb           TTTAAAATCTAT-CGCAAAGCCTATCAAAA-----------ATCCTTACCTTTGTTGCGC 1338
UA802FucT          TTTAAAATCTAT-CACAAAGCTTATCACAA-----------ATCCTTACCTTTGTTGCGT 1287
UA948fucTa         TTTAAAATCTATACGCAAAGCTTATCAAAA-----------GTCCTTACCCTTGTTGCGT 1347
UA1111FucTa        TTTAAAATCTAT-CGCAAAGCTTATCAAAA-----------ATCCTTGCCTTTGTTGCGT 1272
UA1111FucTb        TTTAAAATCTAT-CGCAAAGCCTATCAAAAGCCTATCAAAAATCCTTACCCTTATTGCGC 1334
UA1182             TTTAAAATCTAT-CGCAAAGCTTATCAAAA-----------ATCCTTACCTTTGTTGCGT 1424
                  * ********** * ****   ** ** **             ***** ** **  * **

NCTC11637          GTAATAAGGAGGTGGGTTAAAAAATAA--------------------------------- 1365
NCTC11639          GCCATAAGGAGATGGGTTAAAAAATTGGGTTTGTAA------------------------- 1437
26695FucTa         GCGGTGAGAAAGTTGGTTAAAAAATTGGGTTTG---------------------------- 1275
26695fuctB         ACCATAAGGAGATGGGTTAAAAAG------------------------------------ 1428
J99FucTa           GCCATAAGGAGATGGGTTAAAAAATTGGGTTTGTGA------------------------ 1310
J99FucTb           ACCATAAGGAGATGGGTTAAAAAATAA--------------------------------- 1365
UA802FucT          GCCATAAGGAGATGGGTTAAAAAATTGGGTTTGTAA------------------------ 1323
UA948fucTa         GCCATAAGGAGGT-GGTTAGAAAAT-AGGTTTGTAAAACCAATCAAACCCCTTGCGCTAT 1405
UA1111FucTa        GCCATAAGGAGATGGGTTAAAAAGTAA--------------------------------- 1299
UA1111FucTb        GCCATAA----------------------------------------------------- 1341
UA1182             GCGGCGAGAAAGTTGATTAAAAAATTGGGTTTGTAA------------------------ 1460
                        *

NCTC11637          ----------------------
NCTC11639          ----------------------
26695FucTa         ----------------------
26695fuctB         ----------------------
J99FucTa           ----------------------
J99FucTb           ----------------------
UA802FucT          ----------------------
UA948fucTa         CATCGCAGACGCTACTTTCCTAA 1428
UA1111FucTa        ----------------------
UA1111FucTb        ----------------------
UA1182             ----------------------
```

B.

```
NCTC11637           MFQPLLDAFIESASIEKMASKSPPP--LKIAVANWWGDE--EIKEFKKSTLYFILSQHYT 56
NCTC11639FucTa      MFQPLLDAYVESASIEKMASKSPPP--LKIAVANWWGDE--EIKEFKNSVLYFILSQRYT 56
26695FucTa          MFQPLLDAFIESASIEKMASKSPPPP-LKIAVANWWGDE--EIKEFKKSVLYFILSQRYA 57
26695FucTb          MFQPLLDAFIESASIEKMVSKSPPPP-LKIAVANWWGDE--EIKEFKKSVLYFILSQRYA 57
J99FucTa            MFQPLLDAFIESTPIKKKITFKSPPPPLKIAVANWWGG----AEEFKKSTLYFILSQRYT 56
J99FucTb            MFQPLLDAYTDSTRLDETDYKPPLN----IALANWWPLDKRESKGFRRFILYFILSQRYT 56
UA802FucT           MFQPLLDAFIESASIKKMPLSYPP---LKIAVANWWGG----AEEFKKSAMYFILSQRYT 53
UA948FucTa          MFQPLLDAFIDSTHLDETTHKPP----FKCSLANWWPLKNSEKKGFRDFILTLLLKQRYK 56
UA1111FucTa         MFQPLLDAFIESAPLKKWPLNLPP---LKIAVANWWGDE--EIKKFKKSVLYFILSQHYT 55
UA1111FucTb         MFQPLLDAYVESASIEKMASKSPPP--LKIAVANWWGDE--EIKEFKKSVLYFIFSQRYT 56
UA1182FucT          MFQPLLDAYIESASIEKITSKSPPP--LKIAVANWWGDE--EVEEFKKNILYFILSQHYT 56
                    ********: :*: :.:     .       ::*****       : *:   : :::.*:*

NCTC11637           ITLHRNPDKPADIVFGNPLGSARKILSYQNTKRIFYTGENESPNFNLFDYAIGFDELDFR 116
NCTC11639FucTa      ITLHQNPNEFSDLVFGNPLGSARKILSYQNAKRVFYTGENESPNFNLFDYAIGFDELDFN 116
26695FucTa          ITLHQNPNEFSDLVFSNPLGAARKILSYQNTKRVFYTGENESPNFNLFDYAIGFDELDFN 117
26695FucTb          ITLHQNPNESSDLVFSNPLGAARKILSYQNTKRVFYTGENESPNFNLFDYAIGFDELDFN 117
J99FucTa            ITLHQNPNEPSDLVLGSPIGSARKILSYQNTKRVFYTGENEVPNFNLFDYAIGFDELDFR 116
J99FucTb            ITLHQNPNEPSDLVFGSPIGSARKILSYQNTKRVFYTGENEVPNFNLFDYAIGFDELDFR 116
UA802FucT           ITLHQNPNEPSDLVFGSPIGAARKILSYQNTKRVFYAGENEVPNFNLFDYAIGFDELDLR 113
UA948FucTa          ILLQHNPNEPSDLVFGNPLEQARKILSYQNTKRVFYTGENEVPNFNLFDYAIGFDELDFN 116
UA1111FucTa         ITLHRNPDKPADIVFGNPLGSARKILSYQNAKRVFYTGENEVPNFNLFDYAIGFDELDFR 115
UA1111FucTb         IALHQNPNEFSDLVFSNPLGSARKILSYQNAKRVFYTGENEVPNFNLFDYAIGFDELDFR 116
UA1182FucT          ITLHQNPNEPSDLVFGSPIGSARKILSYQNAKRVFYTGENESPNFNLFDYAIGFDELDFR 116
                    * *::**:: :*:*:..*:  *********:**:**:**** ***************:.

NCTC11637           DRYLRMPLYYDRLHHKAESVNDTTAPYKIKGNSLYTLKKPSHCFKENHPNLCALINNESD 176
NCTC11639FucTa      DRYLRMPLYYDRLHHKAESVNDTTAPYKLKDNSLYALKKPSHCFKEKHPNLCAVVNDESD 176
26695FucTa          DRYLRMPLYYAHLHYKAELVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESD 177
26695FucTb          DRYLRMPLYYAHLHYEAELVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESD 177
J99FucTa            DRYLRMPLYYASLHYKAESVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESD 176
J99FucTb            DRYLRMPLYYASLHYKAESVNDTTAPYKLKDNSLYALKKPSHHFKENHPNLCAVVNDESD 176
UA802FucT           DRYLRMPLYYDRLHHKAESVNDTTAPYKIKPDSLYTLKKPSHHFKEKHPHLCAVVNDESD 173
UA948FucTa          DRYLRMPLYYAYLHYKAMLVNDTTSPYKLK--ALYTLKKPSHKFKENHPNLCALIHNESD 174
UA1111FucTa         DRYLRMPLYYAYLHYKAELVNDTTSPYKLQPDSLYALKKPSHHFKENHPNLCAVVNNESD 175
UA1111FucTb         DRYLRMPLYYDRLHHKAESVNDTTSPYKLKDNSLYTLKKPSHQFKENHPNLCAVVNDESD 176
UA1182FucT          DRYLRMPLYYDRLHHKAESVNDTTSPYKLKPDSLYALKKPSHHFKENHPNLCAVVNNESD 176
                    ********** **::*  *****:***::  :**:****** ***:**:***::::***

NCTC11637           PLKRGFASFVASNANAPMRNAFYDALNSIEPVTGGGAVKNTLGYKVGNKSEFLSQYKFNL 236
NCTC11639FucTa      PLKRGFASFVASNPNAPIRNAFYDALNSIEPVTGGGSVRNTLGYNVKNKNEFLSQYKFNL 236
26695FucTa          LLKRGFASFVASNANAPMRNAFYDALNSIEPVTGGGSVRNTLGYKVGNKSEFLSQYKFNL 237
26695FucTb          LLKRGFASFVASNANAPMRNAFYDALNSIEPVTGGGSVRNTLGYKVGNKSEFLSQYKFNL 237
J99FucTa            PLKRGFASFVASNPNAPIRNAFYDALNSIEPVTGGGSVKNTLGYNVKNKSEFLSQYKFNL 236
J99FucTb            PLKRGFASFVASNPNAPIRNAFYDALNSIEPVTGGGSVKNTLGYNVKNKSEFLSQYKFNL 236
UA802FucT           PLKRGFASFVASNPNAPKRNAFYDALNSIEPVTGGGSVKNTLGYKVGNKNEFLSQYKFNL 233
UA948FucTa          PWKRGFASFVASNPNAPIRNAFYDALNAIEPVASGGSVKNTLGYKVKNKNEFLSQYKFNL 234
UA1111FucTa         PLKRGFASFVASNPNAPRRNAFYEALNAIEPVAGGGSVKNTLGYNVKNKSEFLSQYKFNL 235
UA1111FucTb         PLKRGVVSFVASNANAPMRNAFYDALNSIEPVTGGGSVKNTLGYNVKNKSEFLSQYKFNL 236
UA1182FucT          PLKRGFASFVASNPNAPKRNAFYDVLNSIEPVIGGGSVKNTLGYNIKNKSEFLSQYKFNL 236
                      ***..******.*** *****:.**:**** .**:*:*****:: **.**********

NCTC11637           CFENSQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYVRY 296
NCTC11639FucTa      CFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFKNFDEAIDYIKY 296
26695FucTa          CFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIKY 297
26695FucTb          CFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIKY 297
J99FucTa            CFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVCDFKNFDEAIDYVRY 296
J99FucTb            CFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVCDFKNFDEAIDYVRY 296
UA802FucT           CFENSQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFKNFDEAIDYVRY 293
UA948FucTa          CFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIRY 294
UA1111FucTa         CFENTQGYGYVTEKIIDAYFSHTIPIYWGSPSVAKDFNPKSFVNVHDFNNFDEAIDYIRY 295
UA1111FucTb         CFENSQGYGYVTEKILDAYFSHTIPIYWGSPSVAKDFNPKEFVNVHDFNNFDEAIDYIKY 296
UA1182FucT          CFENSQGYGYVTEKIIDAYFSHTIPIYWGSPSVAQDFNPKSFVNVCDFKDFDEAIDHVRY 296
                    ****:**********:*****************:*****.**** **::******:::*
```

```
NCTC11637            LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYHNN---PFIFY 353
NCTC11639FucTa       LHTHKNAYLDMLYENPLNTLDGKAYFYQNLSFKKILAFFKTILENDTIYHDN---PFIFC 353
26695FucTa           LHTHPNAYLDMLYENPLNTLDGKAYFYQDLSFKKILDFFKTILENDTIYHKFST-SFMWE 356
26695FucTb           LHTHPNAYLDMLYENPLNTLDGKAYFYQDLSFKKILDFFKTILENDTIYHNN---PFIFY 354
J99FucTa             LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYHDN---PFIFY 353
J99FucTb             LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYHDN---PFIFY 353
UA802FucT            LHTHPNAYLDMLYENPLNTLDGKAYFYQDLSFKKILDFFKTILENDTIYHNN---PFVFY 350
UA948FucTa           LHAHQNAYLDMLYENPLNTIDGKAGFYQDLSFEKILDFFKNILENDTIYHCNDAHYSALH 354
UA1111FucTa          LHTHPNAYLDMHYENPLNTIDGKAYFYQNLSFKKILDFFKTILENDTIYHDN---PFIFY 352
UA1111FucTb          LHTHPNAYLDMLYENPLNALDGKAYFYQDLSFKKILAFFKTILENDTIYHKSST-SFMWE 355
UA1182FucT           LHTHPNAYLDMLYENPLNTLDGKAYFYQNLSFKKILDFFKTILENDTIYHDN---PFIFY 353
                     **:* ****** *******:**** ***:***:*** ***.**********

NCTC11637            RDLNEPLVSIDN--------LRINYDNLRVNYDDLRVNYDDLRVNYDDLRINYDDLRINY 405
NCTC11639FucTa       RDLNEPLVTIDD--------LRVNYDDLRVNYDDLRINYDDLRVNYDDLRINYDDLRVNY 405
26695FucTa           YDLHKPLVSIDD--------LRVN--------------YDDLRVN--------------- 379
26695FucTb           RDLHEPLISIDD--------LRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNY 406
J99FucTa             RDLNEPLVAIDD--------LRVNYDDLRVNYDDLRVNYDDLRVN--------------- 390
J99FucTb             RDLNEPLVAIDDLRVN-YDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVN- 411
UA802FucT            RDLNEPLVSIDD--------LRADYNNLRADYNNLRADYNNLRADYNNLR---------- 392
UA948FucTa           RDLNEPLVSVDDLRR-DHDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRRDR 413
UA1111FucTa          RDLNEPSVSIDG--------LRVNYDDLRVNYDDLRVNYDDLRVN--------------- 389
UA1111FucTb          CDLDEPLASIDD--------LRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVN- 406
UA1182FucT           RDLNEPLISIDDDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNYDDLRVNY 413
                     **.:*  ::*.        ** :               *::**.:

NCTC11637            DDLRIN---------------------YERLLQNASPLLELSQNTSFKIYRKIYQKSLPL 444
NCTC11639FucTa       DDLRVNYDDLRINYDDLRVNYDDLRVNYERLLSKATPLLELSQNTTSKIYRKAYQKSLPL 465
26695FucTa           -----------Y----------------DRLLQNASPLLELSQNTTFKIYRKAYQKSLPL 412
26695FucTb           DDLRVNYDDLRVNYDDLRVNYDDLRVNYDRLLQNASPLLELSQNTTFKIYRKAYQKSLPL 466
J99FucTa             ----------------------------YDRLLQNASPLLELSQNTTFKIYRKAYQKSLPL 423
J99FucTb             ----------------------------YDRLLQNASPLLELSQNTTFKIYRKAYQKSLPL 444
UA802FucT            ------------------------ADYDRLLQNRSPLLELSQNTTFKIYHKAYHKSLPL 427
UA948FucTa           DD--------------------------LRRDHERLLSKATPKTPLLKSIRKAYQKSLPL 447
UA1111FucTa          ----------------------------YERLLQNASPLLELSQNTTFKIYRKAYQKSLPL 422
UA1111FucTb          ----------------------------YERLLQNASPLLELSQNTSFKIYRKAYQKPIKN 439
UA1182FucT           DDLRVNYDDLRVNYDDLRVNYDDLRVNYERLLQNASPLLELSQNTTFKIYRKAYQKSLPL 473
                                                 *   :       .:..  *  :* *:*.:

NCTC11637            LRVIRRWVKK------------------ 454
NCTC11639FucTa       LRAIRRWVKKLGL--------------- 478
26695FucTa           LRAVRKLVKKLGL--------------- 425
26695FucTb           LRTIRRWVKK------------------ 476
J99FucTa             LRAIRRWVKKLGL--------------- 436
J99FucTb             LRTIRRWVKK------------------ 454
UA802FucT            LRAIRRWVKKLGL--------------- 440
UA948FucTa           LRAIRRWLENRFVKPIKPLALSSQTLLS 475
UA1111FucTa          LRAIRRWVKK------------------ 432
UA1111FucTb          PYPYCAP--------------------- 446
UA1182FucT           LRAARKLIKKLGL--------------- 486
```

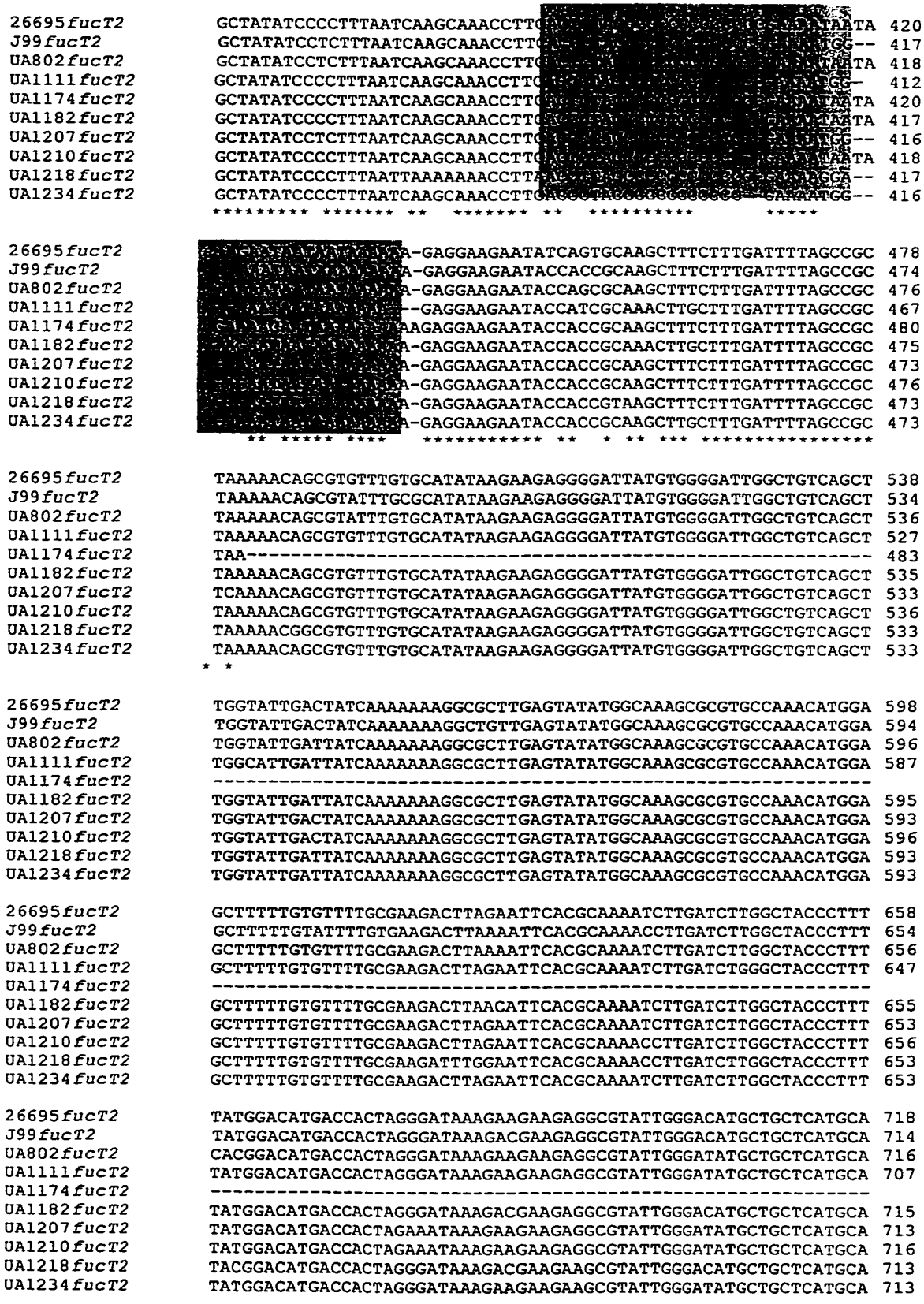
Appendix 2: Alignment of nucleotide and amino acid sequence of all known α(1,2) fucosyltransferases from *H. pylori*. Appendix 2A is the nucleotide sequence alignment and Appendix 2B is the amino acid sequence alignment. In both diagrams the grey boxes indicate the regions of variability in these genes and proteins. The accession numbers to access the sequence data is as follows:

26695*	HP0093/94
J99*	AE001447
UA802	AF076779
UA1111	To be designated
UA1174	AF093830 – This gene is truncated and does not produce full length functional protein under any circumstances.
UA1182*	AF093829
UA1207	AF093832
UA1210	AF093833
UA1218	AF093831
UA1234	AF093828

* denotes that these isolates utilize a translational frameshifting cassette to produce full length functional protein.

A.

```
26695fucT2      ATGGCTTTTAAGGTGGTGCAAATTTGCGGAGGGCTTGGGAATCAAATGTTTCAATACGCT  60
J99fucT2        ATGGCTTTTAAAGTGGTGCAAATTTGCGGGGGGCTTGGGAATCAAATGTTCCAATACGCT  60
UA802fucT2      ATGGCTTTTAAAGTGGTGCAAATTTGTGGGGGGCTTGGGAATCAAATGTTTCAATACGCT  60
UA1111fucT2     ATGGCTTTTAAGGTGGTGCAAATTTGTGGGGGGCTTGGGAATCAAATGTTTCAATACGCT  60
UA1174fucT2     ATGGCTTTTAAGGTGGTGCAAATTTGTGGGGGGCTTGGGAATCAAATGTTTCAATACGCT  60
UA1182fucT2     ATGGCTTTTAAGGTGGTGCGAATTTGTGGAGGGCTTGGGAATCAAATGTTTCAATACGCT  60
UA1207fucT2     ATGGCTTTTAAAGTGGTGCAAATTTGCGGAGGGCTTGGGAATCAAATGTTTCAATACGCT  60
UA1210fucT2     ATGGCTTTTAAGGTGGTGCAAATTTGCGGGGGACTTGGGAATCAAATGTTTCAATACGCT  60
UA1218fucT2     ATGGCTTTTAAGGTGGTGCAAATTTGTGGGGGGCTTGGGAATCAAATGTTCCAATACGCT  60
UA1234fucT2     ATGGCTTTTAAGGTGGTGCAAATTTGCGGGGGGCTTGGGAATCAAATGTTTCAATACGCT  60
                ********** ******* ****** ** ** ***************** *********

26695fucT2      TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATATCACTTCTTTT  120
J99fucT2        TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCCGTGCTATTGGATATCACTTCTTTT  120
UA802fucT2      TTCGCTAAAAGTTTGCAAAAACACCTTAATACGCCCGTGCTATTAGACACTACTTCTTTT  120
UA1111fucT2     TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATATCACTTCTTTT  120
UA1174fucT2     TTCGCGAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATATCACTTCTTTT  120
UA1182fucT2     TTCGCTAAAAGTTTGCAAAAACACTCTAACACGCCTGTGCTATTAGATATTACTTCTTTT  120
UA1207fucT2     TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATATTAGTTCTTTT  120
UA1210fucT2     TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATATTACTTCTTTT  120
UA1218fucT2     TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATACAACTTCTTTT  120
UA1234fucT2     TTCGCTAAAAGTTTGCAAAAACACTCTAATACGCCTGTGCTGTTAGATATTACTTCTTTT  120
                ***** ******************  *** ***** ***** ** ** *  * *******

26695fucT2      GATTGGAGCGATAGGAAAATGCAATTAGAACTTTTCCCTATTGATTTGCCCTATGCGAGC  180
J99fucT2        GATGGGAGCAATAGGAAAATGCAATTAGAGCTTTTCCCTATTGATTTGCCCTATGCGAGC  180
UA802fucT2      GATTGGAGCAATAGGAAAATGCAATTAGAGCTTTTCCCTATTGATTTGCCCTATGCGAAT  180
UA1111fucT2     GATTGGAGCGATAGGAAAATGCAATTAGAACTTTTCCCTATTGATTTGCCCTATGCGAAC  180
UA1174fucT2     GATTGGAGCAATAGGAAAATGCAATTAGAGCTTTTCCCTATTGACTTGCCCTATGCGAGT  180
UA1182fucT2     GATTGGAGTAATAGGAAAATGCAATTAGAACTTTTCCCTATTGATTTGCCCTATGCGAGC  180
UA1207fucT2     GATTGGAGTAATAGGAAAATGCAATTAGAACTTTTCCCTATTGATTTGCCCTATGCGAGC  180
UA1210fucT2     GATTGGAGCGATAGGAAAATGCAATTAGAACTTTTCCCTATTAATTTGCCCTATGCGAGC  180
UA1218fucT2     GATTGGAGCAATAGGAAAATGCAATTAGAGCTTTTCCCTATTGATTTGCCCTATGCGAGT  180
UA1234fucT2     GATTGGAGTAATAGAAAAATGCAATTAGAACTTTTCCCTATTGATTTGCCCTATGCGAGT  180
               *** ****  **** ************** ************ * **************

26695fucT2      GCGAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGACGCGCTC  240
J99fucT2        GCAAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGACGCGCTC  240
UA802fucT2      GCAAAAGAAATCGCTATAGCTAAAATGCAACATCTCCCCAAGTTAGTAAGAGATGCACTC  240
UA1111fucT2     GCAAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGAGGCGCTC  240
UA1174fucT2     GAAAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGAGGTGCTC  240
UA1182fucT2     GCGAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGATGCGCTC  240
UA1207fucT2     GCGAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGACGCGCTC  240
UA1210fucT2     GCGAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGACGCGCTC  240
UA1218fucT2     GAAAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAGAGGCGCTC  240
UA1234fucT2     GAAAAAGAAATCGCTATAGCTAAAATGCAACACCTCCCCAAGCTAGTAAGAAATGTGCTC  240
                *  ****************************** ********* ******** * *  ***

26695fucT2      AAATGCATGGGATTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTG  300
J99fucT2        AAATACATGGGGTTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTA  300
UA802fucT2      AAATACATAGGATTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTG  300
UA1111fucT2     AAATGCATGGGGTTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTA  300
UA1174fucT2     AAATGCATGGGATTTGATAGGGTGAGTCAAGAGATCGTTTTTGAATACGAGCCTGAATTA  300
UA1182fucT2     AAATACATAGGATTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTA  300
UA1207fucT2     AAATGCATGGGATTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTA  300
UA1210fucT2     AAATGCATGGGGTTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTGAATTG  300
UA1218fucT2     AAATACATAGGATTTGACAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTG  300
UA1234fucT2     AAATGCATGGGGTTTGATAGGGTGAGTCAAGAAATCGTTTTTGAATACGAGCCTAAATTG  300
                **** *** ** ***** ************** ********************* ****

26695fucT2      CTAAAGCCAAGCCGCTTGACTTATTTTTTTGGCTATTTCCAAGATCCACGATACTTTGAT  360
J99fucT2        TTAAAGCCAAGCCGCTTGACTTATTTTTATGGCTATTTTCAAGATCCACGATATTTTGAT  360
UA802fucT2      TTAAAGCCAAGCCGTTTGACTTATTTTTTTGGCTATTTCCAAGATCCACGATATTTTGAT  360
UA1111fucT2     TTAAAGCCAAGCCGCTTGACTTATTTTTATGGCTATTTTCAAGATCCACGATACTTTGAT  360
UA1174fucT2     TTAAAGCCAAGCCGCTTGACTTATTTTTTTGGCTATTTCCAAGATCCACGATATTTTGAT  360
UA1182fucT2     CTAAAGCCAAGCCGCTTGACTTATTTTTATGGTTACTTCCAAGATCCACGATATTTTGAT  360
UA1207fucT2     TTAAAGCCAAGCCGCTTGACTTATTTTTATGGTTACTTCCAAGATCCACGATATTTTGAT  360
UA1210fucT2     TTAAAGCCAAGCCGCTTGACTTATTTTTATGGCTATTTTCAAGATCCACGATATTTTGAT  360
UA1218fucT2     TTAAAGCCAAGCCGTTTGACTTATTTTTTTGGCTATTTCCAAGATCCACGATATTTTGAT  360
UA1234fucT2     TTAAAGACAAGCCGCTTGACTTATTTTTATGGCTATTTTCAAGATCCACGATATTTTGAT  360
                 ***** ******* ************** *** ** ** ************** ******
```



```
26695fucT2      ATCTTGTCAGCATGGCATTATCGCTAATAGCACTTATAGCTGGTGGGCGGCCTATTTGAT 778
J99fucT2        ATCTTGCAAGCATGGCATTATCGCTAACAGCACTTATAGCTGGTGGGCGGCTTATTTGAT 774
UA802fucT2      ATCTTGCAAGCATGGCATTATCGCTAATAGCACTTATAGCTGGTGGGCGGCTTATTTGAT 776
UA1111fucT2     ATCTTGCAAGCATGGCATTATCGCTAATAGCACTTATAGCTGGTGGGCGGCTTATTTGAT 767
UA1174fucT2     ------------------------------------------------------------
UA1182fucT2     ATCGTGTCAGCATGGCATTATCGCTAACAGCACTTATAGTTGGTGGGCGGCCTATTTGAT 775
UA1207fucT2     ATCTTGCAAGCATGGCATTATCGCTAATAGCACTTATAGCTGGTGGGCGGCTTATTTGAT 773
UA1210fucT2     ATCCTGTCAACATGGCATTATCGCTAATAGCACTTATAGCTGGTGGGCGGCTTATTTGAT 776
UA1218fucT2     ATCTTGTCAGCATGGCATTATCGCTAACAGCACTTATAGCTGGTGGGCGGCTTATTTGAT 773
UA1234fucT2     ATCTTGCAAGCATGGCATTATCGCTAACAGCACTTATAGCTGGTGGGCGGCCTATTTGAT 773

26695fucT2      AGAAAATCCAGAAAAAATCATTATTGGCCCCAAACACTGGCTTTTTGGGCATGAGAATAT 838
J99fucT2        AAACAATCCAGGAAAAATCATCATTGGCCCCAAACACTGGCTTTTTGGGCATGAAAACAT 834
UA802fucT2      GGAAAATCCAGAAAAAATCATTATTGGCCCCAAACACTGGCTTTTTGGGCATGAAAATAT 836
UA1111fucT2     AAAAAATCCAGAAAAAATCATTATTGGCCCCAAACACTGGCTTTTTGGGCATGAAAATAT 827
UA1174fucT2     ------------------------------------------------------------
UA1182fucT2     AGAAAATCCAGAAAAAATCATCATTGGCCCCAAACACTGGCTTTTTGGGCATGAAAATAT 835
UA1207fucT2     AAACAATCCAGAAAAAATCATCATTGGCCCCAAACACTGGCTTTTTGGGCATGAAAATAT 833
UA1210fucT2     AGAAAATCCAGAAAAAATCATCATTGGCCCCAAACACTGGCTTTTTGGGCATGAAAATAT 836
UA1218fucT2     AAACAATCCAGAAAAAATCATTATTGGCCCCAAACACTGGCTTTTTGGGCATGAGAATAT 833
UA1234fucT2     AAACAATCCAGAAAAAATCATTATTGGCCCCAAACACTGGCTTTTTGGGCATGAGAATAT 833

26695fucT2      CCTTTGTAAGGAGTGGGTGAAAATAGAATCCCATTTTGAGGTAAAATCCCAAAAGTATAA 898
J99fucT2        CCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTGAAATCCCAAAAGTATAA 894
UA802fucT2      TCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTAAAATCCCAAAAATATAA 896
UA1111fucT2     CCTTTGTGAGGAGTGGGTGAAAATAGAATCCCATTTTGAGGTGAAATCCCAAAAGTATAA 887
UA1174fucT2     ------------------------------------------------------------
UA1182fucT2     CCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTGAAATCCCAAAAGTATAA 895
UA1207fucT2     CCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTGAAATCCCAAAAGTATAA 893
UA1210fucT2     CCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTGAAATCCCAAAAGTATAA 896
UA1218fucT2     CCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTAAAATCCCAAAAGTATAA 893
UA1234fucT2     CCTTTGTAAGGAATGGGTGAAAATAGAATCCCATTTTGAGGTGAAATCCCAAAAGTATAA 893

26695fucT2      CGCTTAA 905
J99fucT2        CGCTTAA 901
UA802fucT2      CGCTTAA 903
UA1111fucT2     CGCTTAA 894
UA1174fucT2     -------
UA1182fucT2     CGCTTAA 902
UA1207fucT2     CGCTTAA 900
UA1210fucT2     CGCTTAA 903
UA1218fucT2     CGCTTAA 900
UA1234fucT2     CGCTTAA 900
```

B.

```
26695FucT2      MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSDRKMQLELFPIDLPYAS 60
J99FucT2        MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDGSNRKMQLELFPIDLPYAS 60
UA802FucT2      MAFKVVQICGGLGNQMFQYAFAKSLQKHLNTPVLLDTTSFDWSNRKMQLELFPIDLPYAN 60
UA1111FucT2     MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSDRKMQLELFPIDLPYAN 60
UA1174FucT2     MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSNRKMQLELFPIDLPYAS 60
UA1182FucT2     MAFKVVRICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSNRKMQLELFPIDLPYAS 60
UA1207FucT2     MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDISSFDWSNRKMQLELFPIDLPYAS 60
UA1210FucT2     MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSDRKMQLELFPINLPYAS 60
UA1218FucT2     MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDTTSFDWSNRKMQLELFPIDLPYAS 60
UA1234FucT2     MAFKVVQICGGLGNQMFQYAFAKSLQKHSNTPVLLDITSFDWSNRKMQLELFPIDLPYAS 60
                ******:********************** ******* :*** *:**********:****.

26695FucT2      AKEIAIAKMQHLPKLVRDALKCMGFDRVSQEIVFEYEPKLLKPSRLTYFFGYFQDPRYFD 120
J99FucT2        AKEIAIAKMQHLPKLVRDALKYMGFDRVSQEIVFEYEPKLLKPSRLTYFYGYFQDPRYFD 120
UA802FucT2      AKEIAIAKMQHLPKLVRDALKYIGFDRVSQEIVFEYEPKLLKPSRLTYFFGYFQDPRYFD 120
UA1111FucT2     AKEIAIAKMQHLPKLVREALKCMGFDRVSQEIVFEYEPKLLKPSRLTYFYGYFQDPRYFD 120
UA1174FucT2     EKEIAIAKMQHLPKLVREVLKCMGFDRVSQEIVFEYEPELLKPSRLTYFFGYFQDPRYFD 120
UA1182FucT2     AKEIAIAKMQHLPKLVRDALKYIGFDRVSQEIVFEYEPKLLKPSRLTYFYGYFQDPRYFD 120
UA1207FucT2     AKEIAIAKMQHLPKLVRDALKCMGFDRVSQEIVFEYEPKLLKPSRLTYFYGYFQDPRYFD 120
UA1210FucT2     AKEIAIAKMQHLPKLVRDALKCMGFDRVSQEIVFEYEPELLKPSRLTYFYGYFQDPRYFD 120
UA1218FucT2     EKEIAIAKMQHLPKLVREALKYIGFDRVSQEIVFEYEPKLLKPSRLTYFFGYFQDPRYFD 120
UA1234FucT2     EKEIAIAKMQHLPKLVRNVLKCMGFDRVSQEIVFEYEPKLLKTSRLTYFYGYFQDPRYFD 120
                  ****************:.** :***************:***.******:**********

26695FucT2      AISPLIKQTFTLP[redacted]IKKEEEYQCKLSLILAAKNSVFVHIRRGDYVGIGCQL 180
J99FucT2        AISSLIKQTFTLP[redacted]KKKEEEYHRKLSLILAAKNSVFAHIRRGDYVGIGCQL 178
UA802FucT2      AISSLIKQTFTLP[redacted]NKKEEEYQRKLSLILAAKNSVFVHIRRGDYVGIGCQL 179
UA1111FucT2     AISPLIKQTFTLP[redacted]IKKEEEYHRKLALILAAKNSVFVHIRRGDYVGIGCQL 176
UA1174FucT2     AISPLIKQTFTLP[redacted]NNKKKR---------------------------------- 147
UA1182FucT2     AISPLIKQTFTLP[redacted]IKKEEEYHRKLALILAAKNSVFVHIRRGDYVGIGCQL 179
UA1207FucT2     AISSLIKQTFTLP[redacted]KKKEEEYHRKLSLILAAQNSVFVHIRRGDYVGIGCQL 178
UA1210FucT2     AISPLIKQTFTLP[redacted]NKKEEEYHRKLSLILAAKNSVFVHIRRGDYVGIGCQL 179
UA1218FucT2     AISPLIKKTLTLP[redacted]KKKEEEYHRKLSLILAAKNGVFVHIRRGDYVGIGCQL 178
UA1234FucT2     AISPLIKQTFTLP[redacted]KKKEEEYHRKLALILAAKNSVFVHIRRGDYVGIGCQL 178
                ***.***:*:*****          :*::.

26695FucT2      GIDYQKKALEYMAKRVPNMELFVFCEDLEFTQNLDLGYPFMDMTTRDKEEEAYWDMLLMQ 240
J99FucT2        GIDYQKKAVEYMAKRVPNMELFVFCEDLKFTQNLDLGYPFMDMTTRDKDEEAYWDMLLMQ 238
UA802FucT2      GIDYQKKALEYMAKRVPNMELFVFCEDLKFTQNLDLGYPFTDMTTRDKEEEAYWDMLLMQ 239
UA1111FucT2     GIDYQKKALEYMAKRVPNMELFVFCEDLEFTQNLDLGYPFMDMTTRDKEEEAYWDMLLMQ 236
UA1174FucT2     ---------G----RIPPQAFFDFSR---------------------------------- 160
UA1182FucT2     GIDYQKKALEYMAKRVPNMELFVFCEDLTFTQNLDLGYPFMDMTTRDKDEEAYWDMLLMQ 239
UA1207FucT2     GIDYQKKALEYMAKRVPNMELFVFCEDLEFTQNLDLGYPFMDMTTRNKEEEAYWDMLLMQ 238
UA1210FucT2     GIDYQKKALEYMAKRVPNMELFVFCEDLEFTQNLDLGYPFMDMTTRNKEEEAYWDMLLMQ 239
UA1218FucT2     GIDYQKKALEYMAKRVPNMELFVFCEDLEFTQNLDLGYPFTDMTTRDKDEEAYWDMLLMQ 238
UA1234FucT2     GIDYQKKALEYMAKRVPNMELFVFCEDLEFTQNLDLGYPFMDMTTRDKEEEAYWDMLLMQ 238
                          *:*    :* *..

26695FucT2      SCQHGIIANSTYSWWAAYLIENPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 301
J99FucT2        SCKHGIIANSTYSWWAAYLINNPGKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 299
UA802FucT2      SCKHGIIANSTYSWWAAYLMENPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 300
UA1111FucT2     SCKHGIIANSTYSWWAAYLIKNPEKIIIGPKHWLFGHENILCEEWVKIESHFEVKSQKYNA 297
UA1174FucT2     ------------------------------------------------------------
UA1182FucT2     SCQHGIIANSTYSWWAAYLIENPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 300
UA1207FucT2     SCKHGIIANSTYSWWAAYLINNPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 299
UA1210FucT2     SCQHGIIANSTYSWWAAYLIENPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 300
UA1218FucT2     SCQHGIIANSTYSWWAAYLINNPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 299
UA1234FucT2     SCKHGIIANSTYSWWAAYLINNPEKIIIGPKHWLFGHENILCKEWVKIESHFEVKSQKYNA 299
```

Student Progress and Ciriculum Vitae

Publications

M.A. Monteiro, **D.A. Rasko** and D.E. Taylor. Analysis of Non Lewis antigen containing LPS from *Helicobacter pylori.* In preparation.

D.A. Rasko, T.J. M. Wilson, M. Keelan and D.E. Taylor. Lewis antigen expression by *Helicobacter pylori.* In preparation.

D.A. Rasko, G. Wang, M.A. Monteiro, M.M. Palcic and D.E. Taylor. 2000. Synthesis of mono- and difucosylated Type 1 Lewis antigens by *Helicobacter pylori.* Submitted to European Journal of Biochemistry.

G. Wang, Z. Ge, **D.A. Rasko** and D.E.Taylor. In Press. Lewis antigens in *Helicobacter pylori*: Biosynthesis and phase variation. Molecular Microbiology.

D.A. Rasko, T. J. M. Wilson, D. Zopf and D.E. Taylor. 2000. *Helicobacter pylori* expression and stability of Lewis antigens in serial biopsies. Journal of Infectious Diseases. **181:** 1089-1095.

D.A. Rasko, G. Wang, M. Palcic and D.E. Taylor. 1999. Cloning and characterization of the α1,3/4 fucosyltransferase of *Helicobacter pylori.* Journal of Biological Chemistry. **275**(7):4988-4994.

M.A. Monteiro, B.J. Appelmelk, **D.A. Rasko**, S.O. Hynes, L.L. MaLean, K.H. Chan, F. St. Michael, S.M. Logan, J. O'Rourke, A. Lee, A.P. Moran, D.E. Taylor and M.B. Perry. 1999. Description of the lipopolysaccharide structures of *Helicobacter pylori* genomic strains 26695 and J99, of the *H. pylori* mouse model Sydney strain, of *H. pylori* strain P466 carrying a sialyl Lewis X antigen, and of the *H. pylori* strain UA915 expressing a Lewis B epitope: Classification of the *H. pylori* lipopolysaccharide into glycotype families. European Journal of Biochemistry. **267:** 305-320.

G. Wang, **D.A. Rasko**, R. Sherburne and D.E. Taylor. 1999. Molecular genetic basis for the variable expression of the Lewis Y antigen in *Helicobacter pylori* : analysis of the α(1,2) fucosyltransferase gene. Molecular Microbiology. **31**(4):1265-1274.

D.E. Taylor, **D.A. Rasko**, R. Sherburne, C. Ho and L.D. Jewel. 1998. Lack of correlation between Lewis antigen expression by *Helicobacter pylori* and gastric epithelial cells in infected patients. Gastroenterology. **115:**1113-1122.

M.A. Monteiro, K.H.N. Chan, **D.A. Rasko**, D.E. Taylor, P.Y. Zheng, B.J. Appelmelk, H-P. Wirth, M. Yang, M.J. Blaser, S.O. Hynes, A.P. Moran, and M.B. Perry. 1998. Simultaneous expression of type 1 and type 2 Lewis Blood group antigens by *Helicobacter pylori* lipopolysaccharides: molecular mimicry between *H. pylori* lipopolysaccharides and human gastric epithelial cell surface glycoforms. Journal of Biological Chemistry. **273**(19):11533-11543.

M.A. Monteiro, **D. Rasko**, D.E. Taylor, and M. B. Perry. 1998. Glucosylated *N*-acetyllactosamine O-antigen chain in the lipopolysaccharide from *Helicobacter pylori* strain UA861. Glycobiology. **8**(1):107-112.

Posters

D.A. Rasko, G. Wang, M. Palcic and D.E. Taylor. 1999. Cloning and characterization of an α1,3/4 fucosyltransferase of *Helicobacter pylori*. 10th International Workshop on *Campylobacter*, *Helicobacter* and related organisms. Abstract HP30.

D.A. Rasko, T. J. M. Wilson, D. Zopf and D.E. Taylor. 1999. In vivo Lewis antigen switching by *Helicobacter pylori* 10th International Workshop on *Campylobacter*, *Helicobacter* and related organisms. Abstract HP31.

D.A. Rasko, T.J.M. Wilson, D. Zopf and D.E. Taylor. 1999. *Helicobacter pylori* Lewis antigenic switching in response to carbohydrate therapy. ASM - A Cell Biology Approach to Bacterial Pathogenesis. Abstract #14.

G. Wang, **D.A. Rasko**, P.G. Boulton, R. Sherburne, M.M. Palcic and D.E. Taylor. 1999. Biosynthesis of Lewis Y in *Helicobacter pylori*. Canadian Bacterial Diseases Network General Meeting.

M.A. Monteiro, **D.A. Rasko**, D.E. Taylor, P.Y. Zheng, B.J. Appelmelk, H-P. Wirth, M. Yang, M.J. Blaser, A.P. Moran, and M.B. Perry. 1998. Simultaneous expression of type 1 and type 2 Lewis Blood group antigens by *Helicobacter pylori* lipopolysaccharides. 3rd International Workshop on Pathogenesis and Host response in Helicobacter Infections. Abstract K4.

D.E. Taylor, **D.A. Rasko**, R. Sherburne, C. Ho and L.D. Jewel. 1998. Expression of Lewis antigens in gastric biopsy tissue and comparison with Lewis antigens produced by isolated *Helicobacter pylori* from the same biopsy. 3rd International Workshop on Pathogenesis and Host response in Helicobacter Infections. Abstract K5.

D.A. Rasko, C.M. Szymanski, G.D. Armstrong and D.E. Taylor. (1997). Binding of *Helicobacter pylori* to Lewis Antigens. Second European Meeting on Pathogenesis and Host Response in *Helicobacter pylori* Infections. Irish Journal of Medical Science Vol 166: Supplemental 3. Abstract P40 page 25.

D.E. Taylor, R. Sherburne, and **D.A. Rasko**. (1996). Growth Phase Dependent Production of Lewis X by *Helicobacter pylori*. GUT :34(supplement).

D.A. Rasko, D.E. Taylor, and R. Sherburne. (1996). Growth Phase Dependent Production of Lewis X by *Helicobacter pylori*. UAUC International Conference on Infectious Diseases (Abstract and poster #7B) and Canadian Bacterial Diseases Network General Meeting (Abstract and poster #20).

R. Sherburne, **D.A. Rasko** and D.E. Taylor. (1996). Growth Phase Dependent Production of Lewis X by *Helicobacter pylori*. Canadian Society of Microbiology Meeting. Prince Edward Island.

Awards

1997

FEMS Young Scientist Award

J. Gordin Kaplan Graduate Student Award - Awarded from the University of Alberta, Faculty of Graduate Studies.

1998

Doctoral Research Award from Medical Research Council of Canada (1998-2001)

Alberta Heritage Foundation for Medical Research Studentship (1998-2000)

Walter H. Johns Graduate Fellowship from the University of Alberta (1998)

1999

10th International Workshop on *Campylobacter*, *Helicobacter* and related organisms - Young Scientist Award for Abstracts submitted.

ASM - A Cell Biology Approach to Bacterial Pathogenesis - Student Travel Grant.

Alberta Heritage Foundation for Medical Research Incentive Award

Walter H. Johns Graduate Fellowship from the University of Alberta

Doctoral Research Award from Medical Research Council of Canada (1998-2001)

Classwork

Courses Taken	Grade Received
Physiology 401	8
Medical Microbiology and Infectious Diseases 415	9
Medical Microbiology and Infectious Diseases 520	9
Medical Microbiology and Infectious Diseases 405	8
Medical Microbiology and Infectious Diseases 425	8