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Evidence for Suspended Mismatch Repair During Adaptive Mutation in *Escherichia coli*

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Evidence for Suspended Mismatch Repair During Adaptive Mutation in *Escherichia coli* submitted by Simonne Caroline Longerich in partial fulfillment of the requirements for the degree of Master of Science.

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"He that will avoid trouble must avoid the world."

Robert Burton

Abstract

This thesis provides three types of evidence supporting a novel molecular mechanism for recombination-dependent adaptive reversion of a +1 frameshift mutation in a *lac* gene in *Escherichia coli*, and provides important clues about involvement of post-synthesis mismatch repair (MMR): (i) adaptive reversion sequences are almost all -1 deletions in mononucleotide repeats, whereas growth-dependent reversion sequences are highly heterogeneous; (ii) the sequence spectrum for adaptive *lac* reversion is reproduced during growth if MMR is disabled. These results imply that adaptive mutations form in the absence of functional MMR. MMR could be down-regulated during adaptive mutation in this system. Additionally, a set of plasmids are constructed for use in experiments that test whether the frequency of adaptive reversion to Lac^+ can be depressed by overproducing two mismatch repair proteins, MutS and MutL, in adaptively mutating cells.

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1. INTRODUCTION

Debate over an old question, believed for decades to be solved, has re-emerged recently. The question bears on how mutations form when they are beneficial to the survival of an organism. Popular neo-Darwinian theory states that mutations form randomly, prior to, and independently of an organism's exposure to a selective environment (1, and see 2). Once mutations are made, the selective environment favors the survival of useful mutations only (natural selection). The opposing Lamarckian view is that mutations can be adaptive, their formation being nonrandom and specifically induced, rather than selected, by environmental challenge (3, and see 1).

That a neo-Darwinian explanation (4, and see 1) holds true for bacteria, at least under some circumstances, was demonstrated elegantly by three classical experiments. Luria and Delbrück (5) grew parallel cultures from similar inocula of an *Escherichia coli* strain, B, which is sensitive to the lytic bacteriophage T1, and then plated them on bacteriophage T1-seeded agar plates. They reasoned that if rare spontaneous mutations occur during growth prior to plating, a wide distribution of numbers of mutants in parallel cultures would result because very early mutations would produce many mutant clones, whereas spontaneous mutations occurring later during growth would produce few mutant clones. If instead mutation to phage-resistance were adaptive, occurring only after exposure to bacteriophage, a narrower (Poisson) distribution of numbers of mutants per culture was expected. In a Poisson distribution, most cultures would have a mean number of mutants equal to the number of bacteria plated times the probability that a bacterium generates the necessary

mutation before dying. (A Poisson distribution thus describes the proportion of mutants expected during one generation of growth. Pre-plating mutants arising during multiple generations of growth were expected to produce a Luria-Delbrück distribution – a Poisson distribution modified to account for additional generations). The results of this experiment (see also 6) showed a large variation in number of bacteriophage T1-resistant mutant colonies appearing on the plates, thus establishing that mutations occur randomly during growth of the cultures prior to, and not after, bacteriophage exposure. Subsequently, the Lederbergs (7) invented a method of indirect selection by replica-plating bacterial lawns from a nonselective plate onto a plate selective for bacteriophage T1- or streptomycin-resistance. Because this technique selects siblings of cells pre-existing on the nonselective plate, they were able to show that cells that had never experienced the selective condition (resistant clones remaining on the nonselective plates, identifiable by their correlate position on the replica plate) acquire mutations to phage T1- or streptomycin resistance. A quantitative version of this experiment followed (8) which, together with the evidence above, established firmly that resistance mutations arise spontaneously during growth, prior to selection, and are inherited stably in the absence of selection. Thus, for the next half-century, the Darwin *contre* Lamarck debate was quelled by the establishment of a mutational paradigm: Spontaneous mutations were believed to be formed (i) only in dividing cells, most probably by error during DNA replication and (ii) independently of their usefulness. Regarding the first point, this was believed in part because, except for a few notable exceptions (9, 10, 11, 12), most measured spontaneous rates were generation-dependent, not time-dependent (13, 14). A popular

interpretation of this was that most spontaneous mutation rates result from DNA polymerase error during replication.

In 1988, a publication by Cairns *et al.* (15) sparked a renewal of the battle over whether Lamarckian mutations occur (*e. g.* 2, 16, 17, 18). Cairns *et al.* suggested that because the experiments of Luria and Delbrück (5) and others (6-8) used a lethal selection, which killed any cells not harboring the correct mutation almost immediately, they could not have detected adaptive evolution that requires sufficient experience of the environmental challenge (see also 19). Furthermore, because the phenotypes of phage- and streptomycin-resistance do not appear until several generations after the mutation arises (20), these mutations must have occurred prior to selection. Thus, although early experiments established that some mutations form spontaneously and independently of the selection, they could not exclude the possibility that mutations might also be selection-induced. Max Delbrück seems to have recognized this limitation when he commented:

> "I would ... like to make a point with respect to the use of the word 'spontaneous' as applying to mutation. The word merely means that certain obvious factors which might be suspected of causing the mutations were shown to be without effect. ... It is then an obvious question to ask whether the environment not only selected but also caused the mutations. ... In view of our ignorance of the causes and mechanisms of mutations, one should keep in mind the possible occurrence of specifically induced adaptive mutations. (21)"

What Cairns *et al.* (15, and previously Ryan and colleagues 9, 10) discovered is that when *Escherichia coli* cells are subjected to prolonged nonlethal genetic selection, mutations arise -- (i) after exposure to the selection, (ii) in cells that appear not to be dividing, and (iii) can be found

only in genes whose functions were selected. These mutations, called adaptive mutations, are therefore different from random, spontaneous mutations in growing cells. (The name "adaptive" is used predominantly in the literature according to historical precedence (21) over names like "directed", "Cairnsian", and "selection-induced" mutation[1]).

Deviations from the classical mutation paradigm

Although the mutations described by Cairns *et al.* (15) sparked renewed debate over Lamarckism, previous and other reports of deviations from the Luria-Delbrück paradigm exist (reviewed by 24). For example, Ryan observed that His^+ reversion mutations in *E. coli* occurred both during growth, and continuously over ten days in nondividing, stationary-phase bacteria starved for histidine (11, 9). Mutants arising early (by 2-3 days) on media lacking histidine produced a clonal distribution resembling the Luria-Delbrück mutations that happened during growth prior to selection. However, mutants arising late (after 4 days) produced a nonclonal distribution, and therefore could have arisen among the nondividing bacteria during starvation (9). Numerous artifactual explanations for these observations were postulated, but were discarded following investigation. In a large series of experiments, Ryan accumulated evidence indicating that the His^+ revertants arising during

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[1] Although these names have been used interchangeably in the literature to describe the same mutations, "directed" implies a mechanism whereby the mutations are specifically targeted to the appropriate gene(s) by each unique environmental challenge. "Adaptive" (and "selection-induced") implies only that mutations form in response to the environmental challenge. Because no-one has demonstrated properly that mutants arising after exposure to the selection have not accumulated mutations in unselected genes (but see 22), the name "directed" seems presumptuous. We favor the acronym SLAM (for stressful lifestyle-associated mutations) even over "adaptive" (23), because it does not invoke any specific role for the selection other than to impose stress which is mutagenic by a mechanism not specified in the name.

starvation were not the result of a population of slow-growing mutants, phenotypic lag (9), cell growth, cryptic growth in which every new cell arises at the expense of one dead cell (25), or DNA replication without concomitant cell division (26, 27). Ryan hypothesized finally that the late-arising mutations result either from a "minute" amount of highly error-prone DNA synthesis, but not full replication, or from starvation-induced, unusual base-pair substitutions (27).

Two decades later, Shapiro documented the first thorough examination of genetic changes that occur during selection, but do not occur at a detectable frequency during growth in the absence of selection (19). He studied a strain of *E. coli* that contains the regulatory region of the arabinose operon fused to *lacZ* by an intervening Mu prophage with a temperature-sensitive repressor. Cells in which *ara* becomes fused in-frame to *lacZ* by excision of the intervening Mu segment can utilize lactose to grow only if arabinose is present to induce transcription, and are designated Lac(Ara)$^+$. Cells carrying a nonexcised Mu prophage are both Ara$^-$ and Lac$^-$ [Lac(Ara)$^-$], but produced Lac(Ara)$^+$ colonies after a four to 19 day delay after plating on minimal lactose-arabinose media at 32°C. Thereafter, colonies continued to appear with an increasing rate over time. Lac(Ara)$^+$ mutants arose at a minimum estimated frequency of 1 X 10^{-8} per cell during selection, but were undetectable during nonselective growth prior to plating (<2 X 10^{-10} per cell, 19), suggesting that fusion formation is induced either by starvation, or the selection. The rate-limiting step in appearance of late-arising Lac(Ara)$^+$ colonies is the genetic change, the Mu deletion event, because previously-isolated Lac(Ara)$^+$ cells, and cells into which an intact *araB-lacZ* fusion is transduced, can form colonies within two days on minimal arabinose-lactose medium

(19). Finally, fusion mutants did not arise during starvation in saline solution in the absence of arabinose and lactose. Therefore, *araB-lacZ* fusion mutants appeared only when selected-for in starving cells, and not during starvation *per se*[2]. Shapiro suggested that mutations like Mu deletions arising during nonlethal selective conditions probably abound in nature, but were not found in the classical experiments (5-8) because lethal selections were used which permitted observation of only those mutants occurring prior to selection (19).

The publication by Cairns *et al.* (15) was an attempt to unite various observations of apparent deviations from the mutation paradigm as examples of adaptive mutation. The evidence for adaptive mutation provided by Cairns *et al.* is threefold. They first repeated an experiment performed originally by Ryan (32) in which *Escherichia coli* cells unable to ferment lactose are grown to saturation in complete liquid media, and then spread on minimal-lactose plates. Both Ryan (32) and Cairns *et al.* (15) observed that the resulting distribution of mutants per culture was much narrower than the Luria-Delbrück distribution expected (as calculated by 33). Cairns *et al.* showed that Lac$^+$ revertants of a strain carrying a *lacZ* amber mutation produce a distribution resembling a composite distribution which is generated if mutations form both during growth (to give a Luria-Delbrück distribution--a Poisson distribution modified to represent subsequent generations), and also during stationary phase (to give a Poisson distribution of mutant colonies on plates). The unmodified Poisson part of the composite distribution was caused by Lac$^+$

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2 Further investigations using this system have demonstrated that Lac(Ara)$^+$ mutants do arise under starvation conditions in the absence of arabinose and lactose, but the ones that do have different sized deletions than those arising under selection (28, 29, 30). Furthermore, this system seems to be dependent on Mu transposition functions (31).

colonies that appeared more slowly than Lac$^+$ colonies arising from mutants pre-existing in the culture, suggesting that late-arising colonies arose after arrival on minimal-lactose plates. Furthermore, the late-arising mutant colonies that appeared over days were not produced when Lac$^-$ cells were plated on minimal medium without lactose, but occurred with the same delay after the plates were overlaid with lactose as had been seen when plating directly onto lactose. Thus, appearance of the stationary-phase Lac$^+$ mutants is dependent on the presence of lactose, and not starvation conditions alone. The authors also attempted to show that stationary-phase cells under selection for Lac$^+$ do not accumulate additional, nonadvantageous mutations as measured by failure to make mutations to valine-resistance over time on minimal-lactose plates[3]. These results suggested to the authors that "populations of bacteria, in stationary phase, have some way of producing (or selectively retaining) only the most appropriate mutations" (15).

In a second experiment, Cairns *et al.* used the *araB-lacZ* fusion system described by Shapiro (above, 18). In addition to confirming the results obtained by Shapiro, they also showed that Lac(Ara)$^+$ mutants do not arise in rich media lacking either arabinose or lactose. However, following addition of lactose to a stationary phase culture containing arabinose but not lactose, Lac(Ara)$^+$ mutants begin to accumulate (15). Thus, *araB-lacZ* fusion mutations seemed to be adaptive in that the

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[3] Mutation to valine-resistance has been disputed however as a special case because its expression is dependent on numerous environmental and physiological factors (29, 23). These experiments have been criticized also because valine-resistance was selected in the presence of glucose which is known to suppress mutagenesis (30) by a mechanism now emerging as catabolite repression of a starvation-induced SOS response (31). Therefore, experiments in which valine-resistance was tested as an unselected mutation may not have demonstrated that nonadvantageous mutations do not occur under selection.

selective agent(s) appeared to be required for mutation to Lac(Ara)$^+$ (but see footnote 2 above).

As a third potential example of adaptive mutation in *E. coli*, Cairns *et al.* describe how cells carrying a deletion in the *lacZ* gene, encoding ß-galactosidase, acquire the ability to grow on media containing lactose as the sole carbon source. In fact, *E. coli* harbors a cryptic gene, *ebgA°*, which can be activated transcriptionally by two point mutations (one in a repressor encoded by *ebgR*, and one in *ebgA*). *ebgA*$^+$ encodes a unique lactose-hydrolyzing enzyme (37). Although each point mutation occurs at $<10^{-8}$ per cell division during growth, Lac$^+$ mutants arise after about two weeks in stationary phase on plates containing lactose. Activation of a cryptic gene, *ebgA°*, for use of an otherwise nonhydrolyzable sugar was thus presented as a third piece of evidence by which stationary-phase cells apparently can produce the appropriate mutations for survival of environmental challenge.

Shortly after the report by Cairns *et al.*, Hall reported another example of enhanced excision of a mobile DNA element during selection (38). In this case, the selection was for utilization of the ß-glucoside salicin on MacConkey media. The *bglF*$^+$ gene encodes a ß-glucoside transport protein required for salicin catabolism (39). The rate of excision of an insertion sequence, IS*103*, from within the *bglF* gene was $<2 \times 10^{-12}$ per cell division during unselected growth, but $>10^{-2}$ per cell when excision was advantageous for growth under selection (38). Sal$^+$ mutants were not detected among approximately 3×10^{12} cells incubated for four weeks on MacConkey medium without salicin, suggesting that IS*103* excision is not induced in the absence of selection (24). Therefore, enhanced excision of a

mobile DNA element when advantageous seems not to be unique to Mu DNA.

In a subsequent publication, Hall also showed that base substitution mutations to Trp^+ occur more frequently when they are required for growth (22). Trp^- *E. coli* bacteria plated on tryptophan-limited medium grow into colonies of limited size and then stop dividing. Trp^+ mutants accumulate within these nongrowing Trp^- colonies at an increasing frequency during a few weeks of incubation. A 3- to 30-fold increase over growth-dependent reversion to Trp^+ was seen during starvation. *trp* reversion in (apparently) nondividing cells happened only when tryptophan, but not another amino acid, was depleted and unselected mutations to valine-resistance and lactose-fermentation did not accumulate among the cells under selection. However, when Trp^+ mutants were screened for additional auxotrophic mutations, Hall found 18 times more auxotrophic mutants among the Trp^+ revertants than among other Trp^- cells in the colony. The occurrence of two mutations (*trp* reversion and one auxotrophic mutation) was 600-fold greater than expected if each mutation were an independent event. To explain these results, Hall hypothesized that a sub-population of cells under selection pressure enter a "hypermutable state" in which mutations are made at a high frequency genome-wide. In this case, Trp^+ revertants would be expected to have a higher proportion of unselected mutations than other cells in the colony which never experienced hypermutation (22). If adaptive mutations are generated during such a hypermutable state, their formation would be random, not directed, and thus would appear to fit with Darwinian ideas.

The studies discussed above are important because they were the first to show that mutations occur (i) in the apparent absence of cell division in cells surviving in nutritionally-depleted environments; (ii) at higher (or detectable) frequencies when they are useful for growth; (iii) under starvation conditions only when the selective agent is present; and (iv) are detected exclusively in genes whose functions were selected (except by 22).

Numerous other reports of adaptive mutations, in both prokaryotes (reviewed in 24) and yeast (40, 41), followed those described above. However, despite the accumulating evidence for their existence, the possibility that adaptive mutations might be Lamarckian provoked controversy (*e. g.* 2, 16, 17, 18). The controversy developed partly for historical and sociological reasons (42, 43), partly because of artifactual explanations for observations in some reports (44, 2, 45) and partly, because of the failure to demonstrate a whole or partial molecular mechanism for how adaptive mutations form (46, 43, 47, 48). The controversy over the latter point has been subdued recently because of studies that demonstrate a novel molecular mechanism for the formation of adaptive mutations in one experimental system in *E. coli*.

The assay system

The assay system described by Cairns and Foster (47) is the same system used in all experiments presented in this thesis. In this system, adaptive mutations are measured as reversion to lactose utilization in a particular *lac* strain of *E. coli* K-12. This strain is deleted for the chromosomal *lac-pro* region, but carries a +1 frameshift mutation (CCC to CCCC at position 1039 of *lacI* that is polar on *lacZ*) in a *lacIΩlacZ* fusion

gene located on an F' episome. Transcription is initiated constitutively from the *lacI*^{q} promoter. These bacteria generate Lac^{+} mutant colonies when placed on medium containing lactose as the sole carbon source. The lactose plates also contain an excess of nonrevertible "scavenger" cells added to consume any nonlactose, contaminating sources of carbon. Lac^{+} colonies appearing on day 2 after plating are composed mostly of growth-dependent (Luria-Delbrück) mutants that arise before plating. However, after day 2, Lac^{+} colonies appear continuously over time, and are scored daily. The majority of the late-arising mutants probably form after arriving on lactose media because their distribution tends more towards the Poisson (not modified by growth of clones) at later times after plating (47). As a result, 90-95% of the total number of Lac^{+} mutations scored during the first week after plating happen in apparently nondividing cells under selection (47, 49). The population of *lac* frameshift-bearing cells does not increase or decrease over the course of the experiment as monitored by removing a small plug of agar from between visible Lac^{+} colonies on different days, suspending in liquid, and then plating an aliquot of suspended bacteria on rich media (47). The minimal lactose medium on which cells are plated supports neither apparent growth, nor cryptic growth, of the *lac* frameshift-bearing cells (49). The selective conditions also appear not to be generally mutagenic, because an unselected mutant does not accumulate during selection (49). Lac^{+} mutants do not appear when cells are starved on a similar medium without lactose (47).

This system provides a convenient way to quantify the ability of cells to mutate adaptively, and is thus extremely useful for testing the requirement for specific gene products in adaptive mutagenesis.

Demonstrating that some gene products are required for adaptive, but not growth-dependent spontaneous mutation has led to the elucidation of part of the molecular mechanism that generates adaptive mutations (see below).

Recombination in adaptive mutation

In dividing cells, DNA replication error has been assumed, with exception (see above), to be the source of all spontaneous mutations. For adaptive mutations arising in nondividing cells, in which DNA may not be replicating, another mechanism of mutation was proposed (50). The idea was that perhaps genetic recombination is used to transfer similar but not identical sequence from elsewhere in the genome into the mutant *lacI* gene to restore function. This mechanism is analogous to somatic hypermutation in chicken immunoglobulin genes. In chicken immature B cells, V-region diversity is achieved by recombination between the active *V*-gene, and several "templates" -- partially homologous pseudo-*V* genes located at a separate locus (51). That adaptive reversion might occur using such a recombinational mechanism, is supported by the finding that both functional RecA (47, 52) and RecBC(D) (52) proteins are essential for adaptive mutation in the *lac*-frameshift system. Both RecA and RecBCD proteins are required for recombination in *E. coli* (reviewed by 53).

Besides functioning in recombination, RecA has numerous other activities (reviewed by 53). RecA was tested originally in adaptive mutation for its role in inducing the SOS response (47). The SOS system in *E. coli* is an inducible DNA damage repair regulon (reviewed by 54). RecA protein is activated by an inducing signal, probably single-stranded DNA, to become a co-protease which helps cleave the LexA protein, a

repressor of various downstream genes (including *recA*) whose products function in DNA damage repair. A *recA* derivative of the *lac* frameshift-bearing strain that lacks all RecA co-protease activity, but is partially functional for recombination, is almost completely defective for adaptive mutagenesis (47). A *recA* null mutant cannot mutate adaptively at all (52). Lac^+ reversion rates of *recA* cells during growth are unaffected when compared with $recA^+$ cells (47, 52). Presence of a noncleavable mutant LexA repressor reduced levels of adaptive mutation roughly three-fold, however normal levels of adaptive reversion to Lac^+ could be restored by constitutive (nonrepressed) expression of *recA*. Thus, LexA cleavage seemed to be required only to achieve high levels of *recA* expression, and not de-repression of any other gene(s) in the SOS regulon (47). This observation has been reported recently to be erroneous (55, R. S. Harris and S. M. Rosenberg, personal communication). The supposed $recA^{oc}$ (operator-constitutive) *lexA3* (noncleavable LexA) derivative of the *lac* frameshift-bearing strain was found to be $lexA^+$. In fact, to achieve full levels of adaptive Lac reversion, at least one additional LexA-regulated gene, besides $recA^+$, must be activated by LexA cleavage. Nevertheless, the early results suggested that other functions of RecA besides its co-protease activity, are important for adaptive mutation. One important function is RecA's activity as a strand exchange protein in recombination (52).

The requirement for functional RecBC(D) enzyme for adaptive reversion (52) in *lac* provided further evidence in support of a recombinational mechanism for adaptive mutation. The RecBCD enzyme, also known as exonuclease V, is a heteromultimeric protein that has both helicase and single-strand endonuclease activity which constitute

its double-strand exonuclease activity (56). A model for how the RecBCD enzyme functions in recombination follows (57): RecBCD loads onto DNA exclusively at double-strand breaks and travels along the DNA as exonuclease V. When the RecBCD enzyme reaches a particular nucleotide sequence called Chi, a recombination hotspot, activity of the RecD subunit of RecBCD is proposed to be lost. The RecD subunit is required for RecBCD nuclease activity, and *recD* null mutants have a hyper-recombinagenic phenotype (58-60). The absence of RecD function might cause hyper-recombination because the RecBC(D^-) enzyme may retain only helicase activity for unwinding DNA to produce single-strand ends of opposite polarities (see 61, 62). These single-strand ends could be coated with RecA to promote strand exchange with a homologous (or partially homologous) DNA sequence.

The requirements for RecBCD-mediated recombination parallel those of adaptive mutation (52). Null mutations in *recB* abolish adaptive reversion to Lac^+, and also result in an inability to recombine, whereas a *recD* null mutant is hyper-mutable for adaptive mutation, and also hyper-recombinagenic. Mutations in *recB* and *recD* do not affect growth-dependent mutation rates to Lac^+ as compared with rec^+ strains. More recently, recombination proteins that process strand exchange recombination intermediates (Holliday junctions) have also been shown to be required for adaptive *lac* reversion (55, 63).

The requirement for recombination functions of RecA, RecBC(D), and Holliday junction processing enzymes (55, 63) for adaptive mutation, but not for mutations occurring during growth, indicates that the molecular mechanism by which these two types of mutations form are different, and implies that recombination is part of a novel molecular

mechanism by which the adaptive mutations form. We imagined two ways in which recombination could generate reversion mutations in *lacI* in nondividing cells (52). The first was the templated model described above in which *lacI* reversion occurs by transfer of partially homologous sequence "templates" located elsewhere into *lacI* by recombination (50, 52, 16[Grafen]). Recombination between partially identical DNAs is normally inhibited by the methyl-directed mismatch repair system (64, reviewed by 65, and see below). This inhibition was proposed to be removed by shutting off mismatch repair in cells that are mutating adaptively (50). We also imagined a second, nontemplated mechanism in which the mutations are made by polymerase errors during DNA synthesis associated with recombination (52 and references therein, Chapter 2).

The work presented in this thesis provides three types of further evidence supporting a novel molecular mechanism for adaptive mutation in the *lac* system, and provides important clues about one particular aspect of the mechanism, involvement of methyl-directed mismatch repair.

MutHLSU mismatch repair in *E. coli*

MutHLSU mismatch repair (reviewed by 65) acts to correct mismatched base pairs that result from DNA biosynthetic errors, and from heteroduplex DNA produced during recombination between partially identical sequences. Mismatch correction involves at least ten proteins: MutS, MutL, MutH, MutU (DNA helicase II), single-strand DNA binding protein (SSB), Exonuclease I, Exonuclease VII, RecJ exonuclease (but see 66), DNA Polymerase III holoenzyme, and DNA ligase. The four *mut*$^+$ gene products are essential for this mismatch repair system. Mutations in any one of the *mut* genes results in a 100- to 1000-fold increase in the rate

of spontaneous mutation, which roughly corresponds to the *in vitro* error rate of DNA polymerase III (65). MutS, MutL and MutH, are required for the initial steps of mismatch correction. MutS recognizes and binds directly to transition mispairs, transversion mispairs, or mismatches that are created by insertion/deletion of one to four nucleotides (67). *In vitro*, MutL binds to a complex of MutS bound to DNA when ATP is present (68), and although the function of MutL is unknown, it is proposed to coordinate function of MutS and MutH (65). Both MutH, and the Dam (DNA adenine) methylase responsible for d(GATC) methylation, confer strand discrimination during mismatch repair. (Thus, *dam* null mutants, like *mut*-deficient cells, have an elevated spontaneous mutation frequency). During DNA synthesis, Dam methylase is slow to act such that newly-synthesized DNA remains transiently unmethylated. MutH is an endonuclease that specifically cleaves nonmethylated strand(s) at d(GATC) sequences either 5' or 3' to MutS-, MutL-bound mismatches. During replication d(GATC) sites are hemi-methylated; the old strand is methylated and the new strand is not. The ensuing mismatch correction is thus directed to the new DNA strand, not the template. Removal of the nicked strand probably occurs by unwinding of the DNA helix by DNA helicase II (MutU) (69), displacing single-stranded DNA. Such DNA may be degraded by single-strand dependent exonucleases (66). DNA Polymerase III holoenzyme has the ability to perform resynthesis of the excised strand (70). Because the excision repair tract can be $\geq$ 1000 basepairs (71), the Mut system is known as a long-patch repair pathway.

The Mut system also governs the fidelity of genetic recombination. Normally, DNA sequences that are different by as little as 10-20% do not recombine, even though, *in vitro*, RecA can promote strand transfer

between sequences that are ~30% divergent (72). In mismatch repair-defective mutants, this barrier to recombination between partially homologous (homeologous) DNAs is relaxed. Interspecies recombination between *E. coli* and Salmonella, which share ~80% homology in DNA sequence, increases by one to three orders of magnitude when mismatch repair is inactivated by mutation in one of the four *mut* genes (64). Intrachromosomal duplications formed by recombination between diverged sequences also increase in frequency in the absence of functional Mut proteins (73). In both cases, absence of *mutS* and *mutL* gene products has the greatest effect on increasing the frequency of homeologous recombination. *In vitro,* MutS protein inhibits RecA-catalyzed strand transfer between 97% identical DNAs (74). The inhibitory effect of MutS in this assay is enhanced by the addition of MutL protein (74). The observations reported by Worth *et al.* (74) imply additionally that MutS plus MutL can block the branch migration stage of RecA-catalyzed strand transfer (74).

Mismatch repair functions to maintain the fidelity of metabolic processes in DNA that might otherwise result in genomic instability. It would be important to discover whether mismatch repair function is itself regulated, for example by the physiological state of a cell. If it is regulated, such a discovery would have profound implications for how mutations are thought to form during evolution, and during normal (*e. g.* 50) and abnormal (*e. g.* cancer, see 64) developmental processes in microbes, and in multicellular organisms.

Mismatch repair in adaptive mutation?

The aim of this thesis study was to address two questions: First, are *lac* frameshift adaptive reversions templated or nontemplated? And second, using the assay described, does decreased mismatch repair lead to formation of adaptive reversion mutations in the *lac* assay system described by Cairns and Foster (47, see above)? The possibility that MutHLSU mismatch repair might be down-regulated during adaptive mutation has been suggested in the context of various different mechanistic models for how adaptive mutations form (75, 76, 50, see also 77). Although a number of these models have since been rejected on the basis of their mechanistic context (*e. g.* 78, 46, 75, 50), this thesis study provides two types of evidence in support of suspended mismatch repair during adaptive mutation in *E. coli.* In addition, a third piece of evidence is presented which was obtained by other investigators using a set of plasmids whose construction is described in this thesis.

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2. ADAPTIVE MUTATION BY DELETIONS IN SMALL MONONUCLEOTIDE REPEATS*

A new process of mutation has been discovered that differs in multiple respects from mutations in growing cells (1). The mutations occur only after exposure to a nonlethal genetic selection (2-7), in the apparent absence of cell division (3-5, 7), and have been termed adaptive because their formation has been detected in the genes whose function was selected but not in irrelevant genes (2, 4) [but see (7)]. Adaptive reversion of a *lacZ* frameshift mutation in *E. coli* requires a distinct group of DNA metabolism genes -- the genes for homologous recombination, which are not required for growth-dependent mutation (3, 8). This requirement implies that a different molecular mechanism generates the adaptive mutations, a mechanism that involves recombination.

Possible recombinational mechanisms for adaptive mutation can be divided into two broad classes (8). In the first, templated mutation, blocks of information from preexisting DNA sequences that are similar to those of the mutating gene (templates) are transferred into the gene by recombination (9-11). The preexisting templates need be only partially homologous to the mutating gene (9). In the second class, nontemplated mutation, recombination is required but the mutant sequences occur *de novo* (2, 4, 5, 8, 12, 13). For example, the mutations could result from errors made either by an RNA polymerase (2, 12) or by a DNA polymerase (8, 13), with the errors either indirectly (2, 12, 13) or directly (8) associated with recombination. These models are distinguishable by the sequences of

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the reversion mutations that each predicts (8). If mutations were templated from somewhat similar sequences, then revertants should usually have the same reversion mutation sequence or a small subset of sequences, because few suitable templates would be available. Also, a distinctive pattern of co-transfer of extraneous nucleotide changes near the reversion mutations could occur if the information-donor template or templates were not perfectly identical to the *lac* gene. In chicken immunoglobulin genes, in which somatic hypermutation occurs by this mechanism, such a pattern has been observed (14). If mutant sequences were formed *de novo* and not templated, then the mutations would be expected to occur anywhere in a region that is capable of restoring the correct reading frame for *lacZ* function. Also, only very rarely would they include any extraneous nucleotide changes. These predictions were distinguished by the sequencing of a 276-base pair (bp) region spanning the *lac*$^-$ +1 frameshift mutation among intragenic *lac*$^+$ adaptive revertants (15).

Post-selection reversion mutations of a frameshift mutation (CCC to CCCC) in the *E. coli lacI-lacZ* fusion gene (3) were isolated as Lac$^+$ colonies, arising on days 4 through 6 after plating, as previously described (3, 4, 8) and purified by streaking on minimal lactose medium (8). Figure 1 shows the sequence of a 276-bp region of DNA spanning the frameshift mutation. This region from Lac$^+$ isolates was amplified by polymerase chain reaction (PCR) and sequenced (16). Compensatory frameshift mutations capable of restoring function to the *lacZ* gene product would have to occur between the two stop codons boxed in Figure 1 to avoid creation of a truncated polypeptide chain, stopped in an incorrect reading frame. All of the *lac*$^+$ reversion mutations found are single base deletions

in the small mononucleotide repeats highlighted in Figure 1. Figure 2 shows the distribution of mutations at each site. No extraneous changes were found nearby in any of the adaptive mutants whose *lac* genes were sequenced. The observed spectrum of mutations is characteristic of polymerase errors (17).

We previously found that a hyperrecombinagenic *recD* mutant strain is adaptively hypermutable (8). The hypermutation in this strain appears to occur by the same mutational pathway as that in *rec*$^+$, because in both strains the hypermutation depends on functional *recA* and *recB* recombination genes (8). These dependencies imply that the *recD* mutation does not activate a new route to the formation of adaptive mutants but more intensively uses the same route that operates in *rec*$^+$ strains. Thus, a correlation exists between the ability to recombine DNA and the ability to generate adaptive mutants: The *recD* cells are hyperrecombinagenic (18) and for this reason are hypermutable (8). If our argument is correct, then a similar adaptive mutation spectrum would be seen in *recD* as is seen in *rec*$^+$. In *recD*, as in *rec*$^+$, the intragenic adaptive mutations (15) are only single base deletions, and nearly all occur in the regions of small mononucleotide repeats (Figures 1 and 2). Also, as in *rec*$^+$, no extraneous nucleotide changes were found within the region sequenced.

Spontaneous growth-dependent reversion mutations of the same *lac* frameshift mutation do not require recombination functions (3, 8) and thus presumably occur by a different mechanism, one that does not require recombination. The idea that the mechanisms are different is supported by the observation of a different and much more heterogeneous mutation spectrum in growing cells (Table 1). Whereas only one sort of

adaptive mutation is observed, the growth-dependent mutations include duplications, insertions, and deletions of from 1 to 112 nucleotides, with the smaller deletions not confined to mononucleotide repeats (Table 1). This suggests multiple mechanisms of growth-dependent mutation but a single mechanism of adaptive mutation.

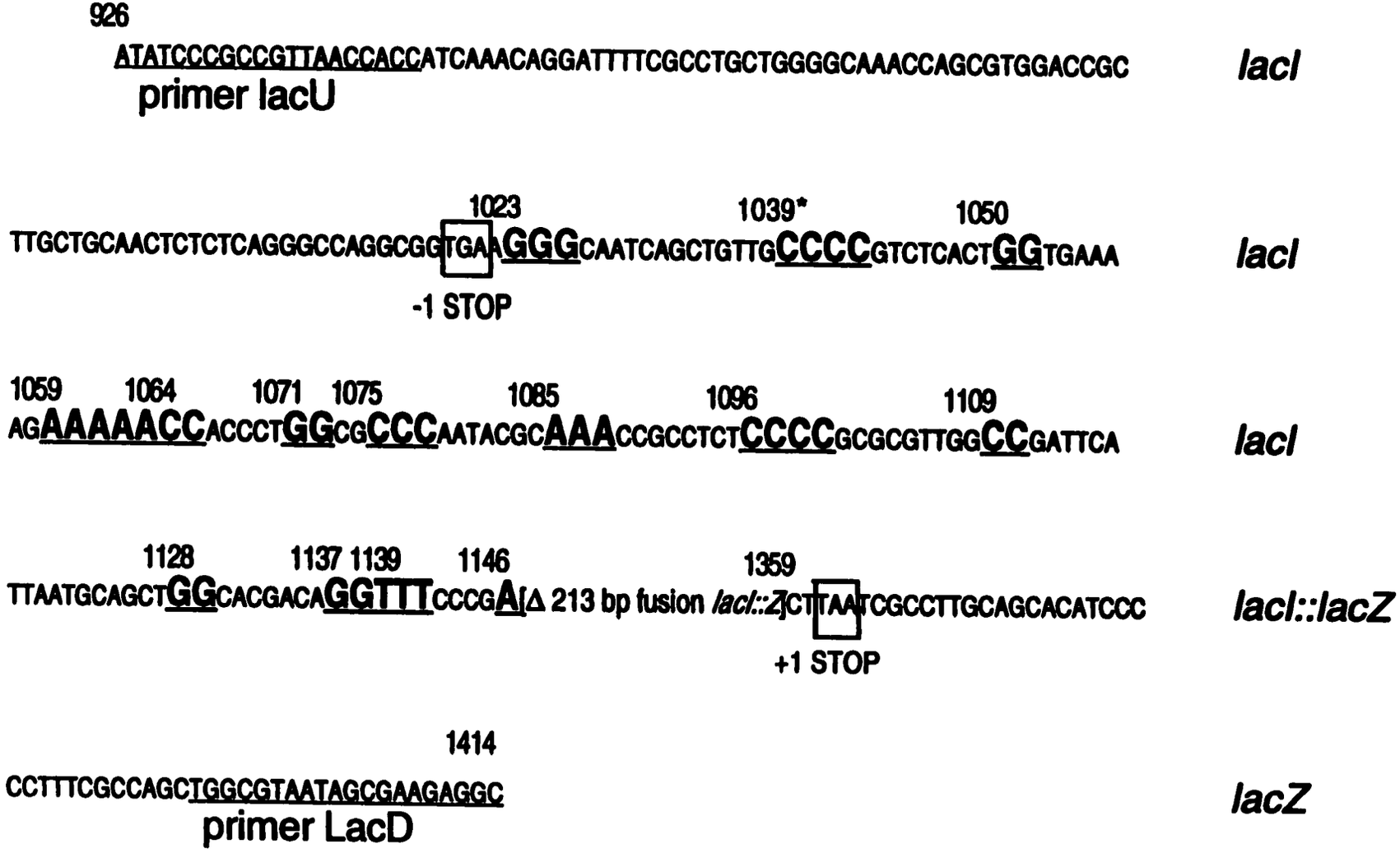
The adaptive mutations that restore Lac^+ function to the +1 frameshift mutation in *rec*$^+$ and *recD* strains are all single base deletions, almost all occurring in small mononucleotide repeats, and are not accompanied by nearby extraneous mutations. Mutations such as these are common errors of DNA polymerases (17), probably caused by slippage of the newly synthesized strand, which results in mispairing with the template during synthesis (19). These results support recombinational models for adaptive mutation that use polymerase error as the mechanism of formation of the mutation. Templated mutation models (9-11) are made improbable by these results.

Recombinational models have been proposed in which polymerase error during normal replicative synthesis (13) and during unusual DNA synthesis events (2, 4) is indirectly associated with recombination. In a model with direct association of synthesis and recombination, we have suggested that repair synthesis associated with recombination could be error-prone (8). This mutagenic recombination model is made tenable by observations of mutation that is associated with normal homologous recombination (20) or sex (or both) (21) in bacteria, yeast, and other fungi. In this model, hyperrecombination would yield hypermutation because of the increased opportunity for faulty synthesis. On the basis of our previous results, we argued that recombination is probably increased because of the presence of double-strand DNA breaks in adaptively

mutating cells (8). Here we modify this view to include a second parameter -- decreased post-synthesis mismatch repair -- that would produce the distinctive mutation spectrum observed and would further increase mutation.

The different mutation spectra for growth-dependent and adaptive mutations could be caused if the polymerase generating the adaptive mutations were different from the normal replicative polymerase and made different mistakes more frequently. However, a second, perhaps additional, explanation appears likely in view of the prevalence of the adaptive mutations in simple repeats. Template-slippage mutations in simple repeats are characteristic of yeast cells (22) and of hereditary colon cancer cells (23-25) that are deficient for post-synthesis mismatch repair. Thus, the hypermutability caused by apparent polymerase errors in *E. coli* cells exposed to selection could also indicate decreased mismatch repair (26). Down regulation of mismatch repair during adaptive mutation has been suggested (6, 9, 27) but not in a context of recombination-dependent, nontemplated adaptive mutations. In both the cancer cells and the in the bacteria exposed to nonlethal selection, the ability to mutate adaptively confers the ability to grow and divide. Mechanistic as well as formal similarities in the two processes may exist (28), which raises the possibility of bacterial model systems for mutagenesis in cancer.

Figure 2-1. The DNA sequence of a region of the *lacI-lacZ* fusion gene that was amplified by PCR. The mutant *lac* gene is the one used previously (3, 4, 8). The *lacI* gene is fused to *lacZ* at position 1146 so that a frameshift mutation of CCC to CCCC at position 1039 (mutation indicated by an asterisk) is polar on *lacZ*, making the cells Lac$^-$. Intragenic *lac*$^+$ reversion mutations could potentially restore the reading frame by deletion or addition anywhere between the two stop codons (boxed), which occur in incorrect reading frames, and would cause translation stops if a compensatory frameshift mutation were to occur before (first box) or after (second box). The region was amplified by PCR with the LacU and LacD primers indicated (16) and then sequenced (16). Adaptive revertants each possess only one single base deletion, all occurring at the underlined bases, all but one of which are small mononucleotide repeats. Nucleotide position numbers are those used by Miller (29), and the +1 frameshift mutation is not numbered.


926
ATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGC
lacI
primer lacU
1023
1039*
1050
TTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCCGTCTCACTGGTGAAA
lacI
-1 STOP
1059
1064
1071
1075
1085
1096
1109
AGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCA
lacI
1128
1137
1139
1146
1359
TTAATGCAGCTGGCACGACAGGTTTCCCGA[Δ 213 bp fusion lacI::Z]CTTAATCGCCTTGCAGCACATCCC
lacI::lacZ
+1 STOP
1414
CCTTTCGCCAGCTGGCGTAATAGCGAAGAGGC
lacZ
primer LacD

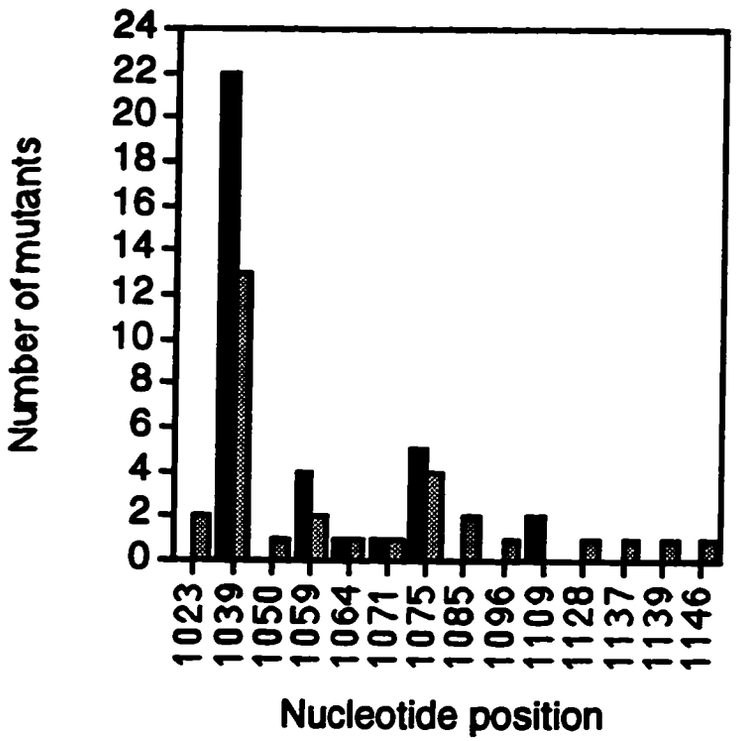


Figure 2-2. Positions of adaptive reversion, single base deletion mutations. Nucleotide positions are shown in Figure 2-1. Black bars indicate *rec*$^+$; gray bars indicate *recD*.

Table 2-1. Spectrum of growth-dependent mutations (30).

Nucleotide position	Strain	
	rec$^+$	*recD*
955		-73
968†		-92
972		-110
979†	-110	-112*
1014†		-13
1016†	-2 with -1 at 1022*	
1018		-24*
1019†		-1
1021		-4
1022		-1; -7‡; -19
1023	-1	-1
1030†		(-1) x 2
1035†	-1	-1; -43
1039	(-1) x 7	(-1) x 13
1042†		-4‡; CG to (CG)2
1043†	(TC)2 to (TC)3	-1
1044†		-1
1045†		-1
1048†		CT to (CT)2
1058†		-1
1059	-1	(-1) x 2; +2
1061		-13
1064		-1
1067	-1	

Table 2-1. (continued)

Nucleotide position	Strain *rec*$^+$	*recD*
1071		-1
1073†		-4
1075	(-1) x 2	(-1) x 5
1078		(-1) x 2
1085		+2; -1
1088		-1
1091	+5	
1092		[(CT)2 to (CT)3] x 2
1095†		-1
1096		-1; +2
1099	+8 (not mononucleotide); [(CG)3 to (CG)4] x 2	
1113	-1	
1117		duplication of 26 (1093-1117) beginning after 1117
1128		-1
1134†		-1
1137		(-1) x 3
1361	duplication of 67 (1082-1361) beginning after 1361	

*These deletions do not restore the correct reading frame, but do disrupt or delete the first stop codon indicated (Figure 2-1), and so are presumably accompanied by a different, frame-shifting mutation upstream of the sequenced region. †Not part of a mononucleotide repeat. ‡ Including bases not in repeat.

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15. The frameshift mutation resides in a *lac* operon that is present on an F' episome in tetracycline-sensitive cells with the chromosomal *lac* region deleted. We identified intragenic reversion mutations by mating the episome into a *lac*-deleted, tetracycline-resistant strain and assaying tetracycline-resistant conjugants for ß-galactosidase activity on X-Gal plates. Out of 109 adaptive revertants, none was found to be extragenic. An additional 10 *lac*$^+$ isolates were sequenced directly, without mating, and all but one (a *recD* isolate) were also found, by sequencing, to be intragenic mutations.

16. Synthetic oligonucleotide primers corresponding to the sequence indicated as primer LacU and complementary to the sequence indicated as primer LacD (Figure 1) (synthesized on an ABI Model 392 Synthesizer, Applied Biosystems, Foster City, CA) were used in asymmetric PCR to amplify single-strand DNA from the 276-bp region for sequencing. The PCR solution consisted of 50 mM KCl, 10 mM tris-HCl (pH 8.6), 2.5 mM MgCl, bovine serum albumin (150 µg/ml), 400 µM deoxynucleoside triphosphates, one primer at 1µM and the other primer at 0.01 µM, and 1.25 units of Taq Polymerase in a total volume of 50 µl, 2 µl of which consisted of bacterial cells suspended in water. These were cycled 40 times at 94°C for 1 min., at 50°C for 1 min., and at 72°C for 1 min. in a RoboCycler 40 (Stratagene). The PCR

products were purified in G50 Spin Columns (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's instructions, primers were removed by isopropanol precipitation followed by a 70% ethanol wash, and products were then sequenced either with the Sequenase 2.0 kit (U. S. Biochemicals) according to the manufacturer's instructions or in an ABI Model 373 Sequencer (Applied Biosystems) with the use of dye-terminator nucleotides and with the prmer used in the PCR at 0.01 μM used to prime the sequencing reactions.

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26. Repeat instability in colon cancers has been shown to occur in mono- (24) and di-nucleotide repeats (23), as deletions only (24), and as deletions and insertions (23). Our selection for reversion of a +1 frameshift mutation could have revealed +2 insertions in di-nucleotide repeats as well as the -1 deletions in mononucleotide repeats, but the former were not observed among adaptive revertants. This would be expected if deletions were a more common template-slippage error in *E. coli* than were insertions or could be due to the presence of more mononucleotide repeats than di-nucleotide repeats in the region (Figure 1). In the human cancer cells and yeast cells that are mismatch repair-defective, only deletions were observed in some

cell lines (22, 24). Thus, the comparison of those systems with ours seems reasonable.

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30. Growth-dependent Lac$^+$ revertants of the +1 frameshift strain were isolated as described (8) (with only one isolate obtained from each culture to avoid duplication in jackpots) and then sequenced (16). For mutations greater than 1 bp, position numbers indicate the first nucleotide affected. For mutations that occurred in more than one isolate, the number of isolates is indicated after x: "x 6" indicates a mutation found in six separate isolates. One *rec*$^+$ isolate has no visible sequence change and so presumably carries an extragenic mutation. It is possible that the few -1 deletions occurring in regions of small mononucleotide repeats are early-appearing adaptive mutations.

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3. ADAPTIVE MUTATION SEQUENCES REPRODUCED BY MISMATCH REPAIR DEFICIENCY*

Adaptive mutations occur in the apparent absence of cell division, in cells exposed to a nonlethal selection (1-6), and have been detected only in genes whose function was selected (refs. 2, 4, 7, but see ref. 6). Thus, these mutations differ from spontaneous mutations in growing cells. In an experimental system that detects reversion of a +1 frameshift mutation in an *Escherichia coli lacI-lacZ* fusion gene (2), the adaptive mutations are further distinguished from the growth-dependent reversions by their molecular mechanism of formation and by their sequences. The adaptive reversions alone require genes of the *E. coli* RecBCD recombination system (8) implying that genetic recombination is part of the molecular mechanism by which they form. Also, the adaptive Lac^+ reversions are almost all single base deletions in mononucleotide repeats, whereas the growth-dependent Lac^+ reversions include large and small insertions, deletions, and duplications, and are not confined to simple repeats (9, 10). This paper addresses the origin of the unusual sequence spectrum of the adaptive reversions of the *lac* +1 frameshift mutation.

The predominance of -1 deletions in small mononucleotide repeats could arise by many possible mechanisms, some examples of which follow.

Deletions in simple repeats are characteristic of DNA polymerase errors (11), thought to be caused by a template slippage mechanism (12).

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Growth-dependent mutations might be different (*i*) because different polymerases with different mistake potentials could be responsible for each (9, 13).

(ii) Perhaps the adaptive mutations form without a DNA polymerase, by unequal sister chromosome recombination in the micro-repeats.

(*iii*) The *lac* frameshift allele resides on an F' episome in cells with the chromosomal *lac* operon deleted (2). Adaptive mutation could depend on sexual transfer replication of the F', and such replication could be induced during adaptive mutation (14-16). Sexual replication is unidirectional, using a leading strand exclusively, and is proposed to produce the -1 deletions in mononucleotide repeats observed in adaptive mutation (16). The lagging strand synthesis, which occurs along with leading-strand synthesis in vegetative replication, was suggested to produce the heterogeneity of the growth-dependent reversion sequences by, e. g., incorrect joinings of Okazaki fragments (16). Data discourage the view that conjugational transfer is a predominant source of adaptive reversion (and thus of its unusual sequence spectrum) (17, 18). However a possible caveat regarding such data has been suggested (15), and it is also possible that transfer replication occurs without actual transfer (16, 18).

(*iv*) A fourth possibility is that both growth-dependent and adaptive mutations result from essentially similar polymerase errors, but, in the case of adaptive mutations, the errors are not subject to the normal postsynthesis mismatch repair (9, 10, 19). This idea is both encouraged and discouraged by the sequences of the adaptive mutations (9, 10). On the one hand, the *lac* adaptive mutations resemble instability of simple repeats which has been observed in *E. coli* (20-23), yeast (24), and in human cells

that are defective in mismatch repair. In humans, hereditary nonpolyposis colon cancer cells display simple repeat instability (25, 26), and 90% of affected individuals examined carry a defect in homologs of either of two *E. coli* mismatch repair genes, *mutS* or *mutL* (e.g., refs. 27 and 28). In *E. coli*, errors that result in small deletions and insertions (of up to four bases) are normally corrected by the MutLSHU mismatch repair system (20). Inactivation of this system -- e. g., by a null mutation in the *mutS* or *mutL* gene -- results in a 10- to 1000- fold increase in the rate of spontaneous mutation during growth (ref. 20; see also Table 1), presumably representing unrepaired polymerase error (e. g., see ref. 21). The preponderance of -1 deletions in mononucleotide repeats is an expected phenotype of inactive mismatch repair (which could perpetuate polymerase errors made during synthesis associated with recombination to produce adaptive mutations; refs. 8-10, 13). Increased frameshift mutation at simple repeats has been observed in other genes in mismatch-repair-defective cells (e. g., refs. 21-23). On the other hand, the absence of +2 insertions in dinucleotide repeats is unexpected in the context of the hypothesis that mismatch repair fails during adaptive mutation. The +2 insertion heteroduplexes are repaired by the MutLSUH system (20), and +2 insertions in dinucleotide repeats are found among growth-dependent reversions of the *lac* frameshift allele (9, 10), suggesting that a DNA polymerase can create +2 insertions in this region.

In this report, hypothesis *iv* is tested by assessing whether the absence of mismatch repair is a sufficient condition for allowing the unusual sequence spectrum observed in adaptive revertants to occur. We find that the growth-dependent Lac^+ reversion spectrum can be made indistinguishable from the adaptive mutation spectrum by eliminating

mismatch repair during growth. This result is not predicted by the first three hypotheses and supports the fourth.

MATERIALS AND METHODS

All derivatives of the *E. coli* strain carrying the *lac* frameshift mutation (2) were constructed by conventional bacteriophage P1 transduction, and a single transductant isolate of each was used in all experiments reported. The mismatch repair-defective derivatives of this strain carry *mutS201*::Tn*5*, *mutL211*::Tn*5*, *dam13*::Tn*9*, *mutH471*::Tn*5*, or *mutU*::Tn*5*. The *lac* +1 frameshift mutation (CCC to CCCC) is at position 1039 of *lacI* (see Figure 1) within a *lacI-lacZ* fusion gene residing on an F' episome. The mutation is polar on *lacZ*, making the cell Lac$^-$. Growth-dependent Lac$^+$ reversion mutations were assayed and isolated as described (8, 9): The earliest possible time after plating when growth-dependent revertant colonies are visible on the selective plates was determined for each mutator strain by assaying two to four separate Lac$^+$ revertant isolates of each. Each growth-dependent revertant was derived from a separate culture to avoid sampling multiple members of a clone. Mutation rates were calculated by using the method of the median (29). Sequencing the Lac$^+$ reversion mutations was done as described (9) after PCR amplification of a region of DNA spanning the +1 frameshift mutation (9). To assay mutator phenotype, occurrence of nalidixic acid-resistant mutants was measured by two methods: (*i*) counting papillae in a zone of clearing around a spot of dry nalidixic acid dropped on 0.1 ml of saturated culture spread on Luria-Bertani (LB) plates and *(ii)* counting colonies in 10-μl drops of saturated cultures spread to ≈2-cm-diameter

circles on nalidixic acid (4 μg/ml) LB plates. Occurrence of streptomycin-resistant mutants was also measured in 10-μl drop/spreads (as above) on streptomycin (200 μg/ml) LB plates. Five positive control (i.e., mutator) strains plated in parallel were the derivatives of the frameshift-bearing cell described above that carry *mutS201*::Tn*5*, *mutL211*::Tn*5*, *dam13*::Tn*9*, *mutH471*::Tn*5*, or *mutU*::Tn*5*. The negative control strain plated in parallel was the *mut*$^+$ frameshift-bearing cell (2).

RESULTS AND DISCUSSION

A prediction of hypothesis *iv*, which is not predicted by the first three hypotheses, is that a failure to repair mismatches resulting from polymerase errors in growing cells might produce a mutation spectrum similar to the adaptive reversion spectrum, including its scarcity of +2 insertions in dinucleotide repeats. We tested this prediction by examining the mutation spectrum of growth-dependent revertants of the *lac* frameshift mutation in cells deficient for either the *mutS* or *mutL* gene product.

Growth-dependent revertants of MutS- or MutL-deficient derivatives of a strain carrying the +1 frameshift mutation in *lac* were isolated as Lac$^+$ colonies arising ≈40 hours after plating and were purified as described (ref. 9; see *Materials and Methods*). The growth-dependent mutation rates for these strains are elevated relative to that of a repair-proficient strain, as expected (Table 1). Figure 1 shows the DNA sequence spanning the frameshift mutation for which the adaptive and growth-dependent reversion mutations were characterized previously. Mutations that restore the reading frame to produce a functional *lacZ* gene product

occur between the two out-of-frame stop codons (boxed in Figure 1). All of the Lac$^+$ growth-dependent reversion mutations in *mutS*-deficient cells and 27 out of 28 isolates from *mutL*-deficient cells are -1 deletions in the same small mononucleotide repeats highlighted in Figure 1 (Figure 2). The mutation spectrum is unlike that of growth-dependent reversion mutations in *mut*$^+$ cells (9, 10) but is indistinguishable from that of the adaptive mutations (refs. 9, 10; see Figure 2). Whereas previous studies have shown elevated frameshift mutation in simple repeats at other loci (21-23), these studies could not address whether exactly the spectrum found in adaptive reversion in our target gene would occur in the absence of mismatch repair during normal growth and DNA replication. Especially distinctive features of this spectrum include the fact that only some of the many mononucleotide repeats present are hotspots and that the dinucleotide repeats are not.

In *E. coli*, nonfunctional mismatch repair can also be caused by a defect in Dam methylase, the enzyme responsible for adenine methylation at d(GATC) sites (33). In cells lacking Dam methylase (*dam* cells), there is no discrimination between template and newly synthesized DNA strands during mismatch repair, resulting in perpetuation of polymerase errors as mutations (33). Thirty-two out of 34 growth-dependent reversions in a *dam* strain are indistinguishable from adaptive mutations (Figure 2), indicating that during adaptive mutation, either insufficiency of mismatch repair or failure of its strand discrimination mechanism could be responsible for generating the peculiar mutation spectrum of -1 deletions in small mononucleotide repeats. The mutation rates of *dam* and *mutL* strains are elevated only modestly above their mismatch repair-proficient parent (Table 1), such that the 1 out of 28 *mutL* and 2 out of 34

dam exceptions to the pattern of -1 deletions in mononucleotide repeats are expected as background mutations -- i.e., those occurring when mismatch repair is functional -- in these strains. See, for example, refs. 30 and 31 for previous reports of quantitative differences in mutability caused by disabling different mismatch-repair genes.

The sequence spectra of Lac$^+$ growth-dependent reversions of *mutS*, *mutL*, and *dam* strains are indistinguishable from the spectrum of Lac$^+$ adaptive reversions in a genotypically *mut*$^+$ strain. This result demonstrates that failure of post-synthesis mismatch repair or its strand-discrimination mechanism during adaptive mutation is capable of generating the unusual sequence spectrum of adaptive mutations. These data support the hypothesis that *E. coli* cells become mismatch repair-defective under starvation conditions, possibly allowing polymerase slippage errors to persist and generate adaptive mutations. This process could cause a high mutation rate, even with minimal DNA synthesis, because the rates of mutation are so enhanced when mismatch repair is nonfunctional.

To assess whether adaptive mutation induces a heritable loss of mismatch repair proficiency, 20 separate Lac$^+$ revertant isolates (9) were assayed for mutator phenotype, the phenotype indicative of mismatch repair dysfunction (20). Two assays were used: spontaneous mutation to nalidixic acid resistance and spontaneous mutation to streptomycin resistance (*Materials and Methods*). The *lac*$^-$ parent served as a nonmutator control strain, and *mutS*, *mutL*, *mutH*, *mutU*, and *dam* derivatives of that parent (see *Materials and Methods*) were used as positive, mutator control strains. Numbers of colonies with the positive control strains differ from those with the negative control, and from all 20

Lac$^+$ isolates by factors of 10-100 with the nalidixic acid assays. With streptomycin, no colonies were seen in the negative control or Lac$^+$ isolates, whereas all of the positive control strains gave one to a few colonies. The *lac*$^-$ parent and 20 Lac$^+$ adaptive revertant isolates do not display mutator phenotype, indicating that once isolated, the adaptive revertants are mismatch repair-proficient. The positive control strains produced mutant frequencies of 10 to 100 times higher (34). These results indicate that the proposed disability of mismatch repair during adaptive mutation would have to be transient, not heritable. We suggest that mismatch repair activity may be regulated and not constitutive.

Means by which mismatch repair could be disabled transiently during adaptive mutation include down-regulation of *mutS, -L, -H,* or-*U* gene expression (35); depletion of any of those gene products due to starvation (19) or due to such high levels of polymerase error that the repair system is saturated (36); transient undermethylation of the DNA due to diminution of the Dam protein before cessation of DNA synthesis during starvation; or transient overmethylation of the unreplicating DNA that blocks action of the MutH endonuclease on DNA (20).

We have suggested a model for adaptive reversion of the *lac* frameshift mutation in which the stress of starvation induces formation of DNA double-strand breaks, which promote RecBCD-mediated recombination (8, 9, 13, 37). RecBCD-mediated recombination is essential for adaptive mutation in this system (8). DNA synthesis associated with recombination could contain errors (8) that persist to form mutations due to the disability of normal post-synthesis mismatch repair. Successful mutation to Lac$^+$ would be rewarded by growth, whereas failure to generate Lac$^+$ would not alleviate induction of double-strand breaks and

might lead ultimately to death in the few cells in the population that are proposed to try this dangerous experiment. A role for the sexual apparatus of the F' in adaptive Lac^+ reversion has been indicated in Salmonella (16) and in *E. coli* (18). We suggest that the role might be in providing high-efficiency double-strand breakage after single-strand nicking of the origin of transfer of the F' (17, 37). This idea is consistent with all of the data, whereas the sexual replication hypothesis described above (16) is inconsistent with our observation that -1 deletions in small mononucleotide repeats can be generated during vegetative growth (leading and lagging strands) simply by the absence of mismatch repair. There are likely to be other, localized, double-strand break sources in the bacterial chromosome (17).

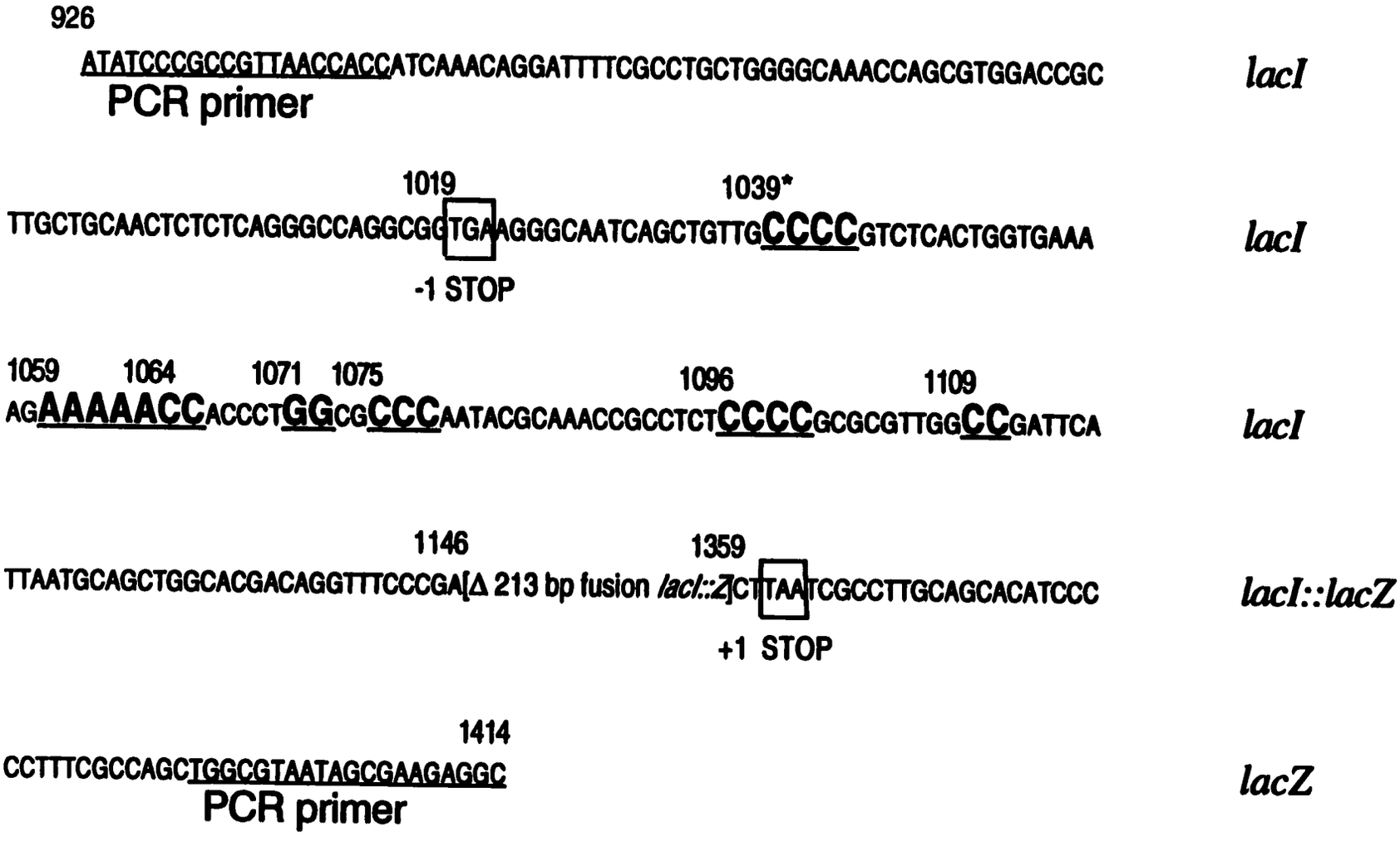
The possibility that mismatch repair-proficiency could be affected by cell physiological inputs would have profound consequences for understanding the regulation of spontaneous mutation in cancers, in normal development (e. g., refs. 35, 38), and in evolution.

Table 3-1. Growth-dependent mutation rates in mismatch repair-defective strains

Genotype	Exp.	Cultures, no.	Revertants in median culture, no.	Revertants per 10^9 cells, median no.	Rate of mutation to Lac^+, mutations • $cell^{-1}$ • $generation^{-1}$
mut+	1	39	1	3.0	8.5×10^{-10}
	2	39	2	3.3	9.0×10^{-10}
mutS	1	39	4	1300	2.3×10^{-7}
	2	39	5	790	1.6×10^{-7}
mutL	1	59	12	24	7.4×10^{-9}
	2	53	19	40	1.0×10^{-8}
dam	1	37	24	130	2.3×10^{-8}
	2	39	18	98	1.9×10^{-8}

Measurement of mutations rates is as described. Different quantitative levels of mutator phenotype in strains carrying different mismatch repair-defective mutations, such as observed here, have been observed (e. g. , refs. 30 and 31). A possible explanation for the higher mutation rate in the *mutS* strain than in *mutL* was suggested by Allen Campbell (Stanford University). Perhaps the MutS protein, which binds the DNA mismatch (20), may kill or remove heteroduplexes that it cannot repair when one of the other components of the system is absent.

Figure 3-1. DNA sequence of a region of the *lacI-lacZ* fusion gene that was amplified by PCR and sequenced as described (9). The mutant *lac* gene (2, 3, 7-10) is a *lacI* gene fused to *lacZ*, such that the frameshift mutation of CCC to CCCC at position 1039 in *lacI* (*) is polar on *lacZ* , making the cells Lac^-. Lac^+ reversion mutations can restore the reading frame by deletion or addition anywhere between two out-of-frame stop codons (boxed). Underlined bases indicate the sites where reversion mutations occur in adaptive mutants. Nucleotide positions are those used by Miller, (32), and the additional cytosine that causes the +1 frameshift is not numbered.


926
ATATCCCGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGC
PCR primer
lacI
1019
1039*
TTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCCGTCTCACTGGTGAAA
-1 STOP
lacI
1059
1064
1071
1075
1096
1109
AGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCA
lacI
1146
1359
TTAATGCAGCTGGCACGACAGGTTTCCCGA[Δ 213 bp fusion lacI::Z]CTTAATCGCCTTGCAGCACATCCC
+1 STOP
lacI::lacZ
1414
CCTTTCGCCAGCTGGCGTAATAGCGAAGAGGC
PCR primer
lacZ

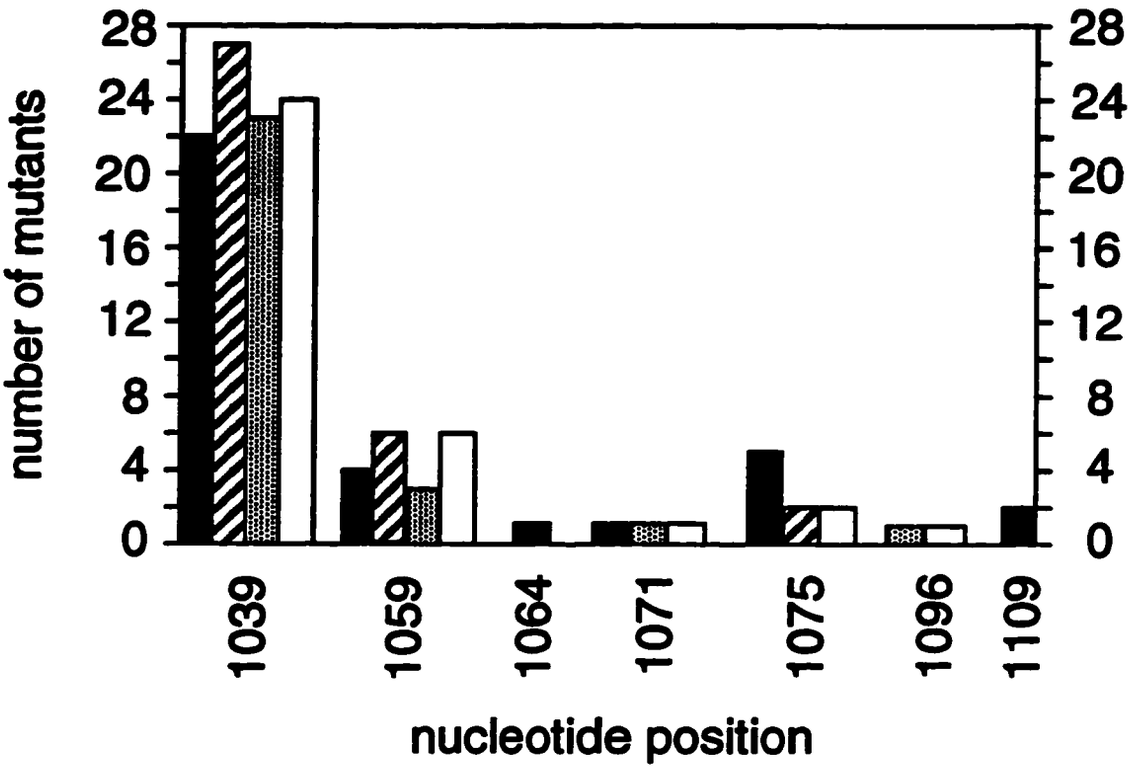


Figure 3-2. Positions of adaptive reversions and of growth-dependent reversions in mismatch repair-defective strains: distribution of the -1 deletions in mononucleotide repeats. All of the growth-dependent reversions in mismatch repair-defective strains are single-base deletions in small mononucleotide repeats, except for two of the 34 *dam* isolates (a single-base deletion at position 1019; a deletion of basepairs 1104-1119), and one of the 28 *mutL* isolates (a duplication of nucleotides 1060-1079). These frequencies of exceptional mutations, 0/35 *mutS*, 1/28 *mutL*, and 2/34 *dam*, are within expectations for the frequencies of normal background mutations that occur beneath the levels of the elevated mutation rates caused by these strains' defects in mismatch repair (Table 3-1). Data for adaptive reversion are from Chapter 2, showing reversions of the mut^+ parent of the *mut* strains used here. Nucleotide positions are shown in Figure 3-1. Black bars represent adaptive mutations. Bars representing growth-dependent *lac* reversion mutations are as follows: hatched, *mutS*; gray, *mutL*; white, *dam*.

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4. OVEREXPRESSION OF *mutS* AND *mutL* IN ADAPTIVE MUTATION

Adaptive reversions of a *lac* +1 frameshift mutation in *Escherichia coli* are almost all -1 deletions in mononucleotide repeats. In contrast, growth-dependent reversions of the same *lac* frameshift mutation are highly heterogeneous, not confined to repeat regions, and include large and small insertions, deletions, and duplications (Chapter 2). However, when MutHLSU mismatch repair is inactive during growth, the sequence spectra of adaptive and growth-dependent mutations are indistinguishable (Chapter 3).

MutHLSU mismatch repair normally corrects mispaired bases resulting from DNA replication error and DNA polymerase proof-reading failure (reviewed by 1). *E. coli* (1-4), yeast (5), and human cells (6-10) that are defective in mismatch repair display instability in simple repetitive sequences where polymerases often make errors (11, 12), probably by a template slippage mechanism (13). The idea that adaptive mutation occurs when mismatch repair is temporarily disabled is supported by -- (i) the prevalence of adaptive mutations in simple repeats (Chapter 2) which resembles the repeat instability of mismatch repair-defective cells, and (ii) the finding that inactivation of mismatch repair is sufficient to alter the reversion sequence spectrum of growth-dependent mutation to a spectrum indistinguishable from adaptive mutation (Chapter 3). The repair disability must be temporary because Lac^+ cells resulting from recombination-dependent adaptive mutations are mismatch repair-proficient (Chapter 3).

In this chapter, I describe the construction of plasmids that were used subsequently to test the hypothesis that mismatch repair is

suspended transiently by limitation of MutS and/ or MutL protein. The experiments for which these plasmids were constructed measure adaptive mutation in the *lac* frameshift-bearing strain when either or both of two mismatch repair proteins, MutS and MutL, are overproduced. MutS and MutL proteins are essential for initiation steps of the *E. coli* major mismatch repair system. If depletion of mismatch repair during adaptive mutation occurs because of a low level or absence of either or both of these proteins, then the frequency of adaptive Lac^+ reversion should decrease when these proteins are overproduced in cells mutating adaptively.

MATERIALS AND METHODS:

Plasmid isolation and bacterial transformations:

Plasmid isolations were performed using one of three methods: (i) Using STET lysis buffer according to the method described by (14); (ii) *CIRCLEPREP* (kit from Bio 101, Inc., La Jolla, CA) miniprep according to the manufacturer's instructions but using noncommercially-made solutions. Glucose-Tris-EDTA (GTE) lysis buffer (pH 8.0), freshly-prepared sodium dodecyl sulfate/NaOH lysis solution, and neutralizing solution were made according to (14). LiCl was used at a final concentration of 3M to remove RNA and single-stranded DNA. Saturated cultures were grown in LBK broth (*e. g.* 15); (iii) Ammonium acetate minipreps were as in (16), except that saturated cultures were grown in LBK or L broth (as LB, 14, but at pH 7.5 and containing NaCl at 0.5 g/L), and RNA was removed only with ribonuclease A (Sigma). For all three protocols, cultures of the plasmid-bearing strains were grown in the presence of the appropriate drug with final concentrations of 100 μg/ml ampicillin, 50 μg/ml

kanamycin, 15 or 25 μg/ml chloramphenicol, or 10 μg/ml tetracycline. In order to obtain high yields of some plasmid DNA, *e. g.* pBR322, chloramphenicol amplification of growing cultures was performed (14). Plasmid DNA was resuspended in either TE buffer (pH 7.5; 14) or water.

Cells were transformed with plasmid DNA either directly, or by electroporation. For direct transformation, cells were made competent by resuspending a 10 ml culture grown in L broth in 5 ml cold 0.05 M $CaCl_2$, leaving in ice for 15 minutes, resuspending pelleted cells in 0.5 ml cold 0.01 M $CaCl_2$, and then either transforming cells immediately, refrigerating overnight in ice and then transforming, or mixing 150 μl of 50% glycerol with the cells to freeze at -80°C for later use. To transform, typically 0.1 ml cells were mixed with 1-10 μl of DNA (~12-20 μg/ml), left on ice for 30-60 minutes, incubated in a 42-45°C water bath for 2 minutes whereupon 0.5 ml of L broth or SOC medium (14) were added to the cells. Cells were incubated in a rotary shaker at 37°C for 30-90 minutes, and then spread on LBH (Luria-Bertani-Hersokowitz, as LB, 14, but containing half as much NaCl) plates containing the appropriate drug(s) at the above concentrations to select for transformed cells. For electroporation (protocol from J. Stone, University of Alberta), 100 ml of SOB medium (14) were inoculated with 0.2 ml of a saturated culture grown in L broth, and incubated in a 37°C rotary shaker until the OD_{600} reached approximately 0.6-1.0. This culture was cooled for 20 minutes on ice, pelleted and rinsed three times in progressively lesser amounts of ice-cold sterile water, and then resuspended in 2 ml of 10% analytical grade glycerol in water. Cells were again pelleted, then resuspended in a minimal volume of 10% glycerol such that a 10^{-2} dilution produces an OD_{600} in the range of 0.125-0.375. Cells either were frozen at -80°C or used immediately. To

electroporate, 25 µl of competent cells were mixed with 1-3 µl of DNA (~12-20 µg/ml) suspended in water (pH 8.0), incubated on ice for exactly one minute, and then transferred to a cold electroporation cuvette (Gene Pulser Cuvette, 0.1 cm electrode gap, Bio-Rad). Cells were electroporated at 1.6 KVolts, 200 ohms, and 25 µFarads, after which cells were removed from the cuvette into 1 ml of SOC and incubated shaking for one hour at 37°C. Following incubation, 0.1-0.5 ml of electroporated cells were spread directly onto selective LBH drug plates as above.

Before further manipulation, transformants that gave rise to colonies on the selective plate were always purified by streaking twice for single colonies on plates with the appropriate drug.

Plasmid constructions:

Four different plasmids expressing the *mutS* gene were used, two expressing *mutS* of *Salmonella typhimurium* strain LT2 and two expressing *mutS* of *Escherichia coli*. pGW1811 (17) is a pBR322 derivative that carries *mutS* of Salmonella, and was obtained from G. Walker. Susan Szigety (S. M. Rosenberg Lab, University of Alberta) constructed pSUE1 by digesting pGW1811 and pKC31 (N. Rao, described in 18) with *Bam*HI and *Hin*dIII restriction enzymes (BRL). Digested DNA was extracted with a 1: 1 mixture of phenol and chloroform, precipitated with 95% ethanol, and resuspended in TE buffer to a concentration of approximately 0.125 µg/µl. 10 µl of each purified digested plasmid DNA was mixed together in a ligation mixture also containing 0.5 mM ATP, 0.5 M Tris-HCl, 0.1 M $MgCl_2$, 0.1 M dithiothreitol, and 1 unit of T4 DNA ligase (Gibco BRL), and was incubated overnight at 16°C. By the $CaCl_2$ method, ligated DNA was used to transform *E. coli* cells of strain ES1481 (Table 4-1, obtained from the

E. coli Genetic Stock Center) that carries a transposon insertion in the *mutS* gene (*mutS215*::Tn*10*). Transformants were selected on LBH kanamycin plates at 37°C. Three positive isolates were purified by streaking and complementation tests were performed as follows: (i) 0.1 ml of saturated culture was spread on LBH rifampicin (50 μg/ml) plates, incubated overnight at 37°C, and rifampicin-resistant colonies counted the next day. Non-mutator strains generate none to a few resistant colonies whereas mutator strains typically generate ~100-1000 resistant colonies. (ii) an LBH plate spread with 0.15 ml of saturated culture, and dotted in the centre with a toothpick-full of nalidixic acid powder, was incubated overnight at 37°C, and nalidixic acid-resistant papillae counted within a central zone of clearing. Non-mutator strains generate none to very few nalidixic acid-resistant papillae. To confirm that the ligation was successful, plasmids were isolated from two positive isolates by the circleprep method, digested with *Bam*HI and *Hin*dIII, and then run on a 1% agarose gel to confirm predicted band sizes. Figure 4-1 is the expected restriction map of pSUE1 for one of these isolates (Strain SL1311, Table 4-1). To show that this plasmid expresses functional MutS, the *mutS* strain ES1481 (Table 4-1) was transformed with pSUE1 and complementation assays were performed as above. pSUE1 complements the mutator phenotype of the host strain ES1481 in both assays.

pMS312 (19) expresses *E. coli mutS* under the control of the bacteriophage λP_L promoter in the pSCC31 vector (derived from a lambda-pBR322 hybrid plasmid; 20), and was obtained from Paul Modrich. pSL6 also expresses *E. coli mutS*. To construct pSL6, restriction digests and ligations were performed as follows: pBR322 (21, 22) was digested with *Cla*I and *Bam*HI and 5' phosphate groups were removed with calf

intestinal alkaline phosphatase (CIAP, Gibco BRL). pKC31 (N. Rao, described in 18) was digested with *Hin*dIII and *Bam*HI and pMS312 (19) was digested with *Cla*I and *Hin*dIII. Digested DNA was run on a 1% agarose gel, and a 3.31 Kb band from the pMS312 digest containing the *mutS* gene, and a 1.96 Kb band containing the kanamycin-resistance gene from pKC31 were cut out of the gel. DNA fragments were recovered as described (23) except that spin filters (Bio 101, Inc., La Jolla, CA) were used in place of punctured eppendorf tubes plugged with glass wool, and ammonium acetate was used in place of sodium acetate. To ligate, digested pBR322, the 3.31 Kb fragment of pMS312, and 1.96 Kb fragments of pKC31 were mixed together and incubated at 51°C for 7 minutes, and then put on ice for 7 minutes. Ligase buffer (as above), 1 mM ATP, and 0.18 units of bacteriophage T4 DNA ligase (Gibco BRL) were added and the mixture was incubated overnight at 16°C. Ligated DNA was used to transform competent cells of a *mut*$^+$ strain, C600 (24), by the $CaCl_2$ method, and cells were plated on LBH kanamycin plates. Out of 36 kanamycin-resistant colonies, 12 were restreaked on LBH kanamycin plates and tested for resistance to ampicillin and tetracycline. All isolates were ampicillin-resistant and tetracycline-sensitive. Plasmid DNA was prepared from 7 isolates and used to transform two *mutS* strains, RSH32 and ES1481 (*mutS215*::Tn*10*), to do complementation analysis. Complementation was assayed by spreading 10 µl of a saturated culture in a ~1 cm diameter circle on a LBH kanamycin plate also containing 4 µg/ml nalidixic acid. At this concentration of nalidixic acid, the above *mutS* strains generate resistant papillae, but *mutS*$^+$ strains do not. All plasmid isolates complemented the *mutS* mutator phenotype. One isolate of the transformed RSH32 strain was frozen into the Rosenberg lab collection as

strain SL1748 (Table 4-1). Agarose gel electrophoresis of restriction digests with *Bam*HI and *Eco*RI confirmed predicted band sizes of the new plasmid. One of the seven candidate plasmids from above was chosen to transform FC40 (strain SL1747, Table 4-1) for use in adaptive mutation experiments. The expected restriction map of pSL6 is shown in Figure 4-2. That SL1747 overproduces MutS 60- to 130-fold (per 150 μg total protein) has been confirmed by Western blot with antibody to MutS (25).

Four plasmids expressing the *mutL* gene were also constructed, two that express Salmonella *mutL*, and two that express *E. coli mutL*. pGW1842 (17) expresses the Salmonella gene ligated into pBR322 and was obtained from Graham Walker. pSL1 was constructed by linearizing pGW1842 at the single *Pst*I site in the gene encoding ß-lactamase and ligating (as above) with pCAT19 (26) fragments obtained from a restriction digest with *Pst*I. Following ligation, DNA was purified by phenol extraction and ethanol precipitation, then resuspended in water (pH 8.0). Plasmid DNA was used to electroporate SMR369 (Rosenberg lab collection, Table 4-1) which is *mutL*-deficient because of a transposon insertion (*mutL211::*Tn*5*). After electroporation, cells were spread on LBH chloramphenicol plates. Out of five chloramphenicol-resistant isolates, all were sensitive to ampicillin indicating that the gene for ß-lactamase was disrupted during ligation. In all isolates, the mutator phenotype of SMR369 was complemented as assayed using the nalidixic acid protocol described above for pSUE1. Plasmid DNA was extracted from these isolates, digested with *Pst*I and *Sal*I, and run on a 1% agarose gel. The band sizes suggested that the isolated plasmids might contain DNA additional to the *CAT* (chloramphenicol acetyl transferase) gene of pCAT19 (26), so in order to shrink it, plasmid DNA from the five isolates was again digested

with *Pst*I, re-ligated as above, and used to electroporate competent cells of strain SMR369. Following restriction digest analysis (with *Kpn*I, *Pst*I, and *Sma*I) of plasmids obtained from chloramphenicol-resistant, nonmutator colonies, and also complementation assays using newly transformed SMR369 cells, one positive plasmid isolate (pSL1) from the above manipulations was used to transform FC40 yielding strain SL1321 (Table 4-1). An expected restriction map of pSL1 is shown in Figure 4-3. The orientation of the *CAT* gene in pSL1 was determined with gel electrophoresis of a *Sma*I restriction digest.

pAL51 (27) expresses *E. coli mutL* under the control of the λP_L promoter in the same pSCC31 vector used to make pMS312 (above) and was obtained from Paul Modrich (Duke University). pSL5 also expresses *E. coli mutL* and was constructed as follows: pBR322 was cut at the single *Pst*I and *Bam*HI sites, and the terminal phosphates removed with CIAP (Gibco BRL) according to the manufacturer's instructions. pKC31 was digested with *Hin*dIII and *Bam*HI, and pAL51 was digested with *Hin*dIII and *Pst*I. Digested DNA from pBR322, pKC31 and pAL51 was run on a 1% agarose gel from which the 1.98 Kb band containing the kanamycin-resistance gene of pKC31, and the 3.92 Kb band containing the *mutL* gene of pAL51 were removed by "freeze-squeeze" (23) as for pSL6. pBR322 DNA fragments, 1.98 Kb pKC31 fragments, and 3.92 Kb pAL51 fragments were mixed together and ligated as for pSL6. Competent cells of strain C600 were transformed by the $CaCl_2$ method and plated on LBH kanamycin medium. Plasmid DNA from ten kanamycin-resistant colonies was extracted and used to transform strain FS1892 (594 *mutL218::*Tn*10*, Table 4-1) in which *mutL* is inactivated by transposon insertion. Transformants were assayed for mutator phenotype by spreading 10 µl of a saturated

culture in a ~1 cm diameter circle on both LBH kanamycin nalidixic acid (4 μg/ml), and LBH kanamycin rifampicin media. All ten plasmids complemented the mutator phenotype of FS1892. One plasmid that also had the predicted band sizes following agarose gel elecrophoresis of a restriction digest with *Hin*dIII and *Pst*I was used to transform FC40 and SL1712, a *mutL218*::Tn*10* derivative of FC40 (yielding strains SL1713 and SL1746 respectively, Table 4-1). The expected restriction map of this plasmid, pSL5, is shown in Figure 4-4. That pSL5 overproduces MutL 20- to 30-fold (per 150 μg total protein) has been confirmed by Western blot with antibody to MutL (25).

pSL*7* expresses both *E. coli mutS* and *mutL*. To construct pSL*7*, pBR322 was digested with *Cla*I and *Pst*I, the terminal phosphates were removed with CIAP as above, and the fragments were extracted with phenol and ethanol-precipitated. A 1.98 Kb fragment of pKC31 carrying the kanamycin-resistance gene from restriction with *Hin*dIII and *Bam*HI, a 3.6 Kb fragment of pSL*6* carrying the *mutS* gene from restriction with *Cla*I and *Bgl*II, and a 3.9 Kb fragment of pAL51 carrying the *mutL* gene from digestion with *Hin*dIII and *Pst*I were removed from a 1% agarose gel by "freeze-squeeze" (23). All pieces were mixed, incubated at 55-58°C for 6 minutes, put on ice for 7 minutes after which the remaining ingredients of the ligation mixture (as above) were added; the ligation mixture was incubated overnight at 16°C. Competent cells of *mut*$^+$ strain C600 (Rosenberg lab collection) were transformed with the ligated DNA and transformants selected on LBH kanamycin medium. Plasmid DNA was isolated and characterized from three kanamycin-resistant colonies. Restriction digest analysis was performed with *Bam*HI and *Hin*dIII, which produced the predicted band sizes for all three plasmid isolates. Each of

these three plasmids was used to transform SL1712 (*mutL*), and SL1786 (*mutS mutL*) to assay for complementation of the mutator phenotype of these strains using the nalidixic acid (4 μg/ml) method described above for pSL5. The expected restriction map of pSL7 is shown in Figure 4-5. That pSL7 overproduces both MutL and MutS (per 150 μg total protein) was confirmed by Western blots (25).

Two additional plasmids that carry the *mutH* gene were used to transform strain FC40 (Table 4-1). pGW1899 (28) was obtained from M. Radman (Institut Jacques Monod, Paris) and expresses the Salmonella *mutH* gene. pGW1899 was used to transform strain FC40 (to produce strain SL653, Table 4-1), and a *mutH* strain (SZ623, Table 4-1) to check for complementation. Complementation tests using the nalidixic acid test described above for pSUE1 were inconclusive. Whether *mutH* is expressed from pGW1899 in these strains is unknown. pAL6 (29) expresses *E. coli mutH* and was obtained form P. Modrich (Duke University). I attempted to transform strain SL591 (yielding strain SL584), Table 4-1) as well as a *mutH* strain (SL613, Table 4-1) to check for complementation. Whether pAL6 expresses *mutH* in these strains is unknown because no transformants of the SL613 strain were obtained. Drug-resistance and Lac$^-$ phenotypes of both SL653 and SL584 were confirmed.

The following control plasmids were used in overexpression studies: pRDK35 (30), pSCC5077 (31, obtained from Martin Marinus), pSL2, pSL3, and pSL4. pRDK35 contains an insertion that disrupts the tetracycline-resistance gene of pBR322, but is otherwise identical to pBR322. pSCC5077 is a low-copy vector which is otherwise identical with pMS312 and pAL51. pSL2 was created by digesting pSUE1 with *Sal*I to

remove the *mutS* gene, and then religating. Religation was performed by diluting ~2 μg of *Sal*I-digested pSUE1 DNA in water (pH 8.0), heating to 45°C, and then immediately placing on ice. Ligase buffer (as above), T4 DNA ligase (Gibco BRL) and adenosine triphosphate (0.5 mM final concentration) were added to make a final volume of 0.2 ml. This mixture was incubated overnight at 16°C. Ligated DNA was used to transform ES1481, a *mutS* strain (Table 4-1). The mutator phenotype of ES1481 on rifampicin medium (assay described above) did not change following transformation, indicating that removal of *mutS* from pSUE1 was successful. Plasmid DNA was isolated from positive transformants and restriction digests performed with *Sal*I and *Eco*RI to confirm the expected plasmid map of pSL2 (Figure 4-6). pSL2 was used to transform both FC29 and FC40 strains, yielding strains SL1322 and SL1323 respectively (Table 4-1).

pSL3 was made by inserting the *CAT* gene-containing *Pst*I fragment of pCAT19 (26) into the single *Pst*I site in the ampicillin-resistance gene in pBR322. Following restriction digestion and agarose gel electrophoresis, the bands containing the appropriate ~1.0 kb *CAT*-containing DNA, and the linearized pBR322 DNA were removed and purified by "freeze-squeeze" (23). Ligations were performed as previously (above) but in a final volume of 7 μl. A *mut*$^+$ strain, C600, was transformed with ligated DNA. Transformants were selected on LBH chloramphenicol, and then screened for resistance to tetracycline and ampicillin. Plasmids were isolated from positive transformants and restriction digests performed with *Pst*I, *Hin*dIII and *Bam*HI to generate the expected restriction map showing the correct orientation of *CAT* in pSL3 (Figure 4-7). Both FC29

and FC40 strains were transformed with pSL3, yeilding strains SL1507 and SL1506 respectively (Table 4-1).

pSL4 contains the kanamycin-resistance gene in pBR322 where it replaces a 346-basepair segment between *Hin*dIII and *Bam*HI sites with a *Hin*dIII-*Bam*HI fragment containing the kanamycin-resistance gene from pKC31. Ligations were performed as above but in a final volume of 10 µl, and ligated DNA used to transform C600. Transformants were selected on LBH kanamycin media. Plasmid DNA was extracted from positive transformants and digested with *Hin*dIII and *Bam*HI to confirm the restriction map expected for pSL4 (Figure 4-8). pSL4 was used to transform strain FC40, yielding strain SL1790 (Table 4-1).

Bacterial Strains:

All plasmid-bearing derivatives of *E. coli* FC40, FC29 (32, Table 4-1) and 594 *mutL218*::Tn*10* strains (Table 4-1) were constructed by $CaCl_2$ transformation of the host strain with the appropriate plasmid. All new FC40- and FC29-derived strains were tested for rifampicin-resistance, lactose-fermentation, and for all drug-resistance markers specific to each plasmid, and transposon where applicable. One isolate of each strain was saved in the Rosenberg bacterial collection.

Standard protocols (33) were used to make λ lysogen derivatives of FC40 and FC29 (to make SL585, SL586, SL608, and SL609, Table 4-1) using wild-type bacteriophage λ (strain SR109 in Rosenberg lab collection). The λ prophage was required in these strains to provide λcI repressor for regulating expression of *mutS* and *mutL* which are both under the control of the λP_L promoter in pMS312 and pAL51. When λP_L is repressed by cI, pMS312 and pAL51 express only low levels of *mutS* and *mutL*,

respectively (19, 27). SL1712 was created by standard phage P1 transduction using a P1 lysate from FS1892 (Table 4-1). Positive transductants were isolated on LBH sodium citrate tetracycline media, purified by streaking twice on the same media, and tested for rifampicin-resistance. Mutator phenotype was confirmed by spreading 10-15 µl of a saturated culture in a ~1 cm diameter circle on nalidixic acid (4 µg/ml) plates, as described above. One isolate was used for all further manipulations. SL1741 was created by disrupting the tetracycline resistance gene in RSH32 according to methods previously described (34). Briefly, RSH32 was lysogenized using standard methods (33) with λTSK (34), a recombinant temperature-sensitive λ-bacteriophage that carries the Tn*10*-*Bgl*II fragment (tetracycline-resistance sequence) disrupted by both streptomycin-resistance cassette, and kanamycin-resistance cassette insertions. Homologous recombination can result in λTSK insertion into the host Tn*10* tetracycline-resistance sequence to produce kanamycin-resistant, streptomycin-resistant, tetracycline-sensitive bacterial lysogens. Lysogens were plated on LBH (no antibiotic) medium and incubated at 42°C to cure the prophage and select temperature-resistant colonies. Two temperature-resistant isolates that were tetracycline-sensitive, kanamycin-sensitive, and streptomycin-resistant were chosen for further manipulation because, upon λTSK excision, the streptomycin-resistance gene must have remained linked to the genomic Tn*10*-sequence within *mutS*. This was confirmed by showing that mutator phenotype co-transduces with streptomycin-resistance when this strain is used as a P1 donor. Out of 20 transductants selected for streptomycin-resistance, all 20 displayed mutator phenotype using the nalidixic acid assay described for the pSUE1 construction, thus confirming linkage.

Adaptive mutation assay:

Adaptive mutation is measured (described by 32) as the frequency of reversion to lactose-fermentation of *lac* strain FC40 and its derivatives (32, Table 4-1), all of which are deleted for the genomic *lac-proB* region, but constitutively transcribe a *lacI-lacZ* fusion gene located on an F' plasmid. The F' *lacI* gene harbors a +1 frameshift mutation (CCC to CCCC) that is polar on *lacZ*, rendering the cells Lac$^-$. FC40 derivatives carrying a test plasmid, either a control vector, or a vector that expresses *mutS* and/or *mutL*, were plated on minimal lactose (0.1-0.2%) plates (as described by 35) containing an antibiotic appropriate for maintaining test plasmids in cells, and in the presence of excess Δ*lac* scavenger cells (derived from FC29, 32, Table 4-1) which carry the appropriate control plasmid vector. The scavenger cells consume any available non-lactose, extraneous carbon sources and cannot revert to Lac$^+$. The appearance of Lac$^+$ mutant colonies was measured over time in days. To monitor viable cells on the lactose plates over time, small plugs of agar were removed from between visible Lac$^+$ colonies, suspended in liquid media (M9, 14, or TB, as in 33 but at pH 7.5), and plated on rifampicin media (LBH or MacConkey lactose), on which the frameshift-bearing cells, but not the scavenger cells, can grow. Rifampicin plates either did contain, or did not contain, an additional drug to select for cells bearing test plasmids.

SUMMARY OF RESULTS AND DISCUSSION:

Multicopy plasmids that express either or both of two mismatch repair genes, *mutS* and *mutL*, were constructed (as described in Materials and Methods) for use in experiments that test whether overproduction of

MutS and/or MutL protein can depress the frequency of adaptive *lac* reversion. MutS and MutL are required during mismatch repair to recognize and bind mismatched bases. MutS recognizes and binds directly to mismatched bases (19). In the presence of ATP, MutL attaches to a MutS-DNA complex *in vitro* (36). During initiation of post-synthesis mismatch repair, MutL is proposed to coordinate binding, and nicking of the unmethylated DNA strand by MutH endonuclease near the closest (5' or 3') d(GATC) site to the MutSMutL-bound mismatch. Previous work suggested that MutL or MutH is rate-limiting in this process when it occurs in growing cells (37).

Plasmids expressing *Salmonella typhimurium mutS* and *mutL* genes: A set of three high-copy pBR322-derived plasmids were used initially for Mut protein overproduction in the adaptive mutation assay described (35, Materials and Methods). pGW1811 (17) expresses *Salmonella typhimurium mutS* and complements the mismatch repair-deficient (mutator) phenotype of an *E. coli* host that harbors a null mutation in *mutS* (17, Materials and Methods). pGW1842 (17), expresses *S. typhimurium mutL* and complements the mutator phenotype of an *E. coli* host with a null mutation in *mutL* (17, Materials and Methods). pRDK35 (30) is a control vector that does not carry any *mut* genes. All three plasmids carry the ampicillin-resistance gene. Therefore, all media used for these experiments contained ampicillin (Materials and Methods) for the purpose of selecting only plasmid-bearing cells. Two problems arose with the use of these plasmids for overexpression studies and are discussed below.

First, I observed that cells carrying pGW1811 (*mutS* overexpresser) lose this plasmid preferentially even if grown in media containing ampicillin. Plasmid retention during growth in ampicillin-containing liquid media was measured by plating dilutions of saturated cultures on solid agar media with and without the antibiotic (Materials and Methods). Cultures of cells carrying the control vector, pRDK35, always had equal or nearly equal viability on media with and without ampicillin. (Compare averages of 1.8 X 10^9 cells/ml on ampicillin media and 1.6 X 10^9 cells/ml on drug-free media calculated from platings of three parallel cultures). In contrast, cultures of cells carrying pGW1811 contained an average of 9.1 X 10^8 cells/ml total, of which 1.1 X 10^7 cells/ml (1.2%) were ampicillin-resistant (averages calculated for four parallel cultures). Therefore, although cells retain the control vectors, they most often lose pGW1811.

That ampicillin-sensitive cells are able to survive and grow in the presence of this drug when a few neighboring cells are ampicillin-resistant can be explained by considering how ampicillin-resistance is achieved. Ampicillin-resistance is conferred by an enzyme, ß-lactamase, that is secreted into the periplasmic space where it hydrolyzes, and thereby detoxifies, ampicillin extracellularly (38, 14). Therefore, if a few (*e. g.* the 1.2% of pGW1811-bearing cells per total cells in cultures from above) plasmid-bearing cells in a growing liquid culture secrete enough ß-lactamase to detoxify most of the antibiotic present, most cells (*e. g.* the other 99% of cells that lost pGW1811) in the culture do not need to harbor a plasmid conferring resistance to ampicillin. Because pGW1811, but not pRDK35, is lost preferentially by growing cells, overproduction of Salmonella MutS appears to be detrimental. Probably, MutS protein is somewhat toxic when overproduced.

The second problem with these plasmids arose with the use of minimal-lactose plates containing ampicillin for measuring adaptive reversion (see Materials and Methods). In one experiment, one saturated culture of strain SL552 (Table 4-1) that had lost 95% of plasmid-bearing cells still gave rise to adaptive mutants at a rate similar to the control strain (SL549) of which virtually all cells in the saturated culture carried plasmids. This observation suggested that the lactose-ampicillin plates were not selecting effectively for growth of only ampicillin-resistant cells. Most probably, the ampicillin was detoxified by the excess scavenger cells (strain SL568, Table 4-1) which carry the control plasmid vector pRDK35, and are plated together with the *lac* frameshift-bearing cells (see Materials and Methods).

The original three plasmids, pGW1811, pGW1842, and pRDK35, are not suited for these overexpression studies. Because the ampicillin-resistance which they confer is not confined to the cell harboring the plasmid, only a few cells secreting ß-Lactamase can allow nearby cells to survive free of plasmid and thereby avoid the apparently undesirable state of MutS-overproduction.

To avoid the problem of plasmid loss, derivatives of the above plasmids were constructed that carry different drug-resistance markers. pSUE1 (Materials and Methods, Figure 4-1) was constructed by inserting a kanamycin-resistance marker into pGW1811 (which expresses *mutS*). Unlike ampicillin-resistance, kanamycin-resistance apparently cannot be transferred to a nearby cell that is genotypically kanamycin-sensitive. Virtually all cells carrying either a control vector (pSL2, Figure 4-6) or pSUE1 (Figure 4-1) are resistant when grown in liquid culture in the presence of this antibiotic (data not shown). Plasmid retention was

measured as for pGW1811 above. pSL1 (Materials and Methods, Figure 4-2) is a derivative of pGW1842 (which expresses *mutL*) that carries the chloramphenicol-resistance marker. Chloramphenicol-resistance is conferred by the presence of a cytosolic enzyme, chloramphenicol acetyl transferase (CAT), which catalyzes modification of chloramphenicol to an inactive form intracellularly (14). Thus, chloramphenicol-resistance is nontransferrable to neighboring sensitive cells. However, pSL1 was found to be unsuitable for the adaptive mutation assay described because presence of chloramphenicol on the minimal-lactose plates used to perform these experiments resulted in poor viability of cells (unpublished observations). The control plasmid for pSL1 is pSL3 (Materials and Methods, Figure 4-7) that carries the *CAT* gene but no *mut* gene.

Plasmids expressing *E. coli mutS* and *mutL* genes: Although the Salmonella Mut proteins are reported to substitute functionally for the homologous *E. coli* proteins (17), novel interactions may result from presence of both Salmonella and *E. coli* Mut homologs in the same cell. If this were the case, then an effect of depressing adaptive mutation by overproducing Salmonella Mut protein(s) in a mismatch repair-proficient *E. coli* cell might be indirect. Additionally, although Salmonella *mut* genes complement respective *mut*-deficiency in *E. coli* using an assay that measures growth-dependent mutation rate (17, Materials and Methods), some other aspect of *mut* function or regulation may differ between these two homologs. This difference may affect an adaptive mutation phenotype caused by *mut* overexpression.

To avoid the potential ambiguities of results obtained using Salmonella *mut* gene overexpression, two low-copy plasmids, pMS312

and pAL51 that express *E. coli mutS* and *mutL*, respectively, were tested in adaptive mutation experiments. *mut* gene expression from both of these plasmids is regulated by the λP_L promoter, and in the absence of λcI repressor which regulates transcription from λP_L, *mut* genes are expressed at a very high level (19, 27, see also 31). In fact, no pMS312 or pAL51 transformants of FC40 were obtained after successive attempts at transformation with these plasmids probably because MutS and MutL proteins, at the levels produced in the nonrepressed state of λP_L, are toxic to cells. In the presence of λcI (supplied by a λ-prophage in SL585 and SL586) the *mut* genes are expressed only at a low level that is sufficient to complement respective *mut*-deficiency of a host cell (19, 27, Materials and Methods). Unfortunately, both plasmids carry the ampicillin-resistance marker such that the potential for plasmid loss (described above) exists. Therefore, the *mutS* and *mutL* genes carried by these plasmids were used to construct pSL5 (Figure 4-4), pSL6 (Figure 4-2), and pSL7 (Figure 4-5) which confer kanamycin-resistance (Materials and Methods). pSL5 expresses *E. coli mutL* and pSL6 expresses *E. coli mutS*. pSL7 expresses both *E. coli mutS* and *mutL*. We thought that overproducing MutL in conjunction with MutS might enhance an inhibitory effect of overproducing MutS or MutL alone during adaptive reversion. Both *mutS* and *mutL* genes are expressed independently of λP_L in these plasmids such that overexpression depends instead on the high-copy number of these plasmids per cell (21). The control plasmid that carries the kanamycin-resistance marker but no *mut* gene is pSL4 (Materials and Methods, Figure 4-8).

These plasmids (pSL4, pSL5, pSL6 and pSL7) have since been used by other investigators in the adaptive mutation experiments for which

they were designed. These experiments test whether MutS and/ or MutL overproduction can depress the frequency of adaptive reversion using the *lac* frameshift assay described (32, see Materials and Methods). Overproduction of MutS and/ or MutL should decrease the frequency of adaptive mutation if adaptive mutations result from a transient deficiency of mismatch repair due to limited amounts of either of these two proteins.

In a previous report, transcript levels of *mutS, mutL,* and *mutH* were found to drop to undetectable levels in late stationary-phase cells (39). Furthermore, MutS and MutH protein levels decrease 10-fold and 3-fold, respectively, whereas MutL protein levels remain constant in late stationary-phase cells. In bacteria starved under the same conditions as in the Lac adaptive mutation assay described (32), levels of MutL protein remain essentially constant whereas the amount of MutS protein drops to undetectable levels (39). The decreased levels of MutS and MutH protein could be due to the relative instability of these proteins in a cell, or due to degradation or inactivation by another gene product(s) which is itself regulated by cell physiology. Because of the decreased amounts of MutS protein under the conditions of adaptive Lac reversion, these results might support the idea that mismatch repair is deficient in bacteria mutating adaptively. However, from these results it was also possible that starving cells do not experience a deficiency in mismatch repair despite low levels of MutS because of a concomitant decrease in DNA replication during stationary phase and starvation. This question is addressed by the adaptive mutation experiments that test the effects of MutS and MutL overproduction.

Previous reports demonstrate a 2-fold (40) to 5-fold reduction in adaptive mutation in strains overproducing MutS plus MutL as compared

with a control strain. These investigators used the same frameshift-bearing parent used in this study, but which instead carries two plasmids: a pBR322-derived plasmid that expresses *E. coli mutS*, and a compatible high-copy plasmid which expresses *E. coli mutL* (41, 40). Plasmids were retained in cells by selecting for carbenicillin-resistance (encoded by the *mutS*-carrying plasmid) and chloramphenicol-resistance (encoded by the *mutL*-carrying plasmid). In one set of these experiments (41, but not the other, 40) however, they observed that overexpressing these two mismatch repair genes also decreased mutation to Lac^+ during growth. The authors therefore argued that reduced levels of mutation to Lac^+ by increased levels of MutS and MutL proteins may not be specific to adaptive mutagenesis. However, when MutS plus MutL were overproduced in a *recG* derivative of the frameshift-bearing strain (a *recG* strain is hypermutable for adaptive mutation, 40, 42), Lac^+ adaptive reversion is decreased to a level below even the control strain. Growth-dependent reversion frequencies remain unaffected (40). Thus, the effect of overproducing MutS and MutL proteins is specific to adaptive mutagenesis in the *recG* strain.

Results obtained by others performing the *mut*-overexpression experiments with the plasmids described in this chapter (pSL4, pSL5, pSL6, and pSL7) show that overproduction of MutL protein (with pSL5 or pSL7) causes a 4-fold decrease in the frequency of adaptive Lac^+ reversion. No change in adaptive reversion frequency results from overproduction of MutS (25). In contrast, Mut protein overproduction does not cause a decrease in spontaneous growth-dependent mutation frequency. Thus, providing more MutS and MutL protein does not decrease mutation frequency generally, but MutL-overproduction affects adaptive

mutagenesis specifically (25). These results indicate that MutL protein either is limiting, or stabilizes another protein that is limiting, during adaptive Lac reversion. These findings are the first to show that mismatch repair is regulated by environmental stimuli.

The results obtained by others using the plasmids described in this chapter support the idea that adaptive mutation occurs when post synthesis mismatch repair is disabled temporarily by depletion of MutL protein. Thus, MutL may be rate-limiting for mismatch repair in both growing cells (as suggested by 37) as well as cells mutating adaptively (25). Discovering if and how the levels of these proteins are regulated, particularly in response to environmental stimuli, would be important for understanding how mutations arise in bacteria, but also in eukaryotes with homologs of Mut proteins that could be regulated similarly. For example in humans, 90% of examined individuals affected with hereditary nonpolyposis colon cancer carry a defect in either a *mutS* or *mutL* homolog (*e. g.* 9, 43). Defect(s) in a human homolog of a putative *E. coli* Mut protein regulator(s) might lead to the same or different cancers.

Table 4-1. ***Escherichia coli*** **strains for use in this study.**

Strain Name	SMR* Collection Number	Relevant Genotype	Reference or Source
C600	127	wild-type	SMR collection
ES1481	685	*mutS215*::Tn*10*	SMR collection
FC40	506	*ara*Δ(*lac-proB*)XIII *thi* Rif^R[F'*lacI33*]	Reference 32
FC29	504	*ara*Δ(*lac-proB*)XIII *thi*[F'Δ(*lacI-lacZ*)]	Reference 32
FCY2	430	wild-type	Reference 44
FS1892	116	594 *mutL218*::Tn*10*	SMR collection
JC9937	173	*rec*+ Su- AB1157 derivative	SMR collection
RSH32	749	FC40 *mutS215*::Tn*10*	SMR collection
SL549	549	FC40[pRDK35]	Materials & Methods
SL550	550	FC40[pGW1842]	Materials & Methods
SL552	552	FC40[pGW1811]	Materials & Methods
SL568	568	FC29[pRDK35]	Materials & Methods
SL584	584	FC40(λ)[pAL6]	Materials & Methods
SL585	585	FC40(λ)[pAL51]	Materials & Methods
SL586	586	FC40(λ)[pMS312]	Materials & Methods
SL591	591	FC40(λ)	Materials & Methods
SL608	608	FC40(λ)[pSCC5077]	Materials & Methods

Strain	SMR #	Genotype	Reference
SL609	609	FC29(λ)[pSCC5077]	Materials & Methods
SL613	613	AB1157(λ) *mutH471*::Tn*5*	Materials & Methods
SL653	653	FC40[pGW1899]	Materials & Methods
SL663	663	FC40[pGW1811] reconstructed	Materials & Methods
SL664	664	SZ622[pGW1811]	Materials & Methods
SL1311	1311	FC40[pSUE1]	Materials & Methods
SL1318	1318	RSH32[pSUE1]	Materials & Methods
SL1320	1320	SZ620[pSL1]	Materials & Methods
SL1321	1321	FC40[pSL1]	Materials & Methods
SL1322	1322	FC29[pSL2]	Materials & Methods
SL1323	1323	FC40[pSL2]	Materials & Methods
SL1506	1506	FC40[pSL3]	Materials & Methods
SL1507	1507	FC29[pSL3]	Materials & Methods
SL1706	1706	594 *mutL218*::Tn*10* [pSL5]	Materials & Methods
SL1712	1712	FC40 *mutL218*::Tn*10*	Materials & Methods
SL1713	1713	FC40[pSL5]	Materials & Methods
SL1741	1741	FC40 *mutS*::Tn*10* -strep	Materials & Methods
SL1746	1746	SL1712[pSL5]	Materials & Methods
SL1747	1747	FC40[pSL6]	Materials & Methods
SL1748	1748	RSH32[pSL6]	Materials & Methods

Strain	SMR #	Genotype	Reference
SL1786	1786	FC40 *mutS*::Tn*10*-strep *mutL218*::Tn*10*	Materials & Methods
SL1790	1790	FC40[pSL4]	Materials & Methods
SL1791	1791	FC40[pSL7]	Materials & Methods
SMR369	369	JC9937 *mutL211*::Tn*5*	SMR collection
SMR437	437	FCY2 *mutL211*::Tn*5*	SMR collection
SMR438	438	FCY2 *mutS201*::Tn*5*	SMR collection
SZ620	620	FC40 *mutL211*::Tn*5*	SMR collection
SZ622	622	FC40 *mutS201*::Tn*5*	SMR collection
SZ623	623	FC40 *mutH471*::Tn*5*	SMR collection

* S. M. Rosenberg

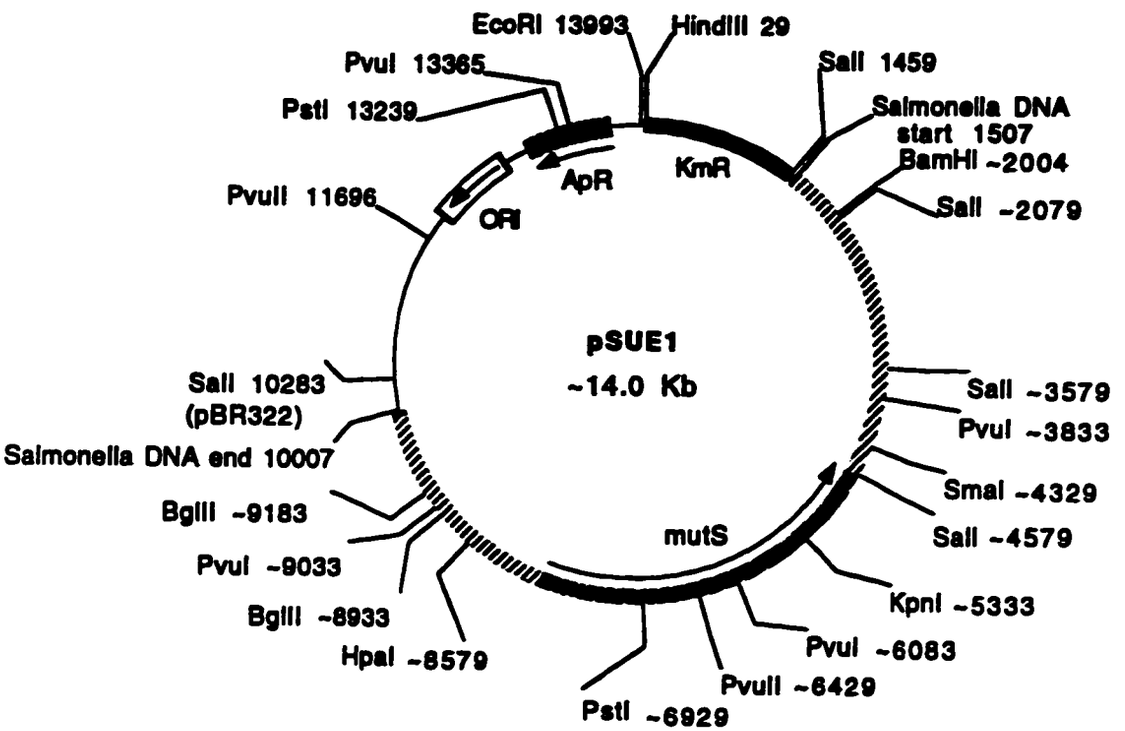

Figure 4-1. Expected restriction map of pSUE1, a high-copy number plasmid that expresses Salmonella *mutS* from its natural promoter. Restriction sites are indicated outside the circle starting from nucleotide position number 1 of pBR322 (18) with approximate locations (estimated from 14) denoted by the symbol (~). The sites indicated are derived from maps of the parental plasmids used to construct pSUE1. Genes are labelled inside the circle and arrows denote the direction of transcription when known. The dark-colored hatched box represents the *mutS* gene of *Salmonella typhimurium*. The light-colored hatched box represents Salmonella DNA flanking the *mutS* gene. Junctions between pBR322 and Salmonella DNA are indicated as sites. ORI, pBR322 origin of replication. ApR, ampicillin resistance gene. KmR, kanamycin resistance gene. (Construction and mapping are described in the text).

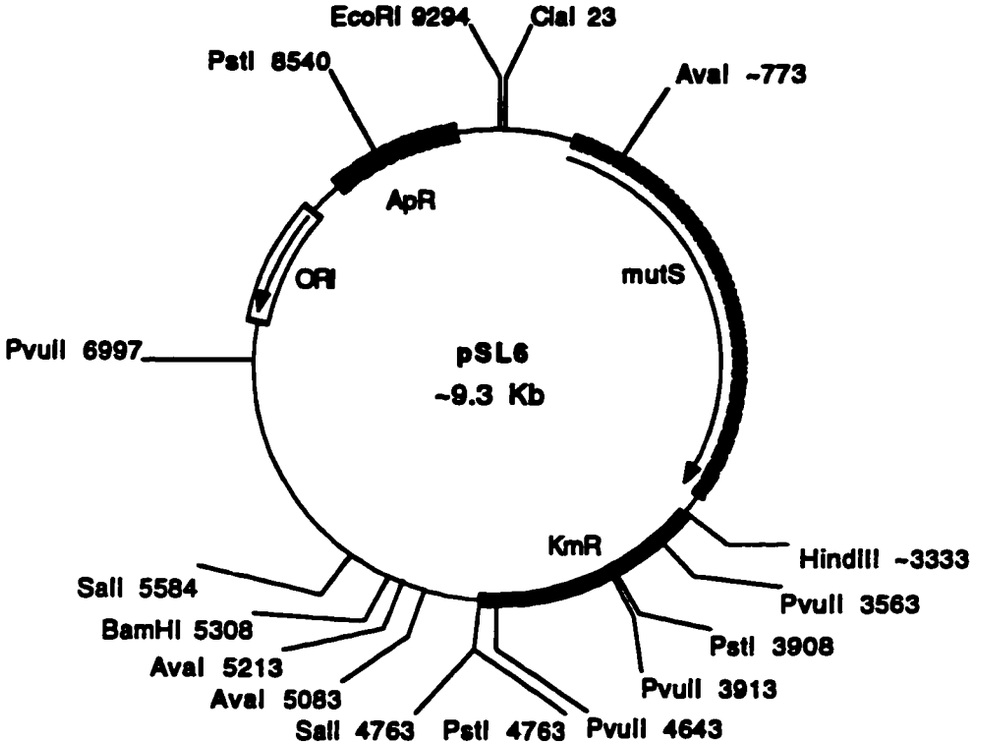

Figure 4-2. Expected restriction map of pSL6, a high-copy number plasmid that expresses the *E. coli mutS* gene from its own promoter. Restriction sites with their positions in basepairs are indicated outside the circle starting at the nucleotide in position number 1 in pBR322 (18). Approximate locations denoted by the symbol (~). Sites indicated are derived from the published partial restriction maps of pMS312 (16), pBR322 (18) and pKC31 (15). Boxes represent the extent of coding sequence of the corresponding gene, and arrows indicate the direction of transcription when known. ORI, pBR322 origin of replication. ApR, ampicillin resistance gene. KmR, kanamycin resistance gene. (Construction and mapping are described in the text).

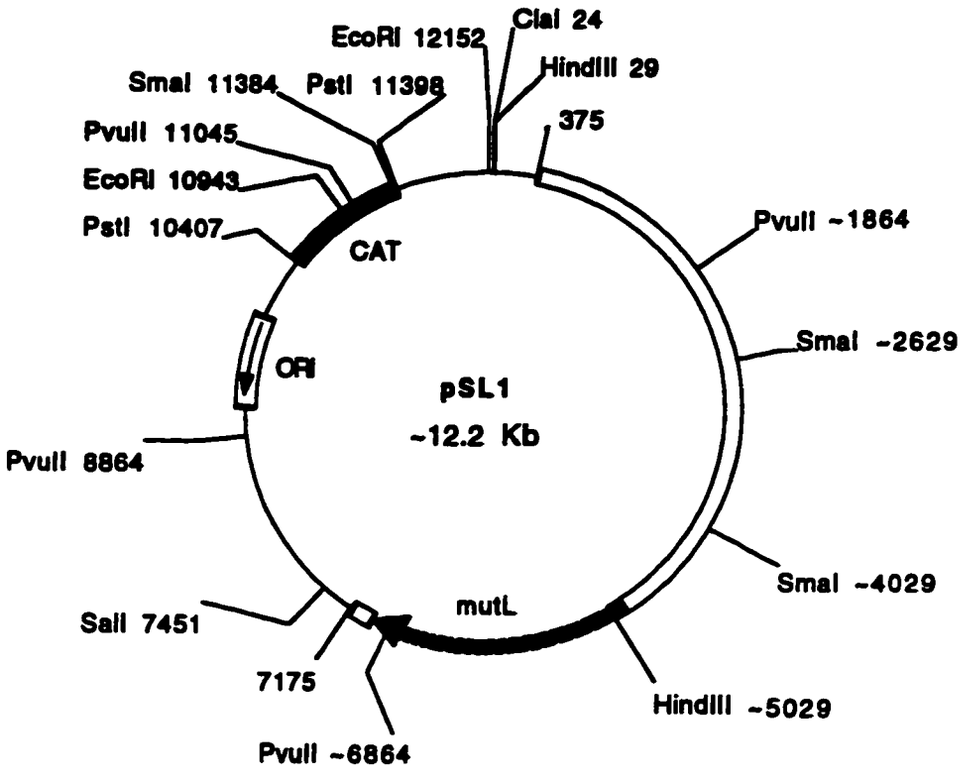

Figure 4-3. Expected restriction map of pSL1, a high-copy number plasmid that expresses Salmonella *mutL* from its natural promoter. Restriction sites are indicated outside the circle starting from the nucleotide in position number 1 in pBR322 (18), with approximate locations denoted by the symbol (~) as estimated from the partial restriction map of pGW1842 (14). All sites indicated are derived from maps of pGW1842 (14), pBR322 (18), and pCAT19 (25). Genes are labelled inside the circle and arrows denote the direction of transcription and extent of coding sequence when known. The dark-colored hatched box represents the *mutL* gene of *Salmonella typhimurium*. The white box represents Salmonella DNA flanking the *mutL* gene. Junctions between Salmonella and pBR322 DNA are indicated as sites (375 and 7175). ORI, pBR322 origin of replication. CAT, chloramphenicol resistance gene. (Construction and mapping are described in the text).

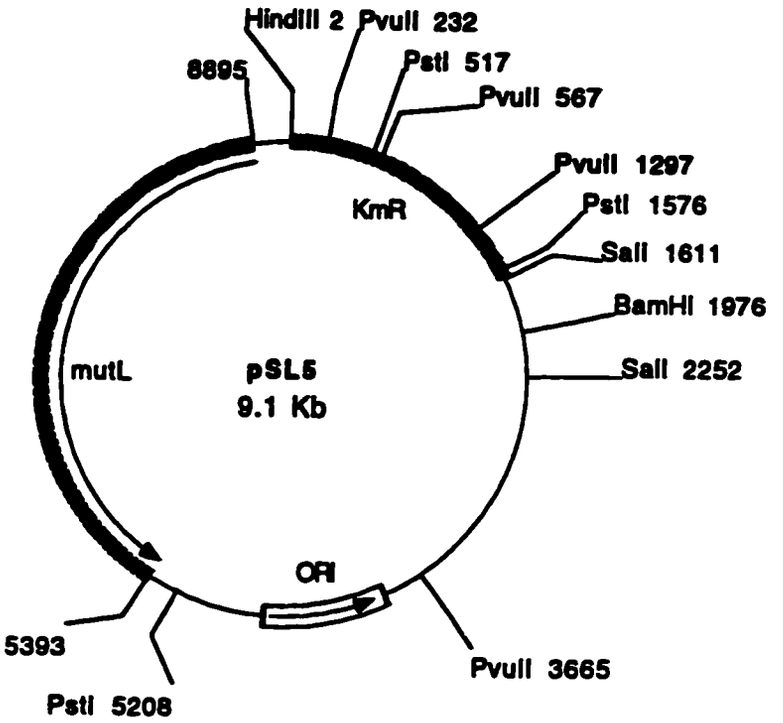

Figure 4-4. Expected restriction map of pSL5, a high-copy number plasmid that expresses *E. coli mutL* from its natural promoter. Restriction sites and their positions in basepairs are indicated outside the circle. Genes are labelled inside the circle. All sites are derived from maps of pBR322 (18), pKC31 (15), and pAL51 (22). Boxes represent the extent of coding sequence for the corresponding gene and arrows show the direction of transcription when known. ORI, pBR322 origin of replication. KmR, kanamycin resistance gene. (Construction and mapping are described in the text).

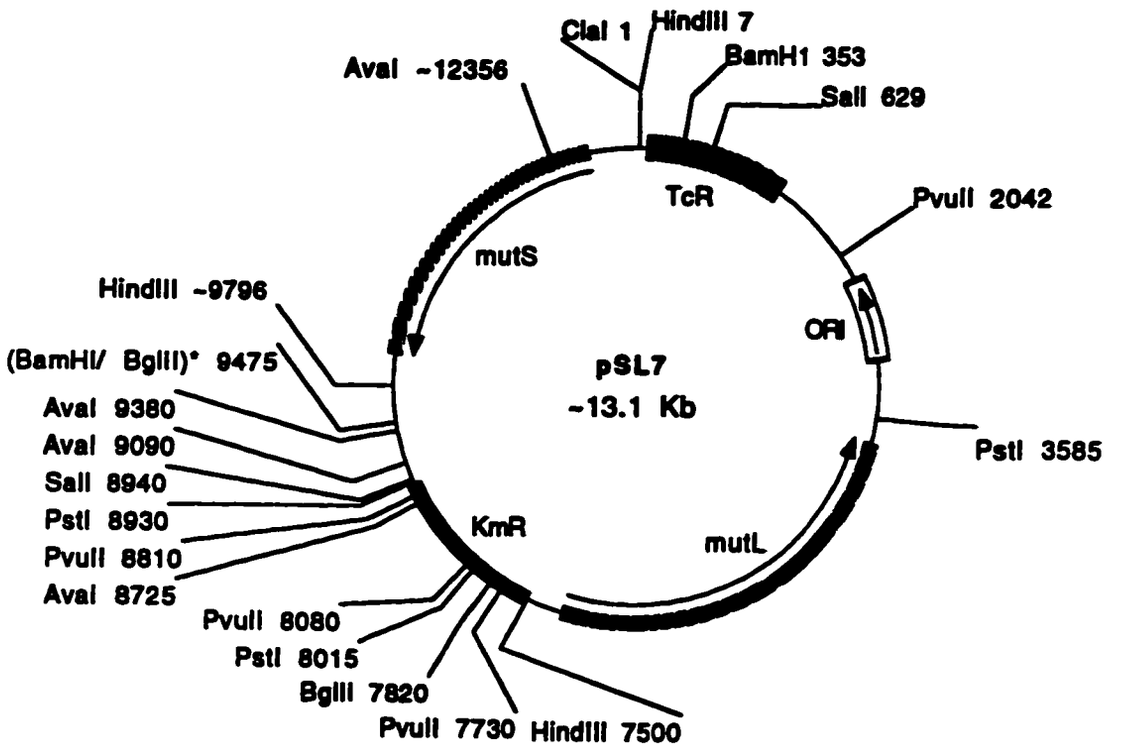

Figure 4-5. Expected restriction map of pSL7, a high-copy number plasmid that expresses *E. coli mutL* and *mutS* from their natural promoters. Restriction sites with their positions in basepairs are indicated outside the circle. All sites indicated are derived from published maps of pBR322 (18), pMS312 (16), pAL51 (22) and pKC31 (15). Position number 9475 marked with an asterisk (*) is the point of ligation between complimentary ends created by *Bam*HI and *Bgl*II digests (see construction information). Approximate locations are denoted by the symbol (~) and are obtained from the published partial restriction map of pMS312 (16). Genes are labelled inside the circle and arrows denote the direction of transcription and extent of coding sequence when known. ORI, pBR322 origin of replication. KmR, kanamycin resistance gene. TcR, tetracycline resistance gene. (Construction and mapping are described in the text).

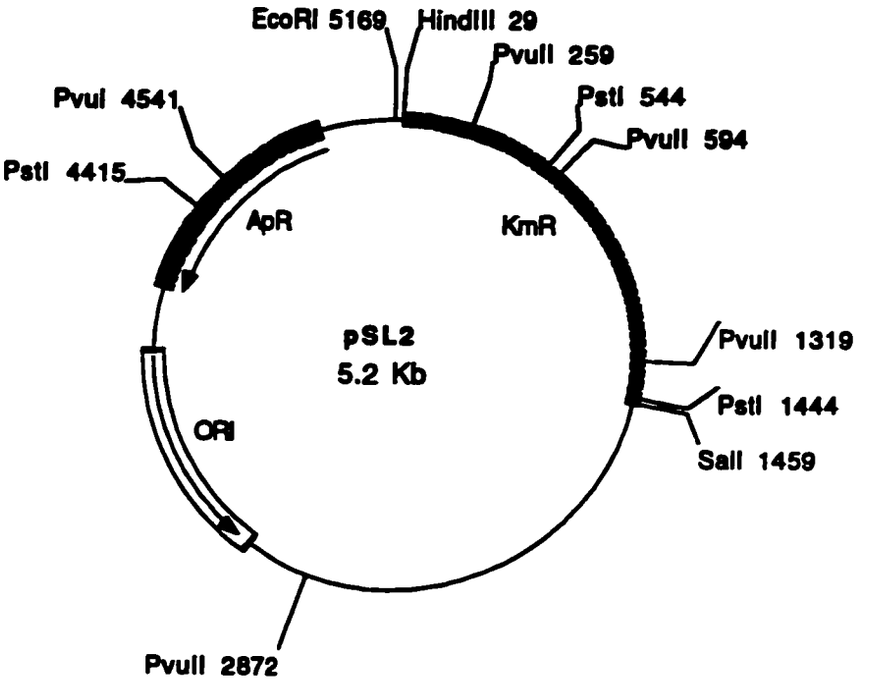

Figure 4-6. Expected restriction map of pSL2, a control plasmid for pSUE1, pSL5, pSL6, and pSL7. pSL2 does not carry any *mut* gene, and is otherwise identical with pSUE1. Restriction sites are indicated outside the circle with a corresponding position number in basepairs beginning from the nucleotide in position number 1 in pBR322 (18). All sites indicated are derived from maps of pBR322 (18), pGW1811 (14), and pKC31 (15). Genes are labelled inside the circle and arrows denote the direction and extent of coding sequence when known. ORI, pBR322 origin of replication. ApR, ampicillin resistance gene. KmR, kanamycin resistance gene. (Construction and mapping are described in the text).

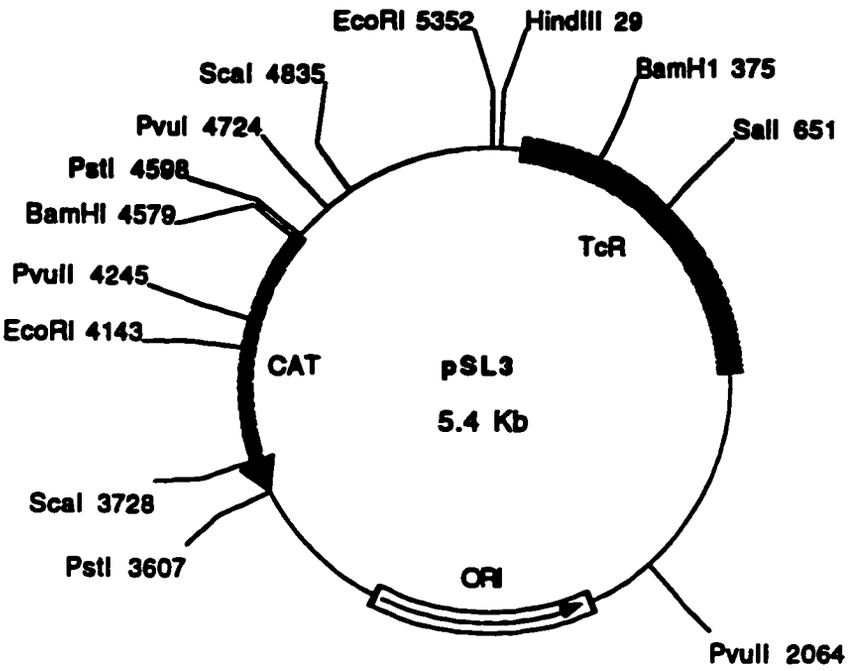

Figure 4-7. Expected restriction map of pSL3, a control plasmid for pSL1, with which pSL3 is identical except for its lack of a *mut* gene. Restriction sites with their positions in basepairs are indicated outside the circle starting from the first nucleotide in pBR322 (18). All were derived from maps of pBR322 (18) and pCAT19 (25). Genes are labelled inside the circle; boxes and arrows denote the extent and direction of coding sequence when known. ORI, pBR322 origin of replication. CAT, chloramphenicol resistance gene. TcR, tetracycline resistance gene. (Construction and mapping are described in the text).

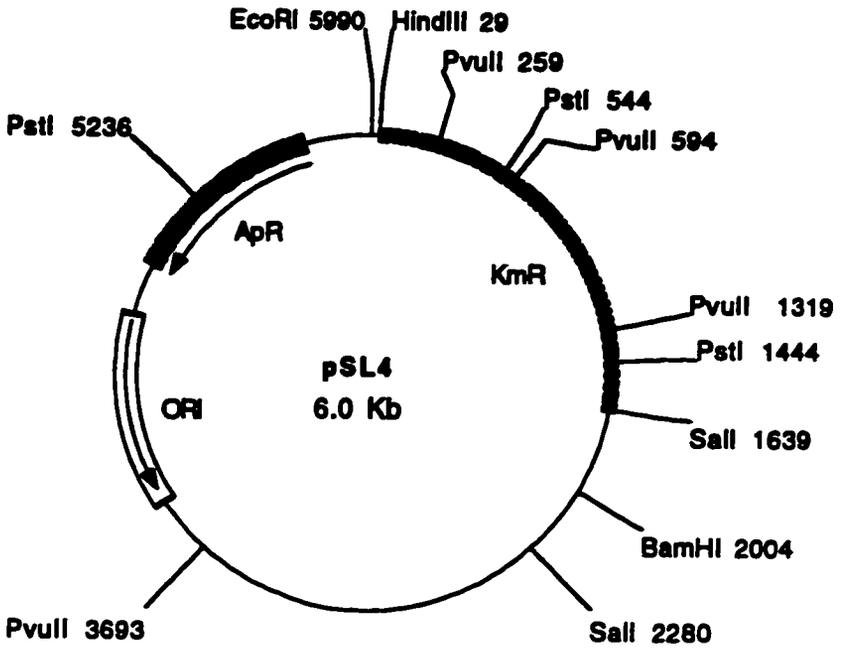

Figure 4-8. Expected restriction map of pSL4 , a control plasmid for pSL5, pSL6 and pSL7. pSL4 does not express any *mut* gene and is otherwise identical with pSL6. Restriction sites with their position in basepairs are indicated outside the circle. All sites are derived from maps of pBR322 (18), and pKC31 (15). Genes are labelled inside the circle; boxes represent the extent of coding sequence and arrows denote the direction of transcription when known. ORI, pBR322 origin of replication. KmR, kanamycin resistance gene. ApR, ampicillin resistance gene. (Construction and mapping are described in the text).

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5. CONCLUSIONS

This thesis study was aimed at addressing two questions: (i) Are adaptive reversion mutations in *lac* templated or nontemplated?; and (ii), does the formation of these reversion mutations occur during a suspension of MutHLSU mismatch repair?

The first question has been answered. Sequencing Lac$^+$ reversion mutations revealed that adaptive mutations are almost exclusively -1 deletions in small runs of mononucleotides (Chapter 2, see also 1). The -1 deletions occur at many different sites and are not associated with extraneous sequence changes that might have been cotransferred from a donor template. These results therefore make templated models highly improbable, and support nontemplated models in which the adaptive mutations are made *de novo*. The sequences in Chapter 2 also yielded four additional important discoveries, one of which addresses the second question.

First, in striking contrast to the adaptive mutations, growth-dependent reversions of the same +1 frameshift mutation in *lac* (2) are a potpourri of big and small deletions, insertions and duplications, and are not confined to repeat regions (Chapter 2, see also 1). The dissimilarity in reversion mutations of adaptive and growth-dependent reversion sequences demonstrates conclusively that adaptive mutations are different from growth-dependent (Luria-Delbrück) mutations.

Second, a derivative of the frameshift-bearing strain that carries a *recD* mutation conferring *recA-*, *recB*-dependent hypermutability for adaptive (but not growth-dependent) mutation (3), has the same unique sequence spectrum of reversion mutations as the *rec*$^+$ parent. This

supports the previous conclusion that *recD* mutants generate adaptive mutations using the same adaptive mutation pathway as a *rec*$^+$ strain. These results, and more recently the requirement for Holliday junction processing enzymes (4, 5), provide further support for the involvement of recombination in the adaptive mutation mechanism.

Third, because small deletions in repeated regions are typical errors made by DNA polymerases (6), the sequence spectrum of adaptive mutations strongly suggests that a polymerase generates adaptive mutations. DNA Polymerase III of *E. coli* has been implicated in this role (7, 8).

Fourth, the adaptive mutation sequences also support the involvement of another system governing DNA metabolism -- MutHLSU mismatch repair. *E. coli* (9-12), yeast (13), and human cancer cells (14-16) defective in mismatch repair display a mutation spectrum similar to the sequence spectrum of adaptive *lac* mutations. Based on these observations, a simple model for how adaptive mutations form in the *lac* frameshift system is that adaptive and growth-dependent mutations form by essentially similar polymerase errors, but that adaptive mutations form in the absence of mismatch repair. We proposed (Chapter 2, see also 1) that mismatch repair might be down-regulated in starving cells during recombination-dependent adaptive mutagenesis. The experiments presented in Chapter 3 and 4 address this hypothesis more directly.

The results in Chapter 3 show that by disabling mismatch repair in growing cells, growth-dependent reversion sequences in *lac* (Table 2-1) are made indistinguishable from adaptive mutations. Thus, the failure of mismatch repair, or its strand discrimination mechanism, is a sufficient explanation for the unique sequence spectrum of adaptive mutations. The

idea that mismatch repair is suspended transiently in starving, adaptively mutating cells is supported by these data. Suspension of mismatch repair must be transient because Lac$^+$ mutants, once isolated and purified, are mismatch repair-proficient (Chapter 3, see also 17). The MutHLSU repair system could be disabled temporarily during adaptive mutation by: (i) down-regulation of *mutH, -L, -S,* or *-U* gene expression (18); (ii) depletion of any of these gene products during starvation (19) possibly *via* saturation of the repair system by copious mismatches (20); or transient under- or overmethylation of DNA to cause either loss of strand discrimination of repair, or blockage of MutH endonucleolytic cleavage (see 9), respectively.

Hypotheses (i) and (ii) are now supported by *mut* gene overexpression studies performed using the plasmids presented in Chapter 4 (21). We thought that if Mut proteins are absent, or present only at very low levels in cells during adaptive mutation, then adaptive mutation might be inhibited in cells overproducing Mut proteins. The results of Harris *et al.* show that overproducing *E. coli* MutL protein in cells mutating adaptively can decrease the rate of adaptive *lac* reversion. Also, *lac* +1 frameshift-bearing cells carrying a *recG* mutation, which confers adaptive hypermutability, are very severely depressed for adaptive reversion when they overproduce *E. coli* MutS plus MutL simultaneously (4). Together, these results suggested that the amount of MutL protein drops during starvation to a level insufficient for normal mismatch repair.

In starving cells, as compared with growing cells, MutS and MutH (but not MutL) protein levels drop 10- fold and 3-fold respectively (22). However, in cells that have overproduced MutL protein from a *mutL*-expressing plasmid, pSL5 or pSL7 (Chapter 4), the levels of MutH and

MutS still decrease after a few days of starvation (21). This suggests that MutL overproduction depresses the frequency of adaptive Lac reversion directly, not by stabilization of MutH or MutS. Though present, most MutL may be in an inactive form such that overproduction supplies enough active MutL for mismatch repair. Therefore, mismatch repair may be regulated physiologically by the availability of functional MutL. MutL may be rate-limiting for mismatch repair in both growing cells (23), and in cells mutating adaptively.

A model for adaptive mutation in the *lac* system (described by 3) has been expanded based on the work in this thesis study (see also 24). In this model, stress due to starvation induces DNA double-strand breaks (DSBs), the substrate for the RecBCD enzyme (see Introduction for a brief description and references). RecBCD loads at these DSBs, and is proposed to promote recombination by acting as a helicase that unwinds the DNA to produce single-strands able to invade a homologous (or partially homologous) DNA molecule. Recombination-associated DNA synthesis (for example, synthesis could be primed by a 3'-ended invading single-strand, see 5), coupled with suspended mismatch repair in these cells, might generate the adaptive mutations. If a cell makes a successful mutation to Lac^+, it will have the ability to divide and grow into a colony using lactose as a carbon source. When no longer starving, the Lac^+ cell will stop accumulating DSBs, and also restore mismatch repair. If a cell is not successful in becoming Lac^+, DSBs will continue to accumulate and cause eventual death of the cell. Because no net death of cells on the selective plates occurs, only a few cells in the population are proposed to ever enter such a hypermutable state (similar to that proposed by Hall, 24, now supported in this system by 17). This mechanism of generating

adaptive mutations supports Darwinian ideas, not Lamarckism, because hypermutation is genome-wide.

Thus far, studying adaptive mutations has led to a further important understanding of how mutations form when they are beneficial to survival. Studying them in the *lac* frameshift assay system used here has also led to the partial description of a novel molecular mechanism of mutagenesis in nongrowing cells.

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APPENDIX A. COMPLETE COLLECTION OF BACTERIAL STRAINS CONSTRUCTED WHILE IN THE ROSENBERG LABORATORY

SMR[1] Collection Number	Strain	Genotype	Parents & Construction Information
540	SL540	FC40 *dnaE486ts* *zae*::Tn*10d*-Cam	P1 NR9779 (bacteria from Roel Schaaper) X #506, screened for temperature-sensitivity
541	SL541	FC40 *zae*::Tn*10d*-Cam	P1 NR9779 (bacteria from Roel Schaaper) X #506, selected for temperature-resistance.
542	SL542	FC40 *mutS1ts* Tn*10* *dnaE486ts* *zae*::Tn*10d*-Cam	P1 NR9779 (bacteria from Roel Schaaper) X #513
543	SL543	FC40 *mutS1ts*::Tn*10* *zae*::Tn*10d*-Cam	P1 NR9779 (bacteria from Roel Schaaper) X #513, selected for temperature-resistance.
544	SL544	FC40 *tnaA*::Tn*10*	P1 #457 X #506, selected for temperature-resistance.
549	SL549	FC40 [pRDK35]	#506 X pRDK35 from SMR[1]-prepared DNA.
550	SL550	FC40 [pGW1842]	#506 X pGW1842 from #421 miniprep, large colonies.
551	SL551	FC40 [pGW1842]	#506 X pGW1842 from #421 miniprep, small colonies.
552	SL552	FC40 [pGW1811]	#506 X pGW1811 from #422 miniprep.
568	SL568	FC29 [pRDK35]	#504 X pRDK35 from SMR[1]-prepared DNA

584	SL584	FC40 (λ) [pAL6]	#591 X pAL6 DNA from Paul Modrich.
585	SL585	FC40(λ) [pAL51]	#591 X pAL51 DNA from Paul Modrich
586	SL586	FC40(λ) [pMS312]	#591 X pMS312 DNA from Paul Modrich.
587	SL587	FC29(λ) [pRDK35]	#592 X pRDK35 from SMR[1]-prepared DNA
591	SL591	FC40 (λ)	#506 X λ#109
592	SL592	FC29 (λ)	#504 X λ#109
594	SL594	FC40(λ) [pRDK35]	#591 X pRDK35 from SMR[1]-prepared DNA
608	SL608	FC40(λ) [pSCC5077]	#591 X pSCC5077 DNA from Paul Modrich.
609	SL609	FC29(λ) [pSCC5077]	#592 X pSCC5077 DNA from Paul Modrich.
613	SL613	AB1157 (λ)*recF143 mutH471*::Tn*5*	#218 X λ#109
614	SL614	594 (λ)*uvrD282*::Tn*5* -Sm (?)	#362 X λ#109 (Sm-resistance was not confirmed).
653	SL653	FC40 [pGW1899]	#506 X pGW1899 from #648 miniprep.
662	SL662	FC40 [pGW1842]	#506 X pGW1842 from #421 miniprep; reconstruction of #550, #551.
663	SL663	FC40 pGW1811]	#506 X pGW1811 from #422 miniprep; reconstruction of #552.

664	SL664	FC40 *mutS201*::Tn*5* [pGW1811]	#622 X pGW1811 from #422 miniprep.
766	SL766	FC40 [pACYC184]	#506 X pACYC184 from #283 minprep.
767	SL767	FC29 [pACYC184]	#540 X pACYC184 from #283 miniprep.
1311	SL1311	FC40 [pSUE1]	#506 X pSUE1 from #1317 miniprep.
1317	SL1317	ES1481 [pSUE1]	#685 X pSUE1 from plasmid ligation.
1318	SL1318	FC40 *mutS215*::Tn*10* [pSUE1]	#749 X pSUE1 from #1317 miniprep.
1319	SL1319	C600 *recD1009* [pSL1]	#130 X pSL1 from miniprep of #369 [pSL1], a strain made during pSL1 plasmid construction but not frozen. The same plasmid prep. was used to construct #1320 and 1321.
1320	SL1320	FC40 *mutL211*::Tn*5* [pSL1]	#622 X pSL1 from pSL1 plasmid construction. (See #1319).
1321	SL1321	FC40 [pSL1]	#506 X pSL1 from pSL1 plasmid construction. (See #1319).
1322	SL1322	FC29 [pSL2]	#504 X pSL2 from miniprep of #685 [pSL2], a strain made during plasmid construction but not frozen. The same plasmid prep. was used to construct #1323.
1323	SL1323	FC40 [pSL2]	#506 X pSL2 from pSL2 plasmid construction. (See #1322).

1324	SL1324	FC29 [pACYC184]	#504 X pACYC184 from miniprep of #283; reconstruction of #766.
1325	SL1325	FC29 [pKC31]	#504 X pKC31 from #333 miniprep.
1495	SL1495	C600 [pBR322]	#127 X pBR322 DNA from Bruce Malcolm.
1506	SL1506	FC40 [pSL3]	#506 X pSL3 from miniprep of C600 [pSL3], a strain made during construction of pSL3, but not frozen. The same plasmid prep. was used to construct #1507.
1507	SL1507	FC29 [pSL3]	#504 X pSL3 from pSL3 plasmid construction. (See #1506).
1582	SL1582	FC40 Lac^+ [pSUE1]	Lac^+ isolate #1 of #1311 obtained from plating on lactose-minimal kanamycin medium. Three additional, separate Lac^+ isolates from this selection are strains #1583, 1584 and 1585.
1583	SL1583	FC40 Lac^+ [pSUE1]	Lac^+ isolate #2 of #1311. (See #1582).
1584	SL1584	FC40 Lac^+ [pSUE1]	Lac^+ isolate #3 of #1311. (See #1582).
1585	SL1585	FC40 Lac^+ [pSUE1]	Lac^+ isolate #4 of #1311. (See #1582).
1586	SL1586	FC40 Lac^+ [pSL2]	Lac^+ isolate #1 of #1323 obtained from plating on lactose-minimal kanamycin medium. Three additional, separate isolates from this selection are strains #1587, 1588 and 1589.
1587	SL1587	FC40 Lac^+ [pSL2]	Lac^+ isolate #2 of #1323. (See #1586).

1588	SL1588	FC40 Lac$^+$ [pSL2]	Lac$^+$ isolate #3 of #1323. (See #1586).
1589	SL1589	FC40 Lac$^+$ [pSL2]	Lac$^+$ isolate #4 of #1323. (See #1586).
1590	SL1590	FC40 Lac$^+$ *mutS215*::Tn*10* [pSUE1]	Lac$^+$ isolate #1 of #1318 obtained from plating on lactose-minmal kanamycin media. Two additional, separate isolates are strains #1591 and 1592.
1591	SL1591	FC40 Lac$^+$ *mutS215*::Tn*10* [pSUE1]	Lac$^+$ isolate #2 of #1318. (See #1590).
1592	SL1592	FC40 Lac$^+$ *mutS215*::Tn*10* [pSUE1]	Lac$^+$ isolate #3 of #1318. (See 1590).
1677	SL1677	FC40 [pT110]= retron	#506 X pT110 from #3383 miniprep.
1678	SL1678	FC40 [pBT339]= retron	#505 X pBT339 from #3384 miniprep.
1679	SL1679	FC40 [pT161]= retron	#506 X pT161 from #3385 miniprep.
1706	SL1706	594 *mutL218*::Tn*10* [pSL5]	#116 X pSL5 from miniprep of C600 [pSL5], a strain made during construction of pSL5, but not frozen. The same plasmid prep. was used to construct #1713 and 1746.
1712	SL1712	FC40 *mutL218*::Tn*10*	P1 #116 X #506
1713	SL1713	FC40 [pSL5]	#506 X pSL5 from pSL5 plasmid construction. (See #1706).

1741	SL1741	FC40 *mutS*::Tn*10*-strep	#749 "Tet-blasted" isolate #1.
1742	SL1742	FC40 *mutS*::Tn*10*-strep	#749 "Tet-blasted" isolate #18.
1746	SL1746	FC40 *mutL218*::Tn*10* [pSL5]	#1712 X pSL5 from pSL5 plasmid construction. (See #1706).
1747	SL1747	FC40 [pSL6]	#506 X pSL6 from miniprep of C600 [pSL6], a strain made during construction of pSL6, but not frozen. The same miniprep was used to construct #1748.
1748	SL1748	FC40 *mutS215*::Tn*10* [pSL6]	#749 X pSL6 from pSL6 plasmid construction. (See #1747).
1786	SL1786	FC40 *mutS*::Tn*10*-strep *mutL218*::Tn*10*	P1 #116 X #1742
1787	SL1787	FC40 *mutS*::Tn*10*-strep *mutL218*::Tn*10*	P1 #116 X #1741
1790	SL1790	FC40 [pSL4]	#506 X pSL4 from miniprep of C600 [pSL4] made during construction of pSL4, but not frozen.
1791	SL1791	FC40 [pSL7]	#506 X pSL7 from miniprep of C600 [pSL7] made during construction of pSL7, but not frozen.
1792	SL1792	FC40 *mutS215*::Tn*10* (λTSK)	#749 X λ#181

3383		AB1157 [pT110]=retron	Strain received from Werner Maas February, 1995 in stab. Overnight was grown in TB ampicillin (Chapter 4) May, 1996, then struck for single colonies on LBH (Chapter 4) twice to purify. A single colony was chosen to make an overnight in TB ampicillin to freeze into SMR collection. Plasmid construction reference: Maas, W. K., Wang, C., Lima, T., Zubay, G. and Lim, D. 1994. *Mol. Microbiol.* 14: 437-441.
3384		AB1157 [pBT339]=retron	Strain received from Werner Maas February, 1995. Frozen into SMR collection as for #3383, except that the overnight was grown in TB kanamycin. See #3383 for reference.
3385		AB1157 [pT161]=retron	Strain received from Werner Maas February, 1995. It was frozen into SMR collection as for #3383. See #3383 for reference.

[1] S. M. Rosenberg

APPENDIX B. LAC+ COLLECTION FOR SEQUENCES IN CHAPTER 2 (ADAPTIVE AND GROWTH-DEPENDENT MUTANTS OF *rec*+ AND *recD* STRAINS)

SMR[1] Collection #	Genotype[2] (Exp.)[3]	Growth-dependent (gd) or Adaptive (a)	Isolate # [culture (gd) or day (a) isolated][4]	Reversion mutation[5] (position)
900	*recD* (SL1)	gd	1 (1)	-1 (1059)
901	*recD* (SL1)	gd	1 (7)	-1 (1035)
902	*recD* (SL1)	gd	1 (10)	-43 (1035)
903	*recD* (SL1)	gd	1 (18)	-1 (1030)
904	*recD* (SL1)	gd	1 (12)	CG to (CG)2 (1042)
905	*recD* (SL1)	gd	1 (13)	-1 (1088)
906	*recD* (SL1)	gd	1 (14)	-1 (1030)
907	*recD* (SL1)	gd	1 (15)	-4 (1073) including bases not in repeat
908	*recD* (SL1)	gd	1 (19)	-92 (968)
909	*recD* (SL1)	gd	1 (20)	-1 (1039)
910	*recD* (SL1)	gd	1 (22)	-1 (1059)
911	*recD* (SL1)	gd	1 (23)	-24 (1018)

continued...

[1] S. M. Rosenberg

[2] Strain background is FC40 except for #1078-1118 and #1125-1130, which are in the CH5180 strain background (Rosenberg lab collection).

[3] Experiment. Initials S. L., S. Longerich; R. S. H., R. S. Harris. Numbers following initials are for the number of the experiment designated by the individual who performed the experiment (designated as such in his/ her notebook).

[4] Culture isolated is for growth-dependent mutants. Day isolated refers to the day of an adaptive mutation experiment after plating where the day of plating is Day 0.

[5] See Table 2-1 for how nucleotide positions are numbered. NF, sequenced but mutation not found. ND, not sequenced.

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
912	*recD* (SL1)	gd	1 (25)	-1 (1048)
913	*recD* (SL1)	gd	1 (28)	-1 (1039)
914	*recD* (SL1)	gd	1 (31)	-1 (1039)
915	*recD* (SL1)	gd	1 (32)	-1 (1023)
916	*recD* (SL1)	gd	1 (33)	-1 (1039)
917	*recD* (SL1)	gd	1 (34)	-1 (1039)
918	*recD* (SL1)	gd	1 (35)	-1 (1075)
919	*recD* (SL1)	gd	1 (36)	-110 (972)
920	*recD* (SL1)	gd	1 (37)	+2 (1059)
921	*recD* (SL1)	gd	1 (38)	-1 (1085)
922	*recD* (SL1)	gd	1 (39)	NF
923	*recD* (SL1)	gd	1 (40)	-1 (1039)
924	*recD* (SL1)	gd	1 (42)	-1 (1039)
925	*recD* (SL1)	gd	1 (43)	-1 (1137)
926	*recD* (SL1)	gd	1 (44)	ND
927	*recD* (SL1)	gd	1 (47)	-1 (1078)
928	*recD* (SL1)	gd	1 (50)	-1 (1095)
929	*recD* (SL1)	gd	1 (52)	-1 (1075)
930	*recD* (SL1)	gd	1 (53)	-1 (1043)
931	*recD* (SL1)	gd	1 (54)	-1 (1071)
932	*recD* (SL1)	gd	1 (57)	-1 (1064)
933	*recD* (SL1)	gd	1 (58)	-1 (1075)
934	*recD* (SL1)	gd	1 (59)	-1 (1039)
935	*recD* (SL1)	gd	1 (60)	-1 (1039)

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
936	*recD* (SL1)	gd	1 (2)	ND
937	*recD* (SL1)	gd	1 (6)	-7 (1022) including bases not in repeat
938	*recD* (SL1)	gd	1 (8)	ND
939	*recD* (SL1)	gd	1 (9)	(CT)2 to (CT)3 (1092)
940	*recD* (RSH25)	gd	1	-1 (1096)
941	*recD* (RSH25)	gd	5	-1 (1022)
942	*recD* (RSH25)	gd	7	-1 (1039)
943	*recD* (RSH25)	gd	9	-1 (1039)
944	*recD* (RSH25)	gd	14	duplication of 26 (1093-1117) beginning after 1117
945	*recD* (RSH25)	gd	15	-13 (1014)
946	*recD* (RSH25)	gd	16	-1 (1039)
947	*recD* (RSH25)	gd	18	-4 (1042) including bases not in repeat
948	*recD* (RSH25)	gd	20	+2 (1096)
949	*recD* (RSH25)	gd	22	CT to (CT)2 (1048)
950	*recD* (RSH25)	gd	23	-1 (1085)
951	*recD* (RSH25)	gd	25	-73 (955)
952	*recD* (RSH25)	gd	27	-13 (1061)
953	*recD* (RSH25)	gd	28	-1 (1137)

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
954	*recD* (RSH25)	gd	29	-1 (1039)
955	*recD* (RSH25)	gd	30	-1 (1134)
956	*recD* (RSH25)	gd	31	-1 (1058)
957	*recD* (RSH25)	gd	32	-19 (1022)
958	*recD* (RSH25)	gd	33	(CT)2 to (CT)3 (1092)
959	*recD* (RSH25)	gd	36	-1 (1019)
960	*recD* (RSH25)	gd	37	-1 (1075)
961	*recD* (RSH25)	gd	38	-4 (1021)
962	*recD* (RSH25)	gd	39	-112 (979)
963	*recD* (RSH25)	gd	43	-1 (1078)
964	*recD* (RSH25)	gd	48	-1 (1044)
965	*recD* (RSH25)	gd	53	-1 (1128)
966	*recD* (RSH25)	gd	55	-1 (1075)
967	*recD* (RSH25)	gd	56	-1 (1137)
968	*recD* (RSH25)	gd	42	ND
969	*recD* (RSH25)	gd	45	ND
970	*rec*+ (SL1)	gd	1 (1)	-1 (1075)
971	*rec*+ (SL1)	gd	1 (3)	ND
972	*rec*+ (SL1)	gd	1 (7)	ND
973	*rec*+ (SL1)	gd	1 (8)	-1 (1039)
974	*rec*+ (SL1)	gd	1 (10)	-1 (1023)
975	*rec*+ (SL1)	gd	1 (13)	+8 (1099) not mono-nucleotide
976	*rec*+ (SL1)	gd	1 (15)	duplication of 67 (1082-1361) beginning after 1361
977	*rec*+ (SL1)	gd	1 (17)	-1 (1075)

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
978	*rec*+ (SL1)	gd	2 (18)	-1 (1059)
979	*rec*+ (SL1)	gd	1 (19)	-1 (1039)
980	*rec*+ (SL1)	gd	1 (20)	NF
981	*rec*+ (SL1)	gd	1 (21)	-110 (979)
982	*rec*+ (SL1)	gd	1 (22)	ND
983	*rec*+ (SL1)	gd	1 (25)	ND
984	*rec*+ (SL1)	gd	1 (27)	ND
985	*rec*+ (SL1)	gd	1 (28)	ND
986	*rec*+ (SL1)	gd	1 (30)	(CG)3 to (CG)4 (1099)
987	*rec*+ (SL1)	gd	1 (32)	(CG)3 to ((CG)4 (1099)
988	*rec*+ (SL1)	gd	1 (33)	ND
989	*rec*+ (SL1)	gd	1 (34)	ND
990	*rec*+ (SL1)	gd	1 (37)	-1 (1035)
991	*rec*+ (SL1)	gd	1 (38)	ND
992	*rec*+ (SL1)	gd	1 (40)	-2 (1016) with -1 (1022)
993	*rec*+ (SL1)	gd	1 (41)	ND
994	*rec*+ (SL1)	gd	1 (43)	+5 (1091)
995	*rec*+ (SL1)	gd	1 (45)	-1 (1039)
996	*rec*+ (SL1)	gd	1 (46)	-1 (1039)
997	*rec*+ (SL1)	gd	1 (47)	ND
998	*rec*+ (SL1)	gd	2 (48)	-1 (1039)
999	*rec*+ (SL1)	gd	1 (49)	NF
1000	*rec*+ (SL1)	gd	1 (50)	ND
1001	*rec*+ (SL1)	gd	1 (51)	ND
1002	*rec*+ (SL1)	gd	1 (52)	ND
1003	*rec*+ (SL1)	gd	1 (55)	ND
1004	*rec*+ (SL1)	gd	1 (58)	+2 (1043)
1005	*rec*+ (SL1)	gd	1 (59)	ND
1006	*rec*+ (SL1)	gd	1 (60)	-1 (1039)
1007	*rec*+ (RSH25)	gd	1	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1008	*rec*+ (RSH25)	gd	3	-1 (1067)
1009	*rec*+ (RSH25)	gd	4	ND
1010	*rec*+ (RSH25)	gd	5	ND
1011	*rec*+ (RSH25)	gd	6	ND
1012	*rec*+ (RSH25)	gd	7	ND
1013	*rec*+ (RSH25)	gd	8	ND
1014	*rec*+ (RSH25)	gd	9	ND
1015	*rec*+ (RSH25)	gd	10	ND
1016	*rec*+ (RSH25)	gd	11	ND
1017	*rec*+ (RSH25)	gd	12	-1 (1113)
1018	*rec*+ (RSH25)	gd	13	ND
1019	*rec*+ (RSH25)	gd	14	ND
1020	*rec*+ (RSH25)	gd	15	ND
1021	*rec*+ (RSH25)	gd	16	ND
1022	*rec*+ (RSH25)	gd	17	ND
1023	*rec*+ (RSH25)	gd	18	ND
1024	*rec*+ (RSH25)	gd	19	ND
1025	*rec*+ (RSH25)	gd	20	ND
1026	*rec*+ (RSH25)	gd	21	ND
1027	*rec*+ (RSH25)	gd	22	ND
1028	*rec*+ (RSH25)	gd	23	ND
1029	*rec*+ (RSH25)	gd	24	ND
1030	*rec*+ (RSH25)	gd	25	ND
1031	*rec*+ (RSH25)	gd	26	ND
1032	*rec*+ (RSH25)	gd	27	ND
1033	*rec*+ (RSH25)	gd	28	ND
1034	*rec*+ (RSH25)	gd	29	ND
1035	*rec*+ (RSH25)	gd	30	-1 (1039)
1036	*rec*+ (RSH25)	gd	31	ND
1037	*rec*+ (RSH25)	gd	32	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1038	*rec*+ (RSH25)	gd	33	ND
1039	*rec*+ (RSH25)	gd	34	ND
1040	*rec*+ (RSH25)	gd	35	ND
1041	*rec*+ (RSH25)	gd	37	ND
1042	*rec*+ (RSH25)	gd	38	ND
1043	*rec*+ (RSH25)	gd	40	(TC)2 to (TC)3 (1043)
1044	*rec*+ (RSH25)	gd	41	ND
1045	*rec*+ (RSH25)	gd	42	ND
1046	*rec*+ (RSH25)	gd	43	ND
1047	*rec*+ (RSH25)	gd	44	ND
1048	*rec*+ (RSH25)	gd	45	ND
1049	*rec*+ (RSH25)	gd	46	ND
1050	*rec*+ (RSH25)	gd	47	ND
1051	*rec*+ (RSH25)	gd	48	ND
1052	*rec*+ (RSH25)	gd	49	ND
1053	*rec*+ (RSH25)	gd	50	ND
1054	*rec*+ (RSH25)	gd	52	ND
1055	*rec*+ (RSH25)	gd	53	ND
1056	*rec*+ (RSH25)	gd	55	ND
1057	*rec*+ (RSH25)	gd	56	ND
1058	*rec*+ (RSH25)	gd	57	ND
1059	*rec*+ (RSH25)	gd	58	ND
1060	*rec*+ (RSH25)	gd	59	ND
1061	*rec*+ (RSH25)	gd	60	ND
1062	*recD* (RSH25)	gd	3	ND
1063	*recD* (RSH25)	gd	10	ND
1064	*recD* (RSH25)	gd	13	ND
1065	*recD* (RSH25)	gd	17	ND
1066	*recD* (RSH25)	gd	24	ND
1067	*recD* (RSH25)	gd	41	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1068	*recD* (RSH25)	gd	44	ND
1069	*recD* (RSH25)	gd	46	ND
1070	*recD* (RSH25)	gd	47	ND
1071	*recD* (RSH25)	gd	49	ND
1072	*recD* (RSH25)	gd	50	ND
1073	*recD* (RSH25)	gd	51	ND
1074	*recD* (RSH25)	gd	52	ND
1075	*recD* (RSH25)	gd	54	ND
1076	*recD* (RSH25)	gd	57	ND
1077	*recD* (RSH25)	gd	58	ND
1078	*rec*+ (SL32)	a	1 (6)	-1 (1039)
1079	*rec*+ (SL32)	a	2 (6)	-1 (1075)
1080	*rec*+ (SL32)	a	3 (6)	-1 (1039)
1081	*rec*+ (SL32)	a	4 (6)	-1 (1075)
1082	*rec*+ (SL32)	a	5 (6)	-1 (1059)
1083	*rec*+ (SL32)	a	6 (6)	-1 (1039)
1084	*rec*+ (SL32)	a	7 (6)	-1 (1039)
1085	*rec*+ (SL32)	a	8 (6)	-1 (1039)
1086	*rec*+ (SL32)	a	9 (6)	-1 (1075)
1087	*rec*+ (SL32)	a	11 (6)	-1 (1064)
1088	*rec*+ (SL32)	a	12 (6)	-1 (1075)
1089	*rec*+ (SL32)	a	13 (6)	-1 (1059)
1090	*rec*+ (SL32)	a	14 (6)	ND
1091	*rec*+ (SL32)	a	15 (6)	-1 (1039)
1092	*rec*+ (SL32)	a	16 (6)	NF
1093	*rec*+ (SL32)	a	17 (6)	ND
1094	*rec*+ (SL32)	a	18 (6)	-1 (1039)
1095	*rec*+ (SL32)	a	19 (6)	-1 (1059)
1096	*rec*+ (SL32)	a	20 (6)	-1 (1039)
1097	*rec*+ (SL32)	a	21 (6)	-1 (1039)

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1098	*rec*+ (SL32)	a	22 (6)	-1 (1059)
1099	*rec*+ (SL32)	a	23 (6)	-1 (1039)
1100	*rec*+ (SL32)	a	24 (6)	-1 (1039)
1101	*rec*+ (SL32)	a	25 (6)	-1 (1039)
1102	*rec*+ (SL32)	a	27 (6)	-1 (1039)
1103	*rec*+ (SL32)	a	27 (6)	-1 (1039)
1104	*rec*+ (SL32)	a	28 (6)	-1 (1132) ?? not included in Science 1994
1105	*rec*+ (SL32)	a	29 (6)	-1 (1039)
1106	*rec*+ (SL32)	a	30 (6)	-1 (1071)
1107	*rec*+ (SL32)	a	31 (5)	-1 (1109)
1108	*rec*+ (SL32)	a	32 (5)	-1 (1039)
1109	*rec*+ (SL32)	a	33 (5)	ND
1110	*rec*+ (SL32)	a	34 (5)	ND
1111	*rec*+ (SL32)	a	35 (5)	ND
1112	*rec*+ (SL32)	a	36 (5)	ND
1113	*rec*+ (SL32)	a	37 (5)	ND
1114	*rec*+ (SL32)	a	38 (5)	ND
1115	*rec*+ (SL32)	a	39 (5)	ND
1116	*rec*+ (SL32)	a	40 (5)	ND
1117	*rec*+ (SL32)	a	51 (5)	ND
1118	*rec*+ (SL32)	a	52 (5)	ND
1119	*rec*+ (SL32)	a	53 (4)	ND
1120	*rec*+ (SL32)	a	54 (4)	ND
1121	*rec*+ (SL32)	a	55 (4)	ND
1122	*rec*+ (SL32)	a	56 (4)	ND
1123	*rec*+ (SL32)	a	57 (4)	ND
1124	*rec*+ (SL32)	a	58 (4)	ND
1125	*rec*+ (SL32)	a	59 (4)	ND
1126	*rec*+ (SL32)	a	60 (4)	ND
1127	*rec*+ (SL32)	a	61 (4)	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1128	*rec*+ (SL32)	a	62 (4)	ND
1129	*rec*+ (SL32)	a	63 (4)	ND
1130	*rec*+ (SL32)	a	64 (4)	ND
1131	*rec*+ (RSH26)	a	1a (4)	ND
1132	*rec*+ (RSH26)	a	1b (4)	ND
1133	*rec*+ (RSH26)	a	2a (4)	ND
1134	*rec*+ (RSH26)	a	2b (4)	ND
1135	*rec*+ (RSH26)	a	3a (4)	ND
1136	*rec*+ (RSH26)	a	3b (4)	ND
1137	*rec*+ (RSH26)	a	4a (4)	ND
1138	*rec*+ (RSH26)	a	4b (4)	ND
1139	*rec*+ (RSH26)	a	5b (4)	ND
1140	*rec*+ (RSH26)	a	6a (4)	ND
1141	*rec*+ (RSH26)	a	6b (4)	ND
1142	*rec*+ (RSH26)	a	8a (4)	ND
1143	*rec*+ (RSH26)	a	8b (4) missing	ND
1144	*rec*+ (RSH26)	a	9a (4)	ND
1145	*rec*+ (RSH26)	a	10a (4)	ND
1146	*rec*+ (RSH26)	a	10b (4)	ND
1147	*rec*+ (RSH26)	a	1a (5)	ND
1148	*rec*+ (RSH26)	a	2a (5)	ND
1149	*rec*+ (RSH26)	a	3a (5)	ND
1150	*rec*+ (RSH26)	a	3b (5)	ND
1151	*rec*+ (RSH26)	a	4a (5)	ND
1152	*rec*+ (RSH26)	a	4b (5)	ND
1153	*rec*+ (RSH26)	a	5a (5)	ND
1154	*rec*+ (RSH26)	a	5b (5)	ND
1155	*rec*+ (RSH26)	a	7a (5)	ND
1156	*rec*+ (RSH26)	a	8a (5)	ND
1157	*rec*+ (RSH26)	a	8b (5)	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1158	*rec*+ (RSH26)	a	9a (5)	ND
1159	*rec*+ (RSH26)	a	9b (5)	ND
1160	*rec*+ (RSH26)	a	1b (6)	-1 (1039)
1161	*rec*+ (RSH26)	a	1c (6)	ND
1162	*rec*+ (RSH26)	a	1d (6)	ND
1163	*rec*+ (RSH26)	a	2a (6)	ND
1164	*rec*+ (RSH26)	a	2b (6)	NF
1165	*rec*+ (RSH26)	a	2c (6)	ND
1166	*rec*+ (RSH26)	a	2d (6)	ND
1167	*rec*+ (RSH26)	a	3b (6)	-1 (1039)
1168	*rec*+ (RSH26)	a	3c (6)	ND
1169	*rec*+ (RSH26)	a	3d (6)	ND
1170	*rec*+ (RSH26)	a	4b (6)	ND
1171	*rec*+ (RSH26)	a	4d (6)	-1 (1109)
1172	*rec*+ (RSH26)	a	5a (6)	-1 (1039)
1173	*rec*+ (RSH26)	a	5b (6)	ND
1174	*rec*+ (RSH26)	a	5c (6)	ND
1175	*rec*+ (RSH26)	a	5d (6)	ND
1176	*rec*+ (RSH26)	a	6a (6)	-1 (1075)
1177	*rec*+ (RSH26)	a	6c (6)	ND
1178	*rec*+ (RSH26)	a	6d (6)	ND
1179	*rec*+ (RSH26)	a	7a (6)	-1 (1064)
1180	*rec*+ (RSH26)	a	7b (6)	ND
1181	*rec*+ (RSH26)	a	7d (6)	ND
1182	*rec*+ (RSH26)	a	8a (6)	-1 (1039)
1183	*rec*+ (RSH26)	a	8d (6)	ND
1184	*rec*+ (RSH26)	a	9a (6)	ND
1185	*rec*+ (RSH26)	a	9b (6)	ND
1186	*rec*+ (RSH26)	a	9c (6)	-1 (1039)
1187	*rec*+ (RSH26)	a	9d (6)	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1188	*rec*+ (RSH26)	a	10a (6)	ND
1189	*rec*+ (RSH26)	a	10b (6)	ND
1190	*rec*+ (RSH26)	a	10c (6)	-1 (1039)
1191	*recD* (SL)	a	D1 (5)	-1 (1023)
1192	*recD* (SL)	a	D2 (5)	NF
1193	*recD* (SL)	a	D3 (5)	-1 (1071)
1194	*recD* (SL)	a	D4 (5)	-1 (1050)
1195	*recD* (SL)	a	D5 (5)	-1 (1039)
1196	*recD* (SL)	a	D6 (5)	-1 (1128)
1197	*recD* (SL)	a	D7 (5)	-1 (1085)
1198	*recD* (SL)	a	D8 (5)	-1 (1039)
1199	*recD* (SL)	a	D9 (5)	-1 (1039)
1200	*recD* (SL)	a	D10 (5)	-1 (1023)
1201	*recD* (SL)	a	D11 (6)	-1 (1064)
1202	*recD* (SL)	a	D12 (6)	-1 (1039)
1203	*recD* (SL)	a	D13 (6)	-1 (1146)
1204	*recD* (SL)	a	D14 (6)	-1 (1042)
1205	*recD* (SL)	a	D15 (6)	-1 (1075)
1206	*recD* (SL)	a	D16 (6)	-1 (1075)
1207	*recD* (SL)	a	D17 (6)	ND
1208	*recD* (SL)	a	D18 (6)	-1 (1039)
1209	*recD* (SL)	a	D19 (4)	ND
1210	*recD* (SL)	a	D20 (4)	-1 (1137)
1211	*recD* (SL)	a	D21 (4)	-1 (1039)
1212	*recD* (SL)	a	D22 (4)	ND
1213	*recD* (SL)	a	D23 (4)	-1 (1075)
1214	*recD* (SL)	a	D24 (4)	-1 (1039)
1215	*recD* (SL)	a	D25 (4)	-1 (1039)
1216	*recD* (SL)	a	D26 (4)	-1 (1085)
1217	*recD* (SL)	a	D27 (4)	-1 (1096)

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1218	*recD* (RSH26)	a	1b (4)	ND
1219	*recD* (RSH26)	a	3a (4)	ND
1220	*recD* (RSH26)	a	7a (4)	ND
1221	*recD* (RSH26)	a	8a (4)	ND
1222	*recD* (RSH26)	a	3b (5)	ND
1223	*recD* (RSH26)	a	4a (5)	ND
1224	*recD* (RSH26)	a	4b (5)	ND
1225	*recD* (RSH26)	a	5a (5)	ND
1226	*recD* (RSH26)	a	6b (5)	ND
1227	*recD* (RSH26)	a	8a (5)	ND
1228	*recD* (RSH26)	a	9b (5)	ND
1229	*recD* (RSH26)	a	10a (5)	ND
1230	*recD* (RSH26)	a	10b (5)	ND
1231	*recD* (RSH26)	a	1a (6)	-1 (1039)
1232	*recD* (RSH26)	a	1b (6)	ND
1233	*recD* (RSH26)	a	2d (6)	-1 (1139)
1234	*recD* (RSH26)	a	3b (6)	ND
1235	*recD* (RSH26)	a	3c (6)	-1 (1039)
1236	*recD* (RSH26)	a	3d (6)	ND
1237	*recD* (RSH26)	a	4a (6)	ND
1238	*recD* (RSH26)	a	4c (6)	ND
1239	*recD* (RSH26)	a	4d (6)	-1 (1059)
1240	*recD* (RSH26)	a	5a (6)	-1 (1039)
1241	*recD* (RSH26)	a	5b (6)	ND
1242	*recD* (RSH26)	a	5c (6)	ND
1243	*recD* (RSH26)	a	5d (6)	ND
1244	*recD* (RSH26)	a	6c (6)	-1 (1059)
1245	*recD* (RSH26)	a	7c (6)	-1 (1039)
1246	*recD* (RSH26)	a	8a (6)	-1 (1075)
1247	*recD* (RSH26)	a	9a (6)	ND

continued...

SMR#	Genotype (Expt.)	gd or a	isolate # (culture/day)	mutation
1248	*recD* (RSH26)	a	9b (6)	ND
1249	*recD* (RSH26)	a	9c (6)	NF-extragenic?
1250	*recD* (RSH26)	a	10a (6)	ND
1251	*recD* (RSH26)	a	10d (6)	-1 (1039)

APPENDIX C. LAC+ COLLECTION FOR SEQUENCES IN CHAPTER 3 (GROWTH-DEPENDENT MUTANTS OF *mut* AND *dam* STRAINS) AND OF *mutD5* AND *dnaQ* GROWTH-DEPENDENT LAC+ REVERTANTS

SMR[1] Collection #	Isolate #[2]	Relevant Genotype[3]	Reversion Mutation[4] (position)	Source[5]
1273	1	*mutS*	-1 (1039)	C. W.
1274	2	*mutS*	-1 (1059)	C. W.
1275	3	*mutS*	-1 (1039)	C. W.
1276	4	*mutS*	-1 (1039)	C. W.
1277	5	*mutS*	-1 (1039)	C. W.
1278	6	*mutS*	-1 (1059)	C. W.
1279	7	*mutS*	-1 (1039)	C. W.
1280	8	*mutS*	-1 (1039)	C. W.
1281	9	*mutS*	-1 (1039)	C. W.
1282	10	*mutS*	-1 (1039)	C. W.
1283	11	*mutS*	-1 (1039)	C. W.
1284	12	*mutS*	-1 (1039)	C. W.
1285	13	*mutS*	-1 (1039)	C. W.
1286	14	*mutS*	-1 (1039)	C. W.
1287	15	*mutS*	-1 (1039)	C. W.
1288	16	*mutS*	-1 (1075)	C. W.
1289	17	*mutS*	-1 (1059)	C. W.
1290	18	*mutS*	-1 (1039)	C. W.

continued ...

[1] Susan M. Rosenberg

[2] Number referred to in polymerase chain reaction notes on protocol, and on sequencing results.

[3] The background genotype in all cases is of the FC40 strain (Rosenberg lab collection).

[4] See Table 2-1 for nucleotide position numbers. NF, sequenced but mutation not found. ND, not sequenced.

[5] A. M. G., Anne M. Galloway. C. W., Cindy Wong.

SMR #	Isolate #	Genotype	Mutation	Source
1291	19	*mutS*	-1 (1039)	C. W.
1292	20	*mutS*	-1 (1059)	C. W.
1293	21	*mutS*	-1 (1039)	C. W.
1294	22	*mutS*	-1 (1039)	C. W.
1295	23	*mutS*	-1 (1075)	C. W.
1296	24	*mutS*	-1 (1059)	C. W.
1297	25	*mutS*	-1 (1039)	C. W.
1298	26	*mutS*	-1 (1039)	C. W.
1299	27	*mutS*	-1 (1039)	C. W.
1300	28	*mutS*	-1 (1039)	C. W.
1328	1	*dam*	-1 (1096)	C. W.
1329	2	*dam*	-1 (1039)	C. W.
1330	3	*dam*	-1 (1039)	C. W.
1331	4	*dam*	-1 (1071)	C. W.
1332	5	*dam*	-1 (1039)	C. W.
1333	6	*dam*	Δ (1104-1119)	C. W.
1334	7	*dam*	-1 (1039)	C. W.
1335	8	*dam*	-1 (1039)	C. W.
1336	9	*dam*	-1 (1039)	C. W.
1337	10	*dam*	-1 (1039)	C. W.
1338	11	*dam*	ND	C. W.
1339	12	*dam*	-1 (1039)	C. W.
1340	13	*dam*	-1 (1039)	C. W.
1341	14	*dam*	-1 (1059)	C. W.
1342	15	*dam*	-1 (1039)	C. W.
1343	16	*dam*	-1 (1059)	C. W.
1344	17	*dam*	-1 (1059)	C. W.
1345	18	*dam*	-1 (1039)	C. W.
1346	19	*dam*	-1 (1039)	C. W.

continued ...

SMR #	Isolate #	Genotype	Mutation	Source
1347	1	*mutL*	-1 (1059)	C. W.
1348	2	*mutL*	-1 (1059)	C. W.
1349	3	*mutL*	-1 (1039)	C. W.
1350	4	*mutL*	-1 (1039)	C. W.
1351	5	*mutL*	-1 (1039)	C. W.
1352	6	*mutL*	-1 (1039)	C. W.
1353	7	*mutL*	-1 (1039)	C. W.
1354	8	*mutL*	-1 (1039)	C. W.
1355	9	*mutL*	-1 (1039)	C. W.
1361	20	*dam*	-1 (1059)	A. M. G.
1362	21	*dam*	-1 (1039)	A. M. G.
1363	22	*dam*	-1 (1039)	A. M. G.
1364	23	*dam*	-1 (1075)	A. M. G.
1365	24	*dam*	-1(1039)	A. M. G.
1366	25	*dam*	-1 (1039)	A. M. G.
1367	26	*dam*	-1 (1039)	A. M. G.
1368	27	*dam*	-1 (1059)	A. M. G.
1369	28	*dam*	-1 (1039)	A. M. G.
1370	29	*dam*	-1 (1039)	A. M. G.
1371	30	*dam*	ND	A. M. G.
1372	31	*dam*	ND	A. M. G.
1373	10	*mutL*	-1 (1039)	A. M. G.
1374	11	*mutL*	-1 (1039)	A. M. G.
1375	12	*mutL*	-1 (1059)	A. M. G.
1376	13	*mutL*	-1 (1039)	A. M. G.
1377	14	*mutL*	-1 (1039)	A. M. G.
1378	15	*mutL*	-1 (1039)	A. M. G.
1379	16	*mutL*	-1 (1039)	A. M. G.
1380	17	*mutL*	-1 (1039)	A. M. G.
1381	18	*mutL*	-1 (1039)	A. M. G.
1382	19	*mutL*	-1 (1039)	A. M. G.
1383	20	*mutL*	-1 (1039)	A. M. G.
1384	21	*mutL*	-1 (1096)	A. M. G.

continued ...

SMR #	Isolate #	Genotype	Mutation	Source
1385	22	*mutL*	1- (1039)	A. M. G.
1386	29	*mutS*	-1 (1039)	A. M. G.
1387	30	*mutS*	-1 (1039)	A. M. G.
1388	31	*mutS*	-1 (1039)	A. M. G.
1389	32	*mutS*	NF	A. M. G.
1390	33	*mutS*	-1 (1039)	A. M. G.
1391	34	*mutS*	NF	A. M. G.
1392	35	*mutS*	-1 (1039)	A. M. G.
1393	36	*mutS*	-1 (1059)	A. M. G.
1394	37	*mutS*	-1 (1039)	A. M. G.
1395	38	*mutS*	ND	A. M. G.
1396	39	*mutS*	ND	A. M. G.
1397	40	*mutS*	ND	A. M. G.
1408	23	*mutL*	-1 (1039)	A. M. G.
1409	24	*mutL*	NF	A. M. G.
1410	25	*mutL*	-1 (1039)	A. M. G.
1411	26	*mutL*	-1 (1039)	A. M. G.
1412	27	*mutL*	NF	A. M. G.
1413	28	*mutL*	NF	A. M. G.
1414	29	*mutL*	NF	A. M. G.
1415	30	*mutL*	-1 (1075)	A. M. G.
1416	31	*mutL*	-1 (1039)	A. M. G.
1417	32	*mutL*	-1 (1039)	A. M. G.
1418	33	*mutL*	-1 (1059)	A. M. G.
1419	34	*mutL*	-1 (1071)	A. M. G.
1420	35	*mutL*	dupl. (1060-1079)	A. M. G.
1421	36	*mutL*	ND	A. M. G.
1422	37	*mutL*	ND	A. M. G.
1423	38	*mutL*	ND	A. M. G.
1424	39	*mutL*	ND	A. M. G.
1425	40	*mutL*	ND	A. M. G.

continued ...

SMR #	Isolate #	Genotype	Mutation	Source
1426	41	*mutL*	ND	A. M. G.
1427	42	*mutL*	ND	A. M. G.
1428	30	*dam*	-1 (1039)	A. M. G.
1429	31	*dam*	-1 (1039)	A. M. G.
1430	32	*dam*	-1 (1039)	A. M. G.
1431	33	*dam*	-1 (1039)	A. M. G.
1432	34	*dam*	-1 (1019)	A. M. G.
1433	35	*dam*	-1 (1075)	A. M. G.
1434	36	*dam*	-1 (1039)	A. M. G.
1435	37	*dam*	-1 (1059)	A. M. G.
1436	38	*dam*	ND	A. M. G.
1437	39	*dam*	ND	A. M. G.
1438	40	*dam*	ND	A. M. G.
1439	41	*dam*	ND	A. M. G.
1440	42	*dam*	ND	A. M. G.
1441	43	*dam*	ND	A. M. G.
1442	44	*dam*	ND	A. M. G.
1443	45	*dam*	ND	A. M. G.
1444	46	*dam*	ND	A. M. G.
1445	47	*dam*	ND	A. M. G.
1446	48	*dam*	ND	A. M. G.
1447	49	*dam*	ND	A. M. G.
1448	50	*dam*	ND	A. M. G.
1449	51	*dam*	ND	A. M. G.
1450	52	*dam*	ND	A. M. G.
1451	53	*dam*	ND	A. M. G.
1452	54	*dam*	ND	A. M. G.
1453	55	*dam*	ND	A. M. G.
1454	56	*dam*	ND	A. M. G.
1455	57	*dam*	ND	A. M. G.
1456	58	*dam*	ND	A. M. G.
1457	59	*dam*	ND	A. M. G.

continued ...

SMR #	Isolate #	Genotype	Mutation	Source
1458	60	*dam*	ND	A. M. G.
1459	61	*dam*	ND	A. M. G.
1460	62	*dam*	ND	A. M. G.
1461	63	*dam*	ND	A. M. G.
1462	64	*dam*	ND	A. M. G.
1475	1	*mutD*[6]	-1 (1039)	A. M. G.
1476	2	*mutD*	-1 (1039)	A. M. G.
1477	3	*mutD*	-1 (1039)	A. M. G.
1478	4	*mutD*	-1 (1039)	A. M. G.
1479	5	*mutD*	-1 (1039)	A. M. G.
1480	6	*mutD*	-1 (1039)	A. M. G.
1481	7	*mutD*	-1 (1039)	A. M. G.
1482	8	*mutD*	-1 (1039)	A. M. G.
1483	9	*mutD*	ND	A. M. G.
1484	10	*mutD*	-1 (1039)	A. M. G.
1485	11	*mutD*	-1 (1039)	A. M. G.
1486	12	*mutD*	-1 (1039)	A. M. G.
1487	13	*mutD*	-1 (1039)	A. M. G.
1488	14	*mutD*	-1 (1039)	A. M. G.
1510	1	*dnaQ*[7]	-1 (1039)	A. M. G.
1511	2	*dnaQ*	-1 (1039)	A. M. G.
1512	3	*dnaQ*	-1 (1039)	A. M. G.
1513	4	*dnaQ*	NF	A. M. G.
1514	5	*dnaQ*	-1 (1039)	A. M. G.
1515	6	*dnaQ*	-1 (1039)	A. M. G.

continued ...

[6] Strain RSH116 (#1398 in SMR collection) is FC40 *mutD5 zae-502*::Tn*10*.

[7] Isolate numbers 1-17 are derived from RSH151 (#1548 in SMR collection) which is FC40 *zae*::Tn*10dCam spq-2 ΔdnaQ903::tet*. Isolate numbers 18-34 are derived from RSH150 (#1547 in SMR collection) which is a second isolate from the construction of RSH151.

SMR #	Isolate #	Genotype	Mutation	Source
1516	7	*dnaQ*	ND	A. M. G.
1517	8	*dnaQ*	-1 (1039)	A. M. G.
1518	9	*dnaQ*	-1 (1039)	A. M. G.
1519	10	*dnaQ*	ND	A. M. G.
1520	11	*dnaQ*	-1 (1023)	A. M. G.
1521	12	*dnaQ*	ND	A. M. G.
1522	13	*dnaQ*	ND	A. M. G.
1523	14	*dnaQ*	-1 (1059)	A. M. G.
1524	15	*dnaQ*	ND	A. M. G.
1525	16	*dnaQ*	-1 (1039)	A. M. G.
1526	17	*dnaQ*	-1 (1059)	A. M. G.
1527	18	*dnaQ*	-1 (1039)	A. M. G.
1528	19	*dnaQ*	-1 (1039)	A. M. G.
1529	20	*dnaQ*	ND	A. M. G.
1530	21	*dnaQ*	-1 (1039)	A. M. G.
1531	22	*dnaQ*	-1 (1039)	A. M. G.
1532	23	*dnaQ*	-1 (1039)	A. M. G.
1533	24	*dnaQ*	-1 (1039)	A. M. G.
1534	25	*dnaQ*	-1 (1039)	A. M. G.
1535	26	*dnaQ*	-1 (1039)	A. M. G.
1536	27	*dnaQ*	ND	A. M. G.
1537	28	*dnaQ*	ND	A. M. G.
1538	29	*dnaQ*	-1 (1039)	A. M. G.
1539	30	*dnaQ*	-1 (1039)	A. M. G.
1540	31	*dnaQ*	-1 (1039)	A. M. G.
1541	32	*dnaQ*	ND	A. M. G.
1542	33	*dnaQ*	-1 (1039)	A. M. G.
1543	34	*dnaQ*	-1 (1059)	A. M. G.

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Education:

1993 -- Bachelor of Science degree with Specialization in Genetics, University of Alberta, Edmonton.

1993 - 1997 -- Master of Science degree, Genetics, Dept. of Biological Sciences, University of Alberta, Edmonton. Co-supervisors: Dr. S. M. Rosenberg (Dept. of Biochemistry), Dr. L. Frost (Dept. of Biological Sciences). NOTE: Dr. Rosenberg was the main advisor and the research was carried out under her direction.

Title of thesis: Evidence for suspended mismatch repair during adaptive mutation in *Escherichia coli*.

Professional Experience:

Summer 1992 -- Full-time summer student in Dr. S. M. Rosenberg's laboratory, Dept. of Biochemistry, University of Alberta. Subject of research: Adaptive mutation in *Escherichia coli.*

September 1992 - April 1993 -- Undergraduate research student in Dr. S. M. Rosenberg's laboratory.

Summer 1993 -- Full-time summer student in Dr. S. M. Rosenberg's laboratory.

September 1993 - present -- M. Sc. program.

Summer 1995 -- Primary health care and research in Tanzania as a volunteer for the Students' International Health Association, University of Alberta. Subject of research: Effect of iron and folate supplementation in pregnant women.

1993 - 1994 -- Teaching assistant for undergraduate (first year) genetics labs (Two labs per term, each with approximately 20 students).

September-December 1994 -- Marking assistant and tutor for undergraduate genetics course.

January - April 1996 -- Teaching assistant for undergraduate (first year) biology labs. (Two labs of approximately 20 students each).

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September 1996 - present -- SIHA Executive, Fundraising Coordinator.

Areas of expertise:

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Awards:

1989 - Alberta Heritage University entrance scholarship.

1989 - Canada Scholarship.

1993 - Alberta Heritage Summer Studentship.

1994 - Myer Horowitz Graduate Scholarship.

Publications:

PAPERS:

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