34398

National Library of Canada | Bibliothèque nationale du Canada

CANADIAN THESES ON MICROFICHE | *THÈSES CANADIENNES SUR MICROFICHE*

NAME OF AUTHOR *NOM DE L'AUTEUR* Arun Kumar Kaundal

TITLE OF THESIS *TITRE DE LA THÈSE* Hormonal Patterns of Seminal Fluid and its Biochemical Analysis

UNIVERSITY/*UNIVERSITÉ* The University of Alberta

DEGREE FOR WHICH THESIS WAS PRESENTED *GRADE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE* Ph.D (Med. Biochemistry)

YEAR THIS DEGREE CONFERRED/*ANNÉE D'OBTENTION DE CE GRADE* 1977

NAME OF SUPERVISOR/*NOM DU DIRECTEUR DE THÈSE* Dr. Saul Zalik

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

DATED/*DATÉ* 29.7.77 SIGNED/*SIGNÉ* A.K. Kaundal

PERMANENT ADDRESS/*RÉSIDENCE FIXÉ* Village Jamsela, P.O. Gadiara Via Baijnath District Kangra Himachal Pradesh, India

NL-91 (3-74)

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

THE UNIVERSITY OF ALBERTA

RIBOSOMAL PROTEINS OF GATEWAY BARLEY AND ITS VIRESCENS MUTANT

by

© KIRPA RAM KOUNDAL

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

PLANT BIOCHEMISTRY

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

FALL, 1977

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Ribosomal Proteins of Gateway Barley and its Virescens Mutant" submitted by Kirpa Ram Koundal in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Biochemistry.

.....
Supervisor

.....

.....

.....

.....

.....
External Examiner

Date July 29, 1977

To my parents

ABSTRACT

The proteins of the 80S cytoplasmic ribosomes isolated from dry embryos of Gateway barley and its virescens mutant were compared by one and two-dimensional electrophoresis. The monosomes of both lines gave similar patterns with 60 basic proteins. After dissociation of the ribosomes of the dry embryos the respective subunits of the normal and mutant had the same number of acidic proteins with similar mobilities. However for the mutant, the basic proteins of the 60S subunit migrated less than those of the normal and lacked three of the proteins present in the normal but had three additional spots. Also the protein patterns for the 40S subunits differed. The mutant lacked three of the proteins present in the normal, but had three additional spots. Despite these differences both homologous and heterologous subunits of the two lines were able to reassociate into monosomes which were active in poly (U)-directed polyphenylalanine synthesis.

The patterns of the basic proteins of the 80S cytoplasmic ribosomes from seedling leaves of the normal and mutant were similar with 60 proteins and they were like those of the 80S ribosomes isolated from the dry embryos. The basic proteins of the chloroplast ribosomes of the two lines gave similar patterns with 53 proteins. However, disc gel electrophoresis of the acidic proteins of the cytoplasmic and chloroplast ribosomes isolated from seedling leaves of the normal and mutant showed differences between the two lines.

ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to Professor Saul Zalik for his valuable guidance and encouragement during the course of my research program and during the preparation of this manuscript. The excellent technical assistance of Mr. Barry Zytaruk is gratefully acknowledged. Also thanks are due to Mr. M.R. Aarbo for calculating the values of sedimentation coefficients and to Mr. Michael Batory for assistance in preparing the plant material.

I would like to express appreciation for valuable suggestions and help received from my colleagues, especially Dr. A.S. Cohen throughout the course of this study. Thanks are expressed to Ms. Georgina French for efficient typing of the manuscript.

Finally, I wish to thank the Government of India for awarding me the National Overseas Scholarship during the course of this investigation.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS AND SYMBOLS

Å	Angstrom
ATP	adenosine 5^{1}-triphosphate
1-D	one-dimensional
2-D	two-dimensional
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetate
EGTA	ethyleneglycol bis (β-aminoethylether) - N,N^{1}-tetracetate
GTP	guanosine 5^{1}-triphosphate
mA	milliamperes
poly (U)	polyuridylic acid
PAGE	polyacrylamide gel electrophoresis
r-RNA	ribosomal ribonucleic acid
S	Svedberg unit of sedimentation velocity
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TEMED	N, N, N^{1}, N^{1}-tetramethyl ethylenediamine
tRNA	transfer ribonucleic acid

INTRODUCTION

Comparative studies of Gateway barley and its virescens mutant in this laboratory have shown that although the seedlings of the mutant were low in pigments (Maclachlan and Zalik, 1963) the deficiency in chlorophyll production was not due to an inadequacy of the chlorophyll precursor δ-aminolevulinic acid (Sane and Zalik, 1970). Chloroplasts of young seedlings of the mutant were abnormal and contained large vesicles (Maclachlan and Zalik, 1963; Jhamb and Zalik, 1973). The total RNA content of leaves of the mutant was less than in the normal (Miller, 1965) and in the juvenile state leucine U-^{14}C incorporation into protein by the mutant was less than for the normal (Sane and Zalik, 1970). More recently it has been found that etioplasts of the mutant lacked crystalline prolamellar bodies (Jhamb and Zalik, 1975), that the mutant lacked a number of soluble and lamellar proteins in the early stages of development and the photoreductive activity of plastids from 4-day old mutant seedlings was only 5% of the normal (Horak and Zalik, 1975). In these studies it has been noted that during the course of development (about 8-day) the chloroplasts of the mutant assumed a normal structure and the mutant acquired the same complement of proteins and pigments as the normal.

Jhamb and Zalik (1973) hypothesized that among the undetected proteins of the mutant in the juvenile state there was probably a key protein which was affected by the nuclear mutation (Stephansen and Zalik, 1971) and might be prerequisite for the formation of other

proteins and pigments.

It has been implied that the production of crystalline prolamellar bodies in etioplasts is related to the presence of ribosomes (Gunning and Jagoe, 1967; Wellburn *et al.*, 1977) and studies from many laboratories have established that both chloroplast and cytoplasmic ribosomes are involved in the synthesis of chloroplast membrane proteins and of RUDP carboxylase (Goodenough and Levine, 1970; Eytan and Ohad, 1970; Kawashima and Wildman, 1972; Hoober, 1970, 1972; Bourque and Wildman, 1973; Bar-Nun and Ohad, 1977). Furthermore, the absence or alteration of specific ribosomal proteins have been related to gene mutations in *E. coli* (Krembel and Apirion, 1968; Funatsu and Wittmann, 1972; Funatsu *et al.*, 1972) and in *Chlamydomonas reinhardi* (Mets and Bogorad, 1972; Davidson *et al.*, 1974; Brügger and Boschetti, 1975).

Thus with respect to the Gateway mutant it was inferred that during the early stages of development it might have a defect or deficiency of chloroplast or cytoplasmic ribosomes. Indeed, unpublished data from this laboratory showed that in comparison with the normal at 2 to 4 days the mutant had fewer ribosomes of both classes and the largest difference appeared to be due to a reduction of chloroplast ribosomes.

The object of the research reported in this thesis is to further these studies. The proteins of the cytoplasmic ribosomes and ribosomal subunits isolated from dry embryos as well as from chloroplast and cytoplasmic ribosomes isolated from seedlings were compared by polyacrylamide gel electrophoresis. Reassociation studies were conducted and in conjunction with these the incorporation abilities of reformed homologous and heterologous monosomes were investigated.

LITERATURE REVIEW

Ribosomes

The ribosome is a complex organelle made up of three molecules of ribonucleic acids, many different protein molecules, and certain monovalent and divalent cations, arranged in two interacting but separable subunits (Kurland, 1972; Wittmann and Stöffler, 1972; Nomura *et al.*, 1974). The ribosome is the site of protein synthesis in all living cells. It carries at least two catalytic sites that participate in this process and also serves as the focus and organizing center of the protein synthesizing apparatus. As such it interacts with other macromolecular components of the apparatus: messenger RNA, aminoacyl and peptidyl t-RNA and numerous protein factors that assist in peptide chain initiation, elongation and termination. The structure and functions of ribosomes have been extensively reviewed in several recent articles (Boulter, 1970; Borst and Goivell, 1971; Kurland, 1971; Boulter *et al.*, 1972; Borst, 1972; Nomura, 1973; Zalik and Jones, 1973).

Studies on ribosomes from various sources have shown that they have many properties in common. From the sedimentation coefficients which have been reported for ribosomes from different species of microorganisms, plants and animals, it appears that ribosomes fall into two distinct classes. Prokaryotic (bacterial and blue-green algae) and certain cytoplasmic organelles (chloroplast and mitochondria), contain a "small" type of ribosome which sediments in the 70S range, while the cytoplasmic matrix of eukaryotic cells (animals and plants) contains a

"large" type of ribosome which sediments in the 80S range.

Chloroplast and Cytoplasmic Ribosomes of Plant Systems

Chloroplast ribosomes can be distinguished from the cytoplasmic ribosomes on the basis of their characteristic sedimentation coefficients as well as the sedimentation coefficients of their subunits and RNA components (Brawerman, 1963; Clark *et al.*, 1964; Boardman *et al.*, 1965; Boardman, 1966; Loening and Ingle, 1967). Several attempts have been made to characterize ribosomes from green algae by their sedimentation properties (Eisenstadt and Brawerman, 1964; Sager and Hamilton, 1967. Rawson and Stutz (1969) determined the presence of 79S and 69S ribosomes in *Chlamydomonas reinhardi*. They found that the ribosomes, particularly the 69S particles, were unstable at a low ratio of divalent to monovalent ions. Hoober and Blobel (1969) demonstrated that chloroplast ribosomes of wild type cells were more sensitive to changes in magnesium concentration than cytoplasmic ribosomes. As the Mg^{2+} concentration was lowered a series of changes occurred in the chloroplast ribosomes which resulted in a progressive lowering of their sedimentation velocity. These changes might be the result either of an alteration in ribosome conformation or of a loss in protein bound to 70S ribosomes. The sensitivity to Mg^{2+} ion concentration together with the susceptibility of chloroplast r-RNA to degradation probably explains the heterogeneity in sedimentation velocity of chloroplast ribosomes reported previously for *Chlamydomonas* (Gillham, 1969; Sager and Hamilton, 1967) and other organisms such as *Euglena* (Eisenstadt and Brawerman, 1964; Smillie *et al.*, 1968). Bourque *et al.* (1971) confirmed the observation of Hoober and Blobel (1969) that chloroplast ribosomes of *Chlamydomonas* can undergo a

progressive decrease in sedimentation velocity in response to sequential reductions in magnesium concentration, prior to their actual dissociation. Similar changes were not observed in the case of cytoplasmic ribosomes. Hoober and Blobel (1969) found that these chloroplastic ribosomes were stabilized and could be isolated in the presence of 20 to 25 mM $MgCl_2$. They were, therefore, able to characterize both the chloroplast and cytoplasmic ribosomes of the Y-I mutant of *Chlamydomonas reinhardi* with regard to the sedimentation rates of the monomers, the subunits and the r-RNA and to obtain electrophoretic patterns of the ribosomal proteins. They have shown that the 68S ribosomes of the Y-mutant contain 23S and 18S RNA. Johnson) and Goodenough and Levine (1970) have presented evidence that 70S ribosomes and the 23S and 16S r-RNA are indeed of chloroplast origin.

Subsequent investigations by Rawson and Stutz (1969) provided clear evidence that intact chloroplast ribosomes from higher plants in fact sediment at 70S and contain 22S and 17S RNA. Both Boardman *et al.* (1966) and Van Kammen (1967) obtained active chloroplast ribosome fractions from tobacco leaves but the preparations were 50% contaminated by cytoplasmic ribosomes. Ribosomes of the cytoplasm can occur bound to the membranes of the endoplasmic reticulum (Blobel and Potter, 1967; Murthy, 1972), to the outer envelope of the nucleus (Whittler *et al.*, 1968; Kellems *et al.*, 1974), to the cell membrane (Glick and Warren, 1969) and to the outer envelope of the mitochondria (Kellems and Butow, 1972, 1974; Kuriyama and Luck, 1973). The ribosomes bound to the endoplasmic reticulum synthesize mainly secretory protein (Redman and Sabatini, 1966; Redman, 1967) and membrane bound proteins (Dallner *et al.*, 1966; Lim and Adams, 1967). Opik (1968) and Payne and Boulter (1969) also suggested

that membrane bound ribosomes preferentially synthesize storage protein in the cotyledons of developing pea and broad bean seeds. Ribosomes are bound to endoplasmic reticulum in part by the nascent protein chain (Adelman *et al.*, 1973).

Chloroplast ribosomes are either attached to the photosynthetic thylakoid membrane or are free in the chloroplast stroma (Chen and Wildman, 1970; Margulies and Michaels, 1974). Thylakoid-bound ribosomes of both algae and higher plants occur in arrangements which suggest polyribosomes (Margulies and Michaels, 1974; Falk, 1969; Clark *et al.*, 1964). A large percentage of chloroplast ribosomes of *Chlamydomonas reinhardi* are recovered bound to isolated chloroplast membranes when completion of nascent chain is prevented with chloramphenicol (Margulies and Michaels, 1974; Chua *et al.*, 1973). Since thylakoid ribosomes are attached to the membranes in part by nascent chains (Chua *et al.*, 1973; Margulies and Michaels, 1974) and carry out amino acid incorporation into products which are released to the membrane it was suggested that they synthesize thylakoid protein (Margulies *et al.*, 1975; Michaels and Margulies, 1975). Only 5% of the chloroplast ribosomes of *Chlamydomonas reinhardi* occur free in stroma and over half of the chloroplast ribosomes are bound to chloroplast thylakoid membranes if completion of nascent polypeptide chains is prevented by chloramphenicol (Margulies and Michaels, 1975).

Hadziyev and Zalik (1970) reported the isolation of ribosomes and polyribosomes from wheat chloroplasts. Using sucrose gradient and analytical ultracentrifuge analysis they showed that polyribosomes from chloroplasts of 4-day old seedlings had mono-, di-, tri-, tetra-, and traces of penta-ribosomes while those from 7-day old seedlings had only

the mono-, di-, and traces of tri-ribosomes. Without Mg^{2+} in the suspending medium the polyribosomes dissociated into ribosomal subunits. In earlier work Hadziyev *et al.* (1969) had shown that the yield of polyribosomes could be increased considerably by using bentonite in the isolation medium. However, they found that bentonite inhibited RNAase only about 50%.

Breen *et al.*, (1971) studied the polysomes from barley tissues. The extracted polyribosomes were characterized in sucrose gradients by their conversion to monosomes when incubated with pancreatic RNAase and by their dependence on adequate amounts of Mg^{2+} during extraction and analysis. The addition of 10 mM DTT was partially effective in preventing the loss of higher polymerized states of polyribosomes at KCl concentrations below 0.4 M. Extraction at KCl concentration greater than 0.4 M and pH 8.0 reduced the amount of ribosomes obtained from the tissue. They observed the dissociation of monosomes into subunits when the tissue was extracted in 0.6 M KCl.

Ramagopal and Hsiao (1973) reported the isolation of polyribosomes from 4-day old maize shoots, using high pH (8.5) isolation media. They studied the effect of pH, bentonite and leaf age on polysomes and concluded that bentonite had no significant effect when polysomes were isolated from very young tissue either at pH 7.5 or 8.5. However, with older leaves bentonite had to be added to the homogenizing buffer to preserve the larger polysomes. High pH media appear to be effective for obtaining polysomes with minimal degradation from ribonuclease rich tissues.

Larkins and Davies (1973) attempted to isolate the polyribosomes from pea seedlings by low speed centrifugation of a post-mitochondr

supernatant made 8 mM in Ca^{2+} concentration. They observed that exogenous and endogenous calcium stimulated polysome degradation.

Later Jackson and Larkins (1976) reported a procedure for extracting polysomes from tobacco leaves. In their study, they ground young, expanded tobacco leaves in high pH and high ionic strength buffers containing EGTA (a divalent cation chelator with high affinity for metallic cations). Their results showed that recovery of polysomes was reduced by the presence of divalent cations (Ca^{2+}) in leaf tissue and this could be overcome by chelations of these ions with EGTA.

Larkins and Davies (1975) isolated and characterized the total population of (free and membrane bound) polysomes from etiolated pea seedlings. They achieved a partial separation of free and membrane-bound polysomes by relatively low speed centrifugation of the homogenate. Based on the r-RNA analysis, they concluded that approximately 45% of the ribosomal material was bound to membrane and the remaining 55% consisted primarily of free polysomes. The free polysomes could be recovered free from membranous material by sedimentation through a dense sucrose pad (700 mg/ml) for varying periods of time up to 90 hr.

Mendiola *et al.* (1970) separated chloroplast and cytoplasmic ribosomes from *Euglena gracilis* by zonal centrifugation. The particles were characterized by their sedimentation rates as well as by their RNA components. Total extracts from the green cells contained 30S, 55S and 89S particles or their aggregates depending upon the Mg^{2+} concentration. They were unable to detect 70S particles from chloroplast preparations. Scott *et al.* (1970) and Avadhani and Buetow (1972) isolated *Euglena* chloroplast ribosomes although their preparations were unstable and

dissociated into subunits when pelleted. Lee and Evans (1970) also found that *Euglena* chloroplast ribosome preparations contained 46S and 55S components which were often present in greater quantity than the monosomes.

Chua *et al.* (1973), isolated a mixture of cytoplasmic (80S) and chloroplast (70S) ribosomes from *Chlamydomonas reinhardi* free of membrane contamination by sedimentation of a post mitochondrial supernatant through a layer of 1.87 M sucrose. These two species of ribosomes were present in the ratio of approximately 3:1. They separated the ribosomes into 80S and 70S fractions by centrifugation at a relatively low speed in a 10-40% (w/v) sucrose gradient containing 25 mM KCl and 5 mM $MgCl_2$.

Extensive physical and chemical characterization has been made of ribosomes from animals, microorganisms and lower plants. In contrast, reasonably complete descriptions of ribosomes from higher plants are limited to peas (Ts'o *et al.*, 1958; Bayley, 1964), white clover (Lyttleton, 1960), spinach (Lyttleton, 1962), and wheat (Lyttleton, 1968; Jones *et al.*, 1972).

In general, estimates of the dimensions of ribosomes in solution obtained by sedimentation velocity and other methods are somewhat larger than those obtained by electron microscope studies. For the 70S ribosomes of the prokaryates the dimensions are 200 x 120 x 170 Å in dry state and 290 x 210 x 120 Å in solution (Spirin and Gavrilova, 1969). There have been several electron microscopic studies on eukaryotic ribosomes from both animals and plants. For some of the plants studied by various investigators, the dimensions of chloroplast and cytoplasmic ribosomes are given in Table 1 which has been extracted from General physical properties of ribosomes (Van Holde and Hill, 1974).

TABLE 1. Electron microscopic studies of chloroplast and cytoplasmic ribosomes

Source	Approx. Sed. Coefficient (S)	Length (Å)	Width (Å)	References
Pea seedlings	80	350	160	Ts'o *et al.* (1958)
	60	420 - 500	180 (calc.)	Bayley (1964)
	40	210 - 250	80	Bayley (1964)
	60	290 - 330	250 - 300	Amelunxen and Spiess (1971)
	40	250 - 300	90 - 130	Amelunxen and Spiess (1971)
Pea and Bean (Cytoplasm)	80	260 ± 10	190 - 200 ± 10	Odintsova *et al.* (1967)
				Bruskov and Odintsova (1968)
				Bruskov and Kiselev (1968)
(chloroplast)	70	220 ± 10	170 ± 10	Odintsova *et al.* (1967)
				Bruskov and Kiselev (1968)
				Bruskov and Odintsova (1968)
Tobacco leaves (Cytoplasm)	80	286 ± 28	222 ± 25	Miller *et al.* (1966)
(chloroplast)	70	268 ± 24	214 ± 20	Miller *et al.* (1966)

Ribosomal Subunits

i. Dissociation

There are now many reports of the dissociation of eukaryotic ribosomes. Chao and Schachman (1956) and Chao (1957) reported the dissociation of yeast ribosomes and Ts'o and Vinograd (1961) and Lomfrom and Glowacki (1962) reported the dissociation of rabbit reticulocyte ribosomes. Petermann (1964) and Tashiro and Siekvitz (1965) isolated the ribosomes from guinea pig liver and dissociated them into their subunits. In these studies dissociation was achieved using buffer high in KCl and low in Mg^{2+}.

Polyvinyl sulphate (PVS) was found to induce dissociation of purified 80S ribosomes from leaves of Chinese cabbage and tobacco as well as rabbit reticulocytes and yeast (Vanyushin and Dunn, 1967). The subunits formed from the ribosomes of different origins were all found to have values of about 34S and 50S. In addition to these subunits, ribosomal preparations from plant leaves and rabbit reticulocytes yielded two faster moving components with values of 70S and 100S.

Martin *et al.* (1969) described the complete dissociation of rat muscle ribosomes into subunits using high concentrations of KCl (0.88 M) at 23°C. They found that 40S subunits at 4°C formed dimers which co-sedimented with 60S subunit in sucrose density gradient centrifugation. Hoober and Blobel (1969) studied the dissociation of chloroplast and cytoplasmic ribosomes of *Chlamydomonas reinhardi* in a buffer containing 25 mM tris-HCl (pH 7.5), 25 mM $MgCl_2$ and 25 mM KCl. The 68S ribosomes dissociated when the Mg^{2+} concentration was lowered to 5 mM whereas the 80S ribosomes dissociated when the Mg^{2+} concentration

was lowered to 1.2 mM. Subunits of 68S *Chlamydomonas* ribosomes sedimented in sucrose gradients containing 0.05 M KCl and 0.01 M tris-HCl (pH 7.5) without Mg^{2+} at 28S and 33S, and subunits of 80S ribosomes at 30S and 43S. Bourque *et al.* (1971) confirmed the observations of Hoober and Blobel (1969) that chloroplast ribosomes of *Chlamydomonas* can undergo progressive decreases in sedimentation velocity in response to sequential reductions in magnesium concentration.

App *et al.* (1971) studied the dissociation of ribosomes from imbibed and nonimbibed rice embryos. Ribosomes from rice embryos were dissociated into ribosomal subunits by systematic reduction of the Mg^{2+} concentration. The ribosomes from imbibed embryos were more easily dissociated than those from nonimbibed embryos. The resistance to dissociation was essentially overcome after 20 min of imbibition at 28°C at which time the ribosomes were active in protein synthesis. Ribosomes from either imbibed or nonimbibed embryos could be dissociated into subunits when the KCl concentration was 0.5 M KCl in the dissociation buffer.

Lin and Key (1971) reported the dissociation of ribosomes from pea root by varying the concentration of KCl to $MgCl_2$ or NH_4Cl to $MgCl_2$ in the presence of dithiothretol. The monosomes dissociated completely into subunits at 0.5 M KCl or NH_4Cl in presence of 5 mM $MgCl_2$. The sedimentation coefficient of the small subunit was more susceptible to change in KCl while the 60S subunit appeared to be more labile in NH_4Cl.

Jones *et al.* (1972) described the dissociation of zonal purified cytoplasmic (80S) and chloroplast (70S) ribosomes from 4.5-day old wheat seedlings, in the presence of the low $MgCl_2$ and high KCl buffer. When

80S ribosomes were centrifuged through 5 to 20% (w/v) sucrose gradients a considerable amount of 80S did not dissociate. From their results they concluded that the 80S ribosomes dissociated into a 60S subunit and a 42S or a 49S species. The chloroplast ribosomes (69S) when sedimented through a similar sucrose gradient dissociated into 31S and 49S subunits.

Cammarano *et al.* (1972a) purified ribosomes (80S) from higher plants (pea, spinach) by zonal centrifugation. Upon dissociation in high KCl buffer with Mg^{2+} at 1.0 mM, the ribosomes dissociated into subunits with sedimentation coefficients of 35S and 55S. In Mg^{2+} free buffer, sedimentation coefficients of the subunits were 27S and 42S.

Chua *et al.* (1973) used high salt buffer to dissociate the cytoplasmic and chloroplast ribosomes of *Chlamydomonas reinhardi* into active subunits of 57S and 37S and 50S and 33S, respectively. Brügger and Boschetti (1975) obtained a direct separation of total ribosomes (70S and 80S) of *Chlamydomonas reinhardi* into four subunits by zonal centrifugation using 200 mM KCl and 2.5 mM Mg^{2+} in the sucrose gradients. They assumed sedimentation coefficients of 50S and 30S for the 70S and 60S and 40S for the 80S ribosomal subunits.

ii. Reassociation

Martin and Wool (1968) and Martin *et al.* (1969) obtained active ribosomal subunits from mammalian tissue using high KCl low Mg^{2+} dissociation media. They suggested that efficient reassociation of the subunits required that the chelating agent (EDTA) be excluded from the isolation and dissociation media.

Using dissociation and hybridization methods Martin and Wool

(1969) and Martin *et al.* (1970) studied the ribosomal subunits from protozoa, fungi, yeast, plants and mammals. In general they found that the small and large subunits of 80S ribosomes of diverse species could reassociate to form hybrid monosomes.

Van der Zeijst *et al.* (1972) for yeast ribosomes and Faben and Tamaoki (1976) using mouse liver ribosomes reported the preparation of active 40S and 60S ribosomal subunits by high salt treatment. The isolated 40S and 60S subunits readily reassociated to form active 80S monosomes when mixed in the ratio of 1:2.

Von der Decken *et al.* (1970) prepared the ribosomal subunits from liver and skeletal muscle of rats and found that the reconstituted hybrid monosomes were active in the synthesis of polyphenylalanine. Cammarano *et al.* (1972b) also obtained synthetically active hybrid monosomes from reassociation of cytoplasmic ribosomal subunits derived from peas and rats.

Jones *et al.* (1973) reported the dissociation and reassociation of wheat chloroplast and cytoplasmic ribosomes. The 70S and 80S ribosomes were dissociated through zonal centrifugation in sucrose gradients containing high KCl to $MgCl_2$. The subunits of the cytoplasmic (80S) ribosomes combined readily to form 80S monomers. Although the subunits of chloroplast ribosomes reassociated to form 70S monomers, there was a relatively large amount of subunits which did not reassociate.

Ribosomal Ribonucleic Acids

Using phenol extraction, Spirin (1961) obtained ribosomal RNA with sedimentation coefficients of 16S and 25S while Glitz and Dekker (1963) reported values of 18S and 24S for the ribosomal RNA isolated

from wheat germ. In comparing the ribosomal RNA by polyacrylamide gel electrophoresis (PAGE), Loening (1968) found that bacteria, actinomycetes, blue green algae and higher plant chloroplasts all had ribosomal RNA with sedimentation coefficients of 16S and 23S. In comparing the ribosomal RNA isolated from bacteria, mammalian tissue and etiolated plants, Click and Tint (1967) and Click and Hackett (1966) found that the sedimentation coefficients of plant ribosomal RNA were 16S to 16.5S and 24.5S to 24.7S, similar to *E. coli* (16.5S and 23.5S).

Hadziyev *et al.* (1968) found that the cytoplasmic high molecular weight RNA's from two species of wheat were the same with sedimentation coefficients of 18S and 25S. Spencer and Whitfeld (1966) found only one high molecular weight RNA (16S) in isolated chloroplast ribosomes from several plant species. However for *Euglena gracilis* Rawson and Stutz (1969) reported two distinctly different ribosomal RNA components for each of cytoplasmic and chloroplast ribosomes with sedimentation coefficients of 20S and 24S, and 17S and 22S, respectively.

Mirault and Scherrer (1971) described a method of electrophoresis of RNA in which ribosomes were applied directly to polyacrylamide gels. Brügger and Boschetti (1975) by applying this technique to analyze ribosomes of *Chlamydomonas reinhardi* found two RNA bands in each of the ribosome classes which they designated as 16S and 23S and 18S and 25S for the 70S and 80S ribosomes respectively.

Plant Ribosomal Proteins

There have been numerous studies on the proteins of bacterial (Kaltschmidt *et al.*, 1967; Moore *et al.*, 1968; Craven *et al.*, 1969; Kurland *et al.*, 1969; Traut *et al.*, 1969; Kaltschmidt and Wittmann,

1970a,b; Mora *et al.*, 1971; Wittmann *et al.*, 1971) and animal (Bielk and Welfle, 1968; Low *et al.*, 1969; Gould, 1970; King *et al.*, 1971; Bickle and Traut, 1974; Sherton and Wool, 1972, 1974; Peeters *et al.*, 1973) ribosomes. However, there is comparatively little information available on plant ribosomal proteins.

Janda and Wittmann (1968) reported a comparison of protein patterns of 70S and 80S ribosomes from various plants, *E. coli* and yeast by disc gel electrophoresis. They found that there is greater similarity between chloroplast ribosomes from various plants than between chloroplast and cytoplasmic ribosomes obtained from the same plant. Odintsova and Yurina (1969) compared the cytoplasmic ribosomal proteins of pea seedlings and beans. They observed differences in their protein patterns by gel electrophoresis. In comparing the protein patterns of cytoplasmic ribosomes of bean and etiolated pea seedlings, they found no differences. The results of Gualerzi and Cammarano (1970) who compared the ribosomal proteins from cytoplasmic and chloroplast ribosomes of several plant species (spinach, lettuce mustard, beet) also showed that chloroplast ribosomal proteins were different than the cytoplasmic ribosomal proteins of the same plant.

Jones *et al.* (1972b) described a mc cation of the two-dimensional PAGE method of Kaltschmidt and Wittmann (1970a). Using this method they found approximately 85 proteins in the wheat leaf cytoplasmic ribosomes and noted that 16 of the proteins migrated towards the anode. The chloroplast (70S) ribosomes contained about 75 proteins which is more than have been reported for prokaryotic ribosomes (Kaltschmidt and Wittmann, 1970b). Upon comparing their ribosomal proteins of chloroplast and cytoplasm from maize and mung beans, Vasconcelos

and Bogorad (1971) concluded that each class of ribosome was distinguishable by the electrophoretic pattern of its proteins.

Nagabhushan *et al.* (1974) studied the electrophoretic separation of the cytoplasmic ribosomal proteins of barley, beans, peas, rye, spinach and of two species of wheat employing the method of Jones *et al.* (1972b). Among the various sp. examined, they found that wheat had the highest number of ribosomal proteins and barley had the least. They also reported that although the numbers and mobilities of the proteins varied to some extent with the species, there was a similarity in their pattern, e.g., beans, peas and spinach were most alike and barley and rye were similar. They obtained a significant positive correlation between the number of chromosomes and the number of basic cytoplasmic ribosomal proteins in the species.

Freyssinet and Schiff (1974) found 75 to 100 cytoplasmic ribosomal proteins in *Euglena gracilis* ranging in molecular weight from 10,200 to 104,000 while the chloroplast ribosomes contained 35 to 42 proteins with molecular weight ranging from 9,700 to 57,900. They also obtained 7 acidic proteins in the chloroplast ribosomes and 2 acidic proteins in the cytoplasmic ribosomes.

Gualerzi *et al.* (1974) compared the cytoplasmic ribosomal proteins of pea seedlings using urea or SDS two-dimensional PAGE. They found 32 intensively stained and 8 faint spots in the small subunit and 44 intensively stained and 11 faint spots in the large subunit by the urea method. By SDS-PAGE, they obtained 35-40 and 50-60 proteins in the small and large subunits, respectively. The majority of the ribosomal proteins of both the small and large subunits had molecular weights between 20,000 to 30,000. Hanson *et al.* (1974) characterized

the chloroplast and cytoplasmic ribosomal proteins of *Chlamydomonas reinhardi* by SDS two-dimensional PAGE. They obtained 22 and 26 proteins in the small and large subunits of chloroplast ribosomes and 26 and 39 proteins in the small and large subunits of cytoplasmic ribosomes, respectively.

Mets and Bogorad (1971, 1972) studied the chloroplast ribosomal proteins of erythromycin resistant mutants of *Chlamydomonas reinhardi*. For a mutant which was under nuclear control, they found alteration of a single protein in the large (52S) subunit and on the basis of electrophoretic mobility they suggested that there was an alteration of the primary sequence of the altered protein. For the mutant which was inherited uniparentally and postulated as being under plastid control, a different protein in the 52S subunit was affected and the alteration was considered to be either a change in the primary sequence or a secondary modification of the protein. Schlanger *et al.* (1972) reported a cytoplasmic gene mutation in *Chlamydomonas* which conferred carbomycin resistance and found that this mutation altered chloroplast ribosome function.

Bourque and Wildman (1973) analyzed the proteins of the 50S subunit of chloroplast ribosomes and found two differences between *Nicotiana tabacum* and *Nicotiana glauca* by PAGE. When they compared the protein pattern of F_1-hybrids of reciprocal crosses, each of the parent proteins were found in both hybrids. Therefore, they concluded that these proteins must be coded by nuclear DNA.

Schlanger and Sager (1974) studied amino acid incorporation of five antibiotic resistant strains of *Chlamydomonas* in order to localize the resistance at the ribosomal subunit level. On the basis of subunit

exchange experiments they found that resistance to streptomycin, neamine and spectinomycin was localized at the 30S subunit, and the 50S subunit was the site of resistance to cleocin and carbamycin. They took these results as evidence that some chloroplast ribosomal proteins are coded by genes in chloroplast DNA. Davidson *et al.* (1974) compared the proteins of 52S subunits of chloroplast ribosomes of wild type and of four ery-MI mutants of *Chlamydomonas reinhardi*. For each mutant, one of the proteins was different from the wild type on the basis of charge and molecular weight. Using PAGE Brügger and Boschetti (1975) compared 70S and 80S ribosomal subunit proteins of streptomycin sensitive and resistant strains of *Chlamydomonas reinhardi* involving both Mendelian and non-Mendelian inheritance. They found 25 and 34 proteins for the 30S and 50S subunits respectively and 31 and 44 proteins for the 40S and 60S subunits. Differences in proteins patterns between strains were observed only in the subunits of the chloroplast ribosomes. From their results they concluded that both nuclear and chloroplast DNA code chloroplast ribosomal proteins. Spiess and Arnold (1975) found the protein pattern of 70S ribosomes of wild type cells of *Chlamydomonas reinhardi* different from that of a Mendelian streptomycin resistant mutant but not from that of a non-Mendelian mutant. On rather tenuous ground they suggested that resistance in the Mendelian mutant was associated with a change in the ribosomal proteins and resistance in the non-Mendelian mutant was due to a change in the ribosomal RNA.

Prokaryotic and Eukaryotic Ribosomal Proteins

Isolation, purification and characterization of the ribosomal proteins of *Escherichia coli* have been achieved in a number of

laboratories (Kaltschmidt *et al.*, 1967; Traut *et al.*, 1969; Hardy *et al.*, 1969; Nomura *et al.*, 1969; Dzionara *et al.*, 1970; Kaltschmidt and Wittmann, 197a; and Kaltschmidt *et al.*, 1970). Kaltschmidt and Wittmann (1970b) who developed the method of two-dimensional PAGE found that the 30S subunits of *E. coli* contained 21 proteins and 50S subunits contained 34 proteins. At present, there is general agreement to the above noted number of ribosomal proteins present in the *E. coli* ribosomal subunits. Different systems of nomenclature had been used by the research groups in Berlin (Kaltschmidt and Wittmann, 1970b), in Uppsala (Kurland *et al.*, 1969), in Madison (Nomura *et al.*, 1969) and in Geneva (Traut *et al.*, 1969). For correlation and comparison of the studies done with ribosomal proteins in different laboratories, the plan agreed to was to co-opt the numbering system used by Kaltschmidt and Wittmann (1970b) based on the mobility of proteins in two-dimensional PAGE (Wittmann *et al.*, 1971).

Dzionara, Kaltschmidt and Wittmann (1970) determined the molecular weight of the isolated proteins from the 30S and 50S ribosomal subunits of *E. coli* by using two independent methods, e.g., SDS-PAGE and equilibrium sedimentation. The results by the two methods agreed well and gave molecular weights ranging from 10,000 to 65,000 for proteins of the 40S subunits and from 9,600 to 31,500 for those of the 50S subunits.

Sun *et al.* (1972) compared the ribosomal proteins from nine sp. of prokaryotes by PAGE in the presence of SDS. From their results they showed that ribosomal proteins from all the species closely resembled each other in number and molecular weight.

Fractionation studies of eukaryotic ribosomal proteins are not as far advanced as those of *E. coli*. However a number of workers have investigated eukaryotic ribosomal proteins by various one- and two-dimensional PAGE techniques (Gould, 1970; Bickle and Traut, 1971; King *et al.*, 1971; Martini and Gould, 1971; Welfle, 1971; Welfle *et al.*, 1971, 1972; Lambertson, 1972; Chatterjee *et al.*, 1973; Pratt and Cox, 1973); Thomas, 1973; Howard *et al.*, 1975).

Bickle and Traut (1971) compared the molecular weight of the ribosomal proteins from two prokaryotes, *E. coli* and *Micrococcus lysodeikticus* and the cytoplasmic ribosomes of *Euglena* and mouse plasmacytoma cells by SDS-PAGE. They found that the average molecular weights of the bacterial ribosomal proteins were substantially smaller than the eukaryotic ribosomal proteins.

Sherton and Wool (1972) determined the number of proteins in rat liver ribosomes and ribosomal subunits by two-dimensional PAGE. They found 30 proteins in the 40S subunit and 39 proteins in the 60S subunits. The 80S ribosomes contained three proteins not present in either subunit thus they estimated that eukaryotic ribosomes contained between 68 and 72 proteins. Howard *et al.* (1975) separated the ribosomal proteins from 40S and 60S subunits of rabbit reticulocytes by two-dimensional PAGE and determined their individual molecular weights. From their studies they identified 32 proteins in the 40S subunit with molecular weights ranging from 8,000 to 39,000 and 39 proteins in the 60S subunit with molecular weights from 9,000 to 50,000. Welfle *et al.* (1972) reported 70 ribosomal proteins in rat liver ribosomes by two-dimensional PAGE. Thirty were found in the small subunit and 39 in the

large subunit. In addition, they also found two acidic proteins in the large subunit. Ishiguro (1974) analyzed the yeast cytoplasmic ribosomal proteins by two-dimensional PAGE methods of Kaltschmidt and Wittmann (1970a) and Mets and Bogorad (1974). By applying these methods, he obtained 28 and 29 proteins in the 40S subunit and 40 and 41 proteins in the 60S subunits, respectively. He reported the molecular weights to be less than 40,000 and 60,000 for the proteins in the small and large subunits, respectively.

Amino Acid Incorporation by Plant Systems

Although protein synthesis by cell free systems from prokaryotes especially *Escherichia coli* has been extensively studied (Tissières *et al.*, 1960, Schlessinger and Gros, 1963; Mangiarotti and Schlessinger, 1966, 1967; Schlessinger *et al.*, 1967; and Sherman, 1972) relatively few similar detailed investigations have been conducted with higher plant material. Protein synthesis in plants may occur on free or membrane bound ribosomes of cytoplasmic, chloroplast or mitochondrial origin (Zalik and Jones, 1973). Thus far, studies have been done mainly with cytoplasmic and chloroplast ribosomes. Cytoplasmic ribosomes isolated from wheat germ have been a preferred material of study. Marcus and Feeley (1966) reported protein synthetic activity of ribosomes isolated from imbibed wheat embryos and poly (U)-directed synthesis by ribosomes isolated from dry embryos in the presence of an ATP energy generating system. Allende and Bravo (1966) and de Groot *et al.* (1967) also found similar results with wheat embryo systems. They found that activity depended upon poly (U), supernatant, Mg^{2+}, K^{+}, GTP and an energy generating system. From their studies on amino acid

incorporation by rice embryos App and Gerosa (1966) also demonstrated an absolute requirement for poly (U), Mg^{2+}, K^{+}, (NH_4^{+}), GTP and supernatant factors. They found that incorporation decreased when the ribosomes were washed with deoxycholate and the incorporation was completely restored when supernatant factors were added. Later App (1969) purified two factors obtained from rice embryo supernatants which were required for *in vitro* polyphenylalanine synthesis. Mehta *et al.* (1969) found that cytoplasmic ribosomes isolated from wheat leaves also had a similar cofactor requirement for amino acid incorporation, moreover, ribosomes obtained by ribonuclease digestion of polyribosomes had negligible activity but it could be increased by the addition of poly (U).

Sissakian *et al.* (1965) studied amino acid incorporation by ribosomes isolated from pea seedling chloroplasts. Boardman *et al.* (1966) working with tobacco leaves and Hadziyev and Zalik (1970) with wheat compared the amino acid incorporating ability of the chloroplast and cytoplasmic ribosomes. Although both groups found higher incorporation rates for chloroplast than cytoplasmic ribosomes, Jones *et al.* (1973) who studied the incorporation of phenylalanine by 80S and 70S wheat leaf ribosomes separated by zonal centrifugation found the rates to be the same. They also observed that the 70S ribosomes were inhibited by chloramphenicol and both 70S and 80S were inhibited by puromycin.

Gulyas and Parthier (1971) found cytoplasmic ribosomes of pea seedlings were activated in their poly (U)-directed synthesis by washing with NH_4Cl, KCl, detergents or Sephadex gel filtration. They suggested the stimulation was due to removal of an inhibitor of the synthesis of phenylalanyl-tRNA.

Tucker and Zalik (1973) studied the phenylalanine incorporation of wheat seedling cytoplasmic ribosomes. When the two subunits of the cytoplasmic ribosomes were mixed in a ratio of 2:1 (60S:40S), they reassociated to form monosomes but the reassociated monosomes were not active in polyphenylalanine synthesis. Lin *et al.* (1973) in comparing phenylalanine incorporation by ribosomes isolated from pea root and corn leaves at different ages found much higher activity for preparations from the younger tissue.

MATERIAL AND METHODS

Chemicals

The chemicals used in this study were reagent grade and were obtained from various suppliers. Acrylamide, N,N^1-methylene bis-acrylamide, N, N, N^1, N^1-tetramethylethylenediamine and 2-mercapto-ethanol were from Eastman Organic Chemicals (Rochester, N.Y., USA). Ammonium persulphate, boric acid, glycine, potassium chloride, sodium ethylenediaminetetraacetic acid, sodium dodecylsulphate, triethanolamine, copper sulphate and phenol reagent were from Fisher Chemicals (Edmonton, Alberta, Canada). Triton X-100, magnesium chloride, potassium hydroxide, urea, sodium acetate, sodium hydroxide, acetic acid and methanol were from Baker Chemical Co., (Phillipsburg, N.J., USA). Ultra pure sucrose was from Schwarz/Mann Division of Becton Dickinson and Co. (Orangeburg, N.Y., USA); and Trizma-base, crystalline bovine serum albumin and coomassie brilliant blue R-250 were from Sigma Chemical Co. (St. Louis, Missouri, USA). Sodium deoxycholate was from K & K Laboratories Inc., (Plainview, N.Y.) and tricine [N-tris(hydroxymethyl) methyl-glycine] was obtained from Calbiochem (Los Angeles, California, USA).

Plant material

Hordeum vulgare c.v. Gateway and its chlorophyll deficient mutant were used in this study. Dry seeds of both lines were cut with a scalpel so as to remove most of the endosperm and the dry embryo ends (unimbibed) were used for most of the studies dealing with cytoplasmic ribosomes.

For simplicity the dry embryo ends will be referred to as embryos.

For studies of chloroplast and cytoplasmic ribosomes, seedlings of both lines were grown under continuous light at 600 ft.c at 22°C. The seeds were grown in vermiculite and prior to harvest, 6 days after planting, the seedlings were chilled at 4°C for 15 min.

Buffer Composition

Extraction buffer: Buffer I (50 mM tricine pH 7.8, 5 mM $MgCl_2$, 40 mM KCl, 5 mM 2-mercaptoethanol)

Resuspension buffer; Buffer II (10 mM tricine, pH 7.8, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol)

Dissociation buffer: (buffer II, containing 400 mM kCl)

Reassociation buffer: Buffer III (25 mM tricine, pH 7.8, 25 mM $MgCl_2$, 25 mM KCl, 5 mM 2-mercaptoethanol)

Grinding buffer: (buffer III, containing 4% triton X-100)

Buffer IV: (25 mM tricine, pH 7.8, 5 mM $MgCl_2$, 25 mM KCl, 5 mM 2-mercaptoethanol)

Isolation of Ribosomes from Embryos

Ribosomes were isolated by a modification of the procedure described by Jones *et al.* (1972). All operations were performed at 4°C. Twenty grams of embryo ends of barley seeds were homogenized 3 times intermittently with a polytron homogenizer (Brinkman Instruments [Canada] Ltd.) type P.T. 200D for about 40-50 seconds in 80 ml of extraction buffer (buffer I). On the basis of many preliminary trials this medium and volume gave the most reproducible preparation of pure ribosomes. The homogenate was clarified by passing through an Acme Superior Juicerator (Acme Juicer, Mfg. Col, Sierra Madre, Calif., USA)

lined with miracloth. The filtrate was brought to a final concentration of 4% Triton X-100 by the addition of 20% Triton X-100 in extraction buffer. The resulting suspension was stirred for 15 min and then centrifuged first at 10,000 rpm in a 970 IEC rotor for 15 min to remove the heavy contaminants and then the supernatant was recentrifuged at 20,000 rpm for 40 min. The supernatant obtained was filtered through miracloth (Calbiochem., La Jolla, Calif., USA) and layered on top of 7 ml of resuspension buffer (buffer II) containing 1 M sucrose in polyallomer (#320823) ultracentrifuge tubes obtained from Beckman Instruments (Palo Alto, Calif., USA). Ultracentrifugation was done at 55,000 rpm for 2.75 hr in a Spinco Ti 60 rotor (Beckman Model L2-65B Ultracentrifuge). The ribosomal pellets were resuspended in 1.5 ml of resuspension buffer and centrifuged at 17,000 rpm for 15 min in a 970 rotor. Ribosomal yield was estimated by determining the OD of the supernatant at 260/280 nm using a Beckman 25 spectrophotometer (Beckman Instruments, Palo Alto, Calif., USA) and the ribosomes were used immediately for further study. This method of extraction normally yielded around 500 A_{260} and 400 A_{260} units of ribosomes from 20 g of normal and mutant embryos, respectively.

Dissociation of Cytoplasmic Ribosomes

In order to dissociate the cytoplasmic ribosomes obtained from the dry embryos, the ribosomes were suspended in dissociation buffer which consisted of buffer II including 400 mM KCl, and 50 A_{260} units (approximately 200 µl) of this preparation were layered on a 7-37% (w/v) linear sucrose density gradient in dissociation buffer in 38.5 ml nitrocellulose tubes (Beckman Instruments #302237, Palo Alto, Calif., USA). The linear

sucrose density gradients were prepared by mixing the high and low sucrose solutions using a density gradient mixer (Buchler Instruments, Fort Lee, N.J., USA). Centrifugation was done at 22,000 rpm for 16 hr in a Beckman SW 27 rotor. After spinning, the tubes were punctured at the bottom and the gradient was pumped at a constant rate of 1 ml/min with a peristaltic pump (LKB-Produkter AB, Stockholm, Sweden) through a flow cell and absorbance at 260 nm was recorded. The linearity of the sucrose gradients was confirmed in two separate runs by adding 0.4 mg/ml (w/v) ATP to the high sucrose solution used in preparing the gradient in one tube. After centrifugation this tube gave a linear scan with OD ranging from 0.95 to 0.05 at 260 nm.

The fractions of subunit peaks denoted in Figure 2 were collected, pooled and stored frozen at -18°C. After four independent preparations the pooled fractions were combined and centrifuged at 55,000 rpm in a Ti 60 rotor for 14 hr to collect the subunit pellets.

Reassociation of Ribosomal Subunits

The isolated subunit pellets were resuspended in dissociation buffer or in reassociation buffer (buffer IV) by gently rubbing with a Q-tip from which most of the cotton had been pulled off and the suspension was clarified by spinning at 17,000 rpm for 15 min in a 970 rotor. The 60S and 40S subunits suspended in either the dissociation or reassociation buffer were combined in the proportion of 1:1 A_{260} units. After incubation for 0.5 hr at room temperature 2 A_{260} units of each mixture was layered on a 5-20% (w/v) sucrose linear gradient in either dissociation or reassociation buffer. The tubes were centrifuged at 26,000 rpm for 4.25 hr at 20°C in an SW 27 rotor. The gradients were

eluted and monitored at 260 nm as already described.

Isolation of Ribosomes from Seedlings

Eighty grams of leaves from 6-day-old barley seedlings were cut with a scissors into 0.5 to 1 cm pieces and were ground in a Waring blender for 1-2 min intermittently using 160 ml of buffer IV containing 4% Triton X-100. The homogenate was clarified by passing through an Acme Superior Juicerator lined with Whatman #1 filter paper. The supernatant was centrifuged at 20,000 rpm for 40 min in a 970 rotor to remove the debris and cell organelles of the resultant supernatant. 29 ml was added to each of the polyallomer tubes and 7 ml of buffer III containing 1 M sucrose was underlayed before centrifugation at 55,000 rpm for 5 hr in a Ti 60 rotor. The ribosome pellets were combined by suspending them in 3 ml of gradient buffer (buffer IV) and after clarifying at 17,000 rpm for 15 min in a 970 rotor, the supernatant was immediately used for separation of 70S and 80S ribosomes.

Separation of Ribosomes into 70S and 80S Monosomes

From the above ribosome preparation 150 A_{260} units (approximately 300 µl) were layered on 38.5 ml of a 7-37% (w/v) linear sucrose gradient in gradient buffer. After centrifugation at 22,000 rpm for 16 hr in a SW 27 rotor, the gradient was eluted and monitored as already described. Absorption was measured at 290 nm rather than 260 nm because of the large quantities of ribosomal material. The fractions corresponding to 70S and 80S ribosome peaks as denoted in Figure 7 were collected, pooled and stored frozen at -18°C.

In order to prevent dissociation of the ribosomes during subsequent centrifugation the Mg^{2+} concentration in the pooled fractions was

raised to 25 mM by addition of appropriate amounts of a 1 M $MgCl_2$ solution. After 5 independent preparations, the pooled fractions were combined and centrifuged to pellet the ribosomes at 55,000 rpm for 10 hr in a Ti 60 rotor.

Analysis of Purity of Ribosomes and Ribosomal Subunits

i. Sucrose density gradient centrifugation

To analyze the purity of the ribosomes and subunits sedimented from the pooled fractions, 2 A_{260} units (approximately 100 µl) of ribosomal material was layered on top of 17 ml of a 5-20% (w/v) linear sucrose gradient prepared in the appropriate buffer and centrifuged at 26,000 rpm for 4.25 hr at 25°C in an SW 27 rotor. The gradients were eluted and scanned at 260 nm as already described. *E. coli* ribosomes (Miles Laboratories, Inc., Elkhart, Indiana, USA) were used for comparison.

ii. Polyacrylamide gel electrophoresis of ribosomal RNA

The purity of ribosomes and ribosomal subunit preparations was also assessed by analysis of their ribosomal RNA components by polyacrylamide gel electrophoresis (PAGE). The procedure of Mirault and Scherrer (1971) based on that of Loening (1969) was used.

Uniform 2.5% (w/v) polyacrylamide gel solution was prepared by mixing 2.0 ml of stock buffer (0.4 M triethanolamine, 0.02 M sodium acetate, and 0.02 M EDTA, pH 7.4), 2.5 ml of 20% acrylamide stock (20 g recrystallized acrylamide, and 1 g bis-acrylamide made to 100 ml with water) and 15.3 ml water in a conical flask kept in ice. The solution was degassed under vacuum for 10 min and 100 µl of each of 10% (w/v)

freshly prepared aqueous ammonium persulphate and TEMED were added in sequence. Electrophoresis was carried out in 9.5 x 0.6 cm (i.d.) quartz tubes which were cleaned with chromic acid and coated with 1:200 (v/v) Kodak Photo-Flo (Eastman Kodak Co., Rochester, N.Y.) aqueous solution.

The polyacrylamide gel solution was swirled and poured to a depth of 8.5 cm in the tubes. The gel solution was overlayed immediately with buffer containing TEMED and ammonium persulphate all in the same concentration used in preparing the gel. To assure good polymerization the gels were kept overnight at room temperature.

The elctrophoresis buffer consisted of stock buffer diluted 1:10, 2.5% glycerol, 0.2% SDS and 0.1% sodium deoxycholate. The gels were prerun at 5 mA/tube for 1 hr at 4°C and 25 µl of ribosomal material containing 0.36 A_{260} units in 1 mM Tris-HCl, (pH 7.4), 1 mM $MgCl_2$, 10% glycerol and 2% SDS was applied. Prior to electrophoresis 100 µl of 0.1% (w/v) bromophenol blue in buffer was added to the upper reservoir and electrophoresis was at 5 mA/tube for 2.5 hr at 4°C. The gels were scanned directly in the tubes at 260 nm using a gel scanning assembly attached to a Beckman model 25 spectrophotometer.

iii. Determination of sedimentation coefficents

Ribosomal samples containing 25 A_{260} units/ml in the suspension medium specified with the figures were centrifuged at 20°C, in a Spinco model E ultracentrifuge fitted with Schlieren optics (Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif., USA), and pictures were taken at 0, 4, 8, 12, 16 and 20 min on Kodak metallographic plates after reaching a speed of 39,460 rpm.

The plates were developed and read with a Gaertner micro-

comparator (Gaertner Sc. Co., Chicago, Ill., USA) by Mr. Morris R. Aarbo, Department of Biochemistry, who calculated the sedimentation coefficients using a computer program.

Ribosomal Proteins

i. Extraction of ribosomal proteins

Proteins were extracted from ribosomes and from ribosomal subunits by acetic acid essentially as described by Jones *et al.* (1972). Pure ribosomal material was resuspended in buffer II or buffer III (pH 7.8) containing 100 mM $MgCl_2$ and two volumes of glacial acetic acid were added. The mixture was stirred for 45 min at 4°C, followed by centrifugation at 24,000 g for 15 min to pellet the ribosomal RNA which was discarded. The supernatant fraction containing the proteins was lyophilized overnight and after lyophilization, the proteins were dissolved in the first-dimensional electrophoresis buffer. Protein concentrations were determined by the procedure of Lowry *et al.*, (1951) using crystalline bovine serum albumin as standard.

ii. Polyacrylamide gel electrophoresis of ribosomal proteins

a. First-dimensional polyacrylamide gel electrophoresis: The methods used were described by Kaltschmidt and Wittmann (1970a) and modified by Jones *et al.* (1972). First-dimensional electrophoresis was carried out in 4% acrylamide gel in 15 x 0.6 cm (i.d.) glass tubes. The gel solution contained 4 g recrystallized acrylamide, 0.133 g bis-acrylamide, 0.800 g EDTA-Na_2, 3.200 g boric acid, 4.866 g trizma (pH 8.6) and 36 g urea made up with water to 99 ml. After degassing the gel solution it was made to 0.3% TEMED and to 0.07% with respect to

ammonium persulphate. The tubes were filled to 13.5 cm with gel solution and then overlayered with water. After the gels were polymerized, the water was sucked off with the aid of rolled filter paper. Pre-electrophoresis was done at 4 mA/tube for 1 hr using the first-dimension electrophoresis buffer containing 6 M urea, 0.15 M borate, 6.5 mM EDTA-Na_2 and 0.12 M trizma (pH 8.6) in both the upper and the lower trough. Then 0.5 mg of ribosomal protein sample (approximately 300 µl) in 10% sucrose was layered on top of the gels and 10 µl of 0.5% methyl green was placed on top of one gel as a marker dye. Electrophoresis was carried out at 5 mA/tube towards the cathode till the marker dye band had reached the bottom of the gel (15-16 hr). The gels were equilibrated in urea-acetate buffer (6 M urea, 0.012 N KOH, pH 4.6) for 20 min before laying them on top of the second-dimensional gel.

One-dimensional polyacrylamide gel electrophoresis of acidic ribosomal proteins differed from the above in that only 150 µg of each protein sample and 10 µl of 0.5% bromophenol blue were layered on top of the gel and the run was made towards the anode. Electrophoresis was stopped when the tracking dye was within 1 cm of the bottom of the gel (10 hr). The gels were stained with 0.1% coomassie brilliant blue R-250 (in 10% methanol and 10% acetic acid) for 3 hr and destained by shaking the gels overnight in the destaining solvent (10% acetic acid and 10% methanol). After complete destaining, the gels were photographed and scanned at 550 nm.

b. Second-dimensional polyacrylamide gel electrophoresis: The apparatus used in the second-dimension was the same as previously described by Jones *et al.* (1972) to accommodate gel slabs of 14 x 14 x 0.6 cm.

The following gel solution was used for the second-dimension: 360 g urea, 180 g acrylamide, 5.0 g bis-acrylamide, 52.3 ml glacial acetic acid, 9.6 ml 5N KOH, 5.8 ml TEMED, and water to make 967.0 ml. The gel solution was deaerated in 250 ml batches for 10 min and polymerization was initiated by the addition of 8.25 ml of 10% freshly prepared ammonium persulphate. The 2-D gel apparatus was placed in a shallow tray and 250 ml of gel solution was poured around the apparatus and allowed to polymerize. After the chamber was sealed about 125 ml gel solution was poured into each chamber. In order to prevent formation of air bubbles in the gel slabs the chambers were filled from the bottom using fine tubing attached to a column. Immediately an equilibrated 1-D gel was laid horizontally on the 2-D gel and additional 2-D gel was poured over the 1-D gel. A piece of plastic 3 mm^2 was inserted into the second-dimension gel before polymerization to mark the end of 1-D gel. The gel solution was kept in ice before pouring and the equipment was kept at 4°C for 1 hr prior to pouring to avoid too rapid polymerization of the acrylamide.

After polymerization, the apparatus was lifted from the tray, cleaned of superfluous gel and put into the cathode buffer container containing 750 ml of 2-D buffer (0.018 M glycine-acetic acid, pH 4.6). Also, 750 ml 2-D buffer was poured into the anode buffer container. After removing the 3 mm^2 piece of plastic, 10 µl of 0.5% methylene blue was applied to the hole as a tracking dye. Electrophoresis towards the cathode was carried out at 120 volts with the current limited to 220 mA at 4°C. Electrophoresis was stopped when the tracking dye reached 1 cm from the bottom of the gel slab (18-20 hr).

Staining and Destaining

After electrophoresis the gel slabs were carefully taken out of the chamber and placed in a tray containing 0.1% coomassie brilliant blue R-250, for 3 hr with occasional agitation. The slabs were placed in a destaining apparatus filled with destaining solvent and were destained electrophoretically by applying 220 mA current for 6 hr.

Finally, the gel slabs were placed in a glass tray and were destained by slow shaking on a mechanical shaker using several changes of destaining solvent over a period of 10-12 hr. After complete destaining the gel slab in the tray containing destaining solvent was illuminated from below on a light box and photographed with a Pentax Spotmatic Camera fitted with a 50 mm lens.

^{14}C-phenylalanine Incorporation Studies

For incorporation studies, the ribosomes and ribosomal subunits from barley embryos were derived as described earlier. These studies were carried out in collaboration with Dr. A.S. Cohen and the results are given in the Appendix.

RESULTS AND DISCUSSION

Isolation of Ribosomes and Ribosomal Subunits from Barley Embryos

i. Characteristics of isolated ribosomes

Each ribosomal preparation was examined for homogeneity by sucrose density gradient centrifugation and by analytical untracentrifugation. Ribosomes isolated from barley embryos sedimented as a single homogenous peak which remained symmetrical throughout the run (Fig. 1).

As seen from Figure 1, there was no evidence of subunits or polysomes in the preparation and the calculated sedimentation value of the monosome was 81S. This was taken as confirmation that the dry (unimbibed) embryo ends of barley seeds yielded only cytoplasmic monosomes. Other research workers using dry embryos of various seeds have reported obtaining only 80S cytoplasmic monosomes (Wolfe and Kay, 1967; App *et al.*, 1971; Gumilevskaya *et al.*, 1975). Ribosomal preparations from the mutant barley embryos gave the same patterns as the normal Gateway both in sucrose density gradient and analytical centrifugation. Thus these preparations were also composed of only 80S monomeric ribosomes, but the yield of ribosomes appeared to be less than that of normal embryos.

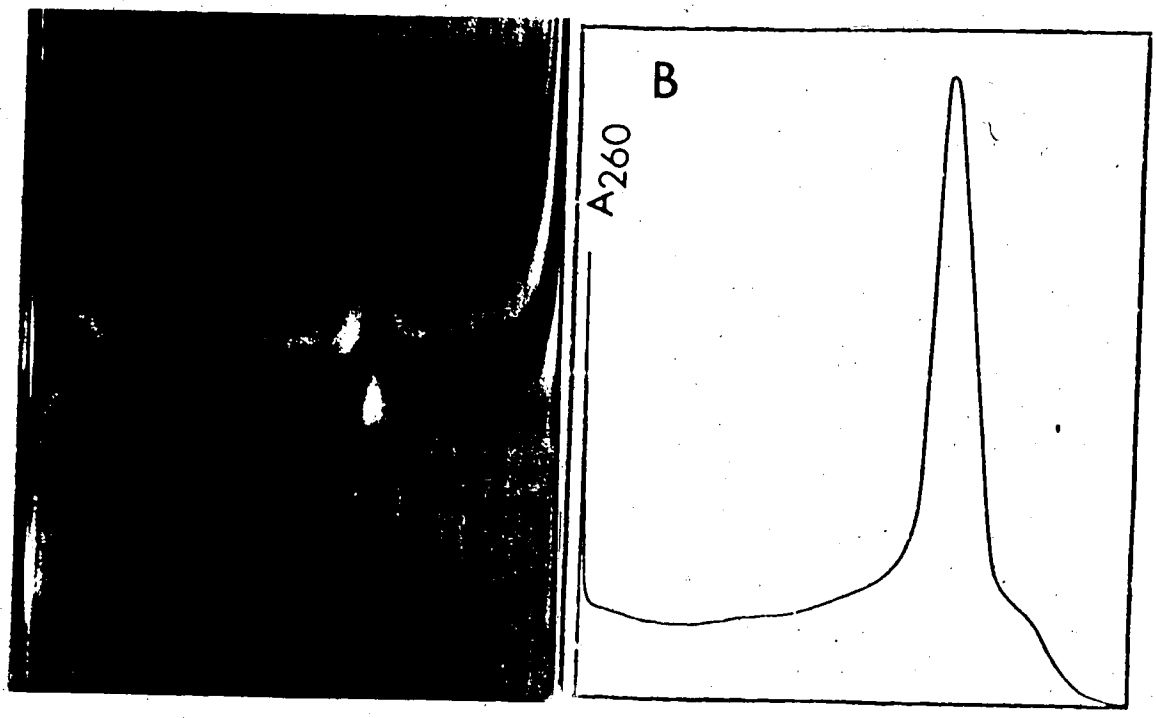
ii. Dissociation of ribosomes and isolation of ribosomal subunits

The purity of the ribosomal preparations that were to be used in dissociation studies was assessed by absorbance readings at 235, 260 and 280 nm (Petermann, 1964). Only those preparations having absorption

FIG. 1. Analytical ultracentrifuge pattern and sucrose gradient analysis of ribosomes isolated from embryos of Gateway barley.

A- the ribosomal pellet in resuspension buffer was centrifuged in the analytical ultracentrifuge and the picture was taken 12 min after reaching a speed of 29,460 rpm at 20°C. Direction of the run from left to right.

B- Sucrose gradient analysis of ribosomal pellet. Two A_{260} units of ribosomal preparation were applied to a 5-20% (w/v) sucrose density gradient in resuspension buffer and centrifuged at 26,000 rpm for 4.25 hr at 25°C in a Beckman SW 27 rotor (small buckets). After centrifugation the eluant was monitored at 260 nm.


B
A260

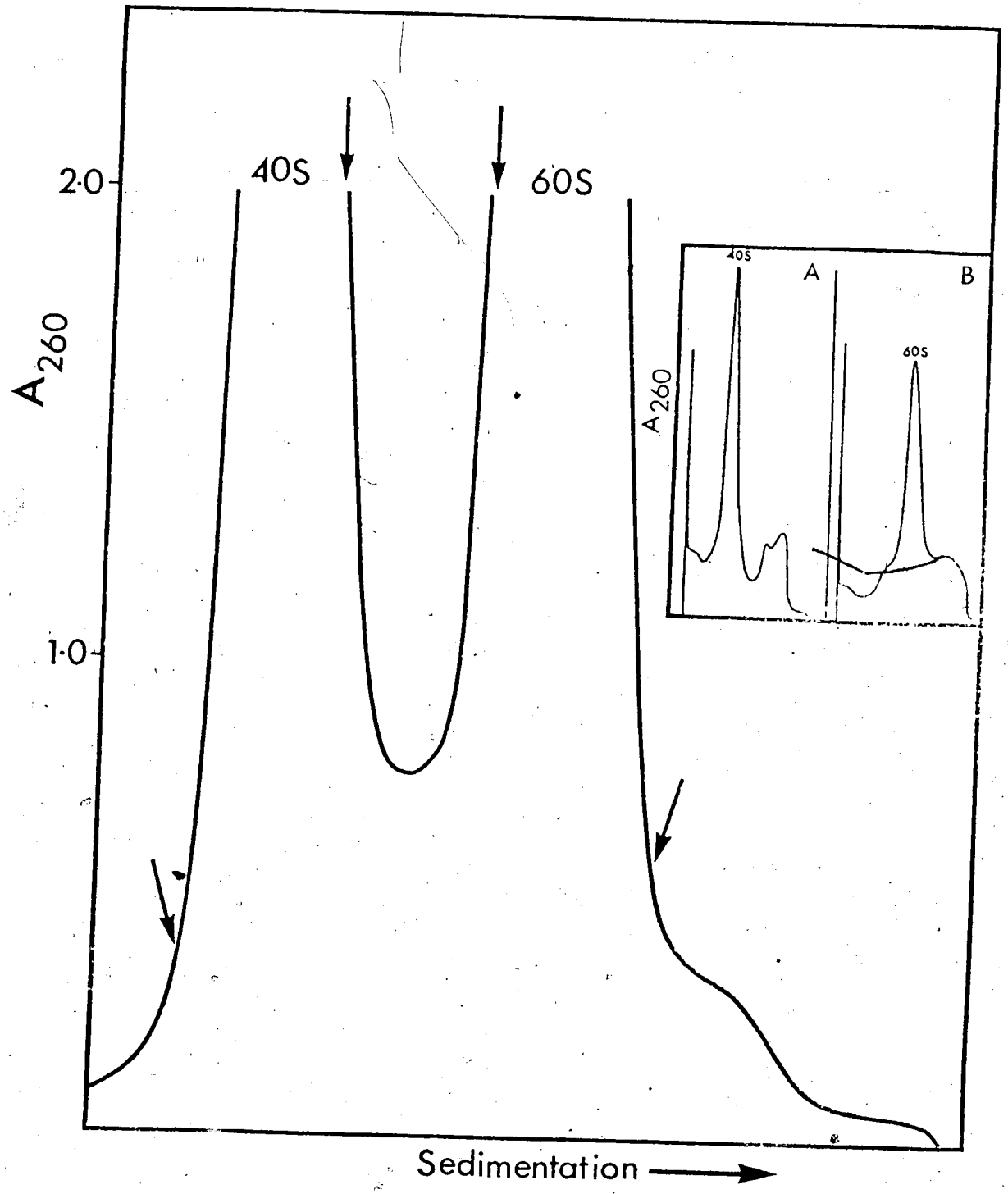
ratios 260:235 and 260:280 greater than 1.40 and 1.80 respectively were used. Using such preparations the optimum conditions in terms of KCl concentration, sucrose gradient and quantity of material to obtain complete dissociation of the ribosomes and isolation of their subunits were determined. From these preliminary studies it was found that complete dissociation and separation of ribosomal subunits in high yield could be obtained consistently by using the procedure given in the methods section. After dissociation of the ribosomes and separation of the subunits on sucrose gradients the separate portions of the 40S and 60S subunits indicated in Figure 2 were collected and pooled for the 6 tubes. As seen from the figure there was complete dissociation of the monosomes and the subunits were well separated. The dissociation pattern of the mutant embryo ribosomes was the same as that of the normal. These methods made it possible to isolate both subunits from the normal and mutant lines of barley in sufficient quantities to carry out further studies.

Analysis of Purity of Ribosomal Subunit Preparations

The insert in Figure 2 shows that purified 40S subunits (A) were almost free of contamination with 60S subunits and purified 60S subunits (B) were free of contamination with 40S subunits. The extent to which the small subunits were contaminated by large subunits was markedly dependent on the ribosomal concentration in the high salt sucrose gradients and could be reduced to almost nil when the amount of ribosomes loaded on the 7-37% (w/v) sucrose gradients did not exceed 50 A_{260} units.

FIG. 2. Sucrose gradient separation of 40S and 60S ribosomal subunits of Gateway barley embryos

Fifty A_{260} units (200 µl) of ribosomes suspended in dissociation buffer were layered on a 7-37% (w/v) linear sucrose gradient in dissociation buffer. After centrifugation at 22,000 rpm for 16 hr at 4°C in SW 27 rotor the eluant was monitored at 260 nm. The portions of the 40S and 60S subunits indicated by the arrows were collected and pooled. The subunits in each fraction were pelleted by centrifugation in a Ti 60 rotor at 55,000 rpm for 14 hr at 4°C. Two A_{260} units of each subunit were applied to a 5-20% (w/v) linear sucrose gradient in buffer including 40 mM KCl, and centrifuged at 26,000 rpm for 4.25 hr at 25°C in an SW 27 rotor. The contents were monitored at 260 nm. Insert A- 40S; B- 60S.


40S
60S
2·0
1·0
A_{260}
Sedimentation
40S
A
B
60S
A_{260}

i. Polyacrylamide gel electrophoresis of ribosomal RNA isolated from individual subunits

To confirm the purity of each subunit preparation, ribosomal RNA from individual subunits was extracted and electrophoresed. Using 2.5% polyacrylamide gels. As indicated in Figures 3A and B each yielded only one RNA component assumed to be 25S and 18S RNA for the large and small subunits respectively. From the electrophoretic patterns it is apparent that these RNA's were still intact after dissociation of the monosomes. Earlier Glitz and Dekker (1963) working with wheat germ reported values of 24S and 18S for the high molecular RNA.

ii. Sedimentation coefficients of ribosomal subunits

The purity and sedimentation coefficients of the ribosomal subunits was determined by analytical ultracentrifuation as described in the Materials and Methods and are given in Figure 4. As seen from Figure 4A the large ribosomal subunit was free from 40S contamination and gave an S value of 59. The small subunit (Fig. 4B) gave a major peak with a sedimentation coefficient of 37S and a minor peak with a sedimentation coefficient of about 60S. This heavier peak was assumed to be a dimer of the small subunit rather than contamination by the large subunit. There are two reasons for this assumption; firstly the purity of the subunits assessed by the sucrose gradients and secondly the absence of any RNA in the small subunit fraction heavier than 18S upon SDS polyacrylamide electrophoresis (Fig. 3B). Gumilevskaya *et al.* (1975) and Faber and Tamakoi (1976) reported the capacity of small subunits from dry pea seeds and rat liver ribosomes respectively to form aggregates.

FIG. 3. Polyacrylamide gel electrophoresis of RNA extracted from ribosomes and ribosomal subunits of Gateway barley.

Fifty µg ribosomes in 1 mM tris-HCl (pH 7.5), 1 mM $MgCl_2$, 10% glycerol and 2% SDS were applied to a uniform 2.5% gel. Electrophoresis was for 2.5 hr at 5 mA per gel. The gels were scanned directly in the quartz tubes at 260 nm.

RNA of Ribosomal subunits from barley embryos:

A- 60S; B- 40S.

RNA of cytoplasmic and chloroplast ribosomes from leaves of barley seedlings:

C- 80S; D- 70S.

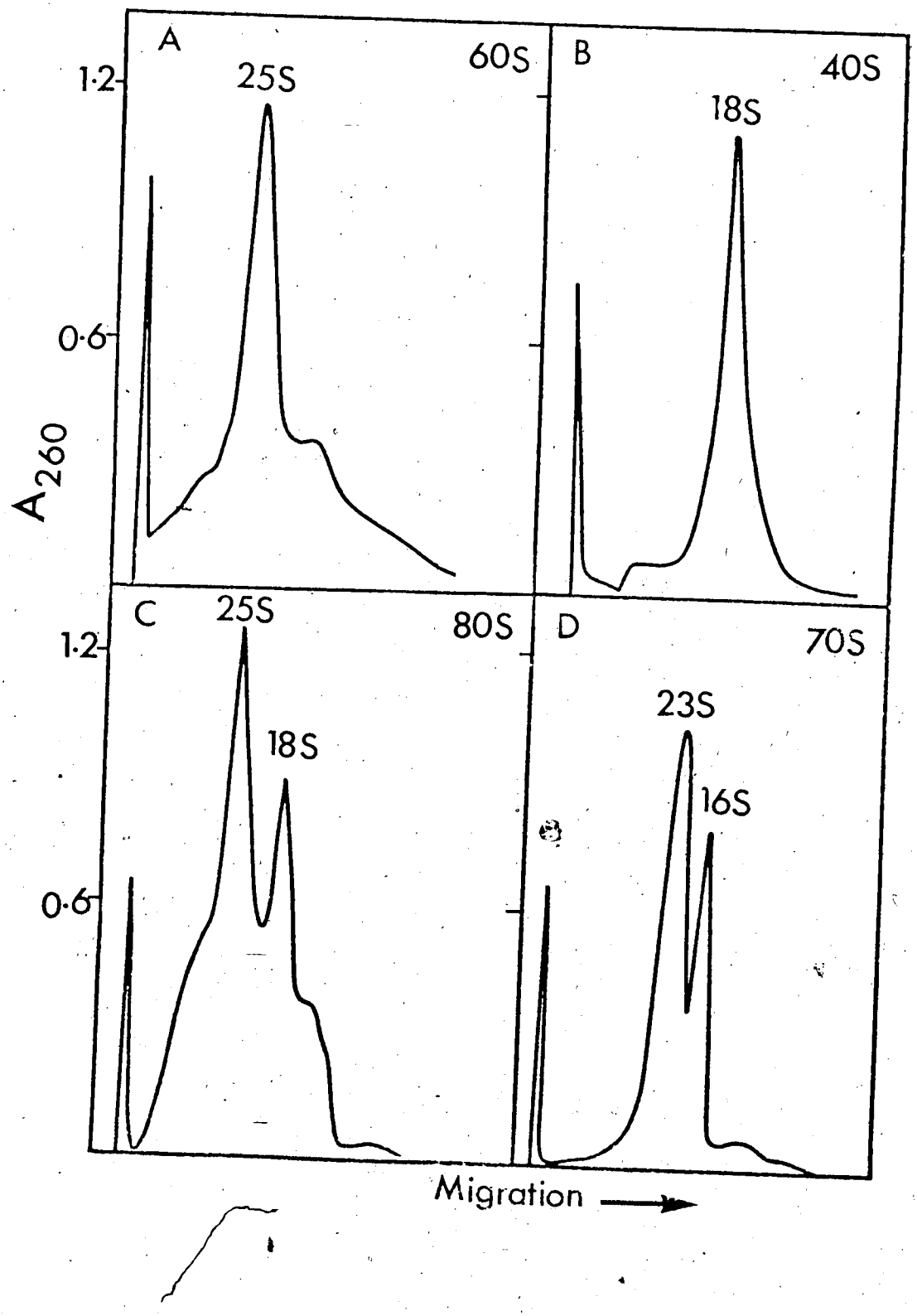
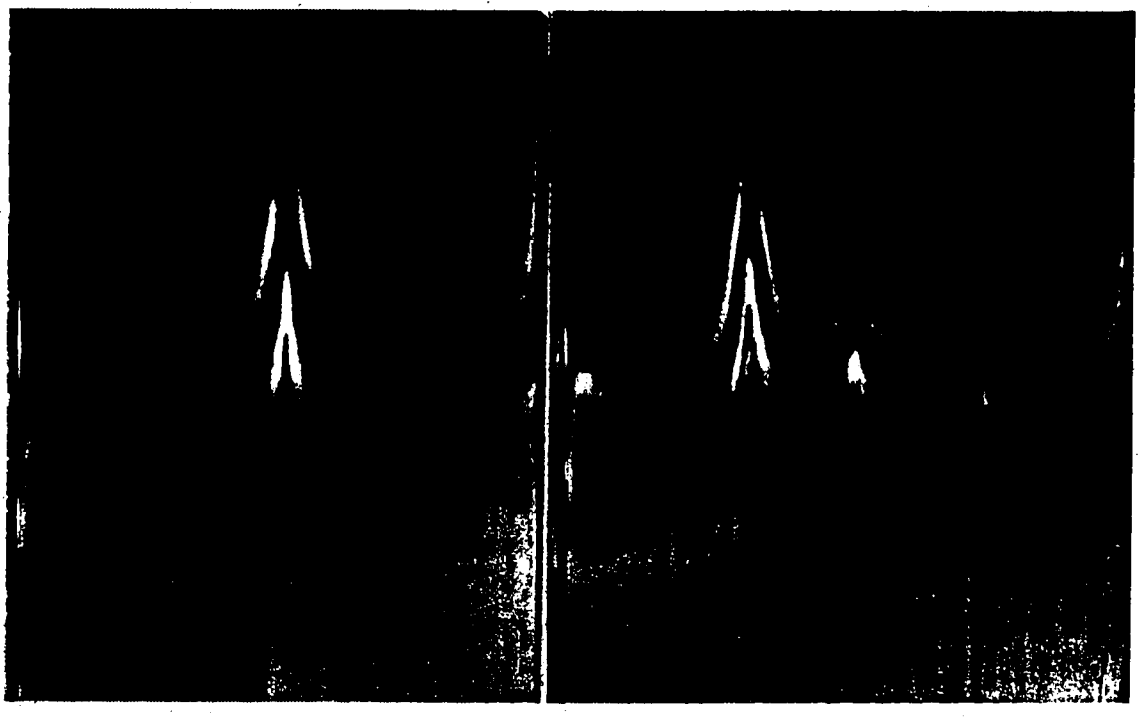

A
60S
25S
B
40S
18S
C
25S
80S
18S
D
70S
23S
16S
1·2
0·6
1·2
0·6
A_{260}
Migration

FIG. 4. Analytical ultracentrifuge pattern of ribosomal subunits isolated from embryos of Gateway barley.

The ribosomal subunit pellets in resuspension buffer were centrifuged in the analytical ultracentrifuge and the pictures were taken 12 min after reaching a speed of 39,460 rpm at 20°C. Direction of the run was from left to right.

A- large subunit; B- small subunit.



Jones *et al.* (1972) carried out dissociation studies on cytoplasmic (80S) ribosomes from wheat seedlings using high KCl to $MgCl_2$ buffer. They concluded that the 80S monomer could dissociate into a large (61S) subunit and a small subunit which might have an S value of either 42S or 49S depending upon the level of Mg^{2+}. The sedimentation coefficients of high salt derived cytoplasmic subunits in the presence of Mg^{2+} reported in this study were higher than those found by Hoober and Blobel (1969) for *Chlamydomonas reinhardi* ribosomes but they were similar to those obtained by Jones *et al.* (1972) for wheat and Chua *et al.* (1973) for *Chlamydomonas reinhardi*. The lower sedimentation coefficients (30S and 43S) obtained by Hoober and Blobel (1969) may have been due to their obtaining the ribosomal subunits at 50 mM KCl and 0.1 mM Mg^{2+} or 50 mM KCl without Mg^{2+}. It is now known that absence of Mg^{2+} in the dissociation buffer results in lower sedimentation values for the ribosomal subunits (Cammarano *et al.*, 1972a; Chua *et al.*, 1973).

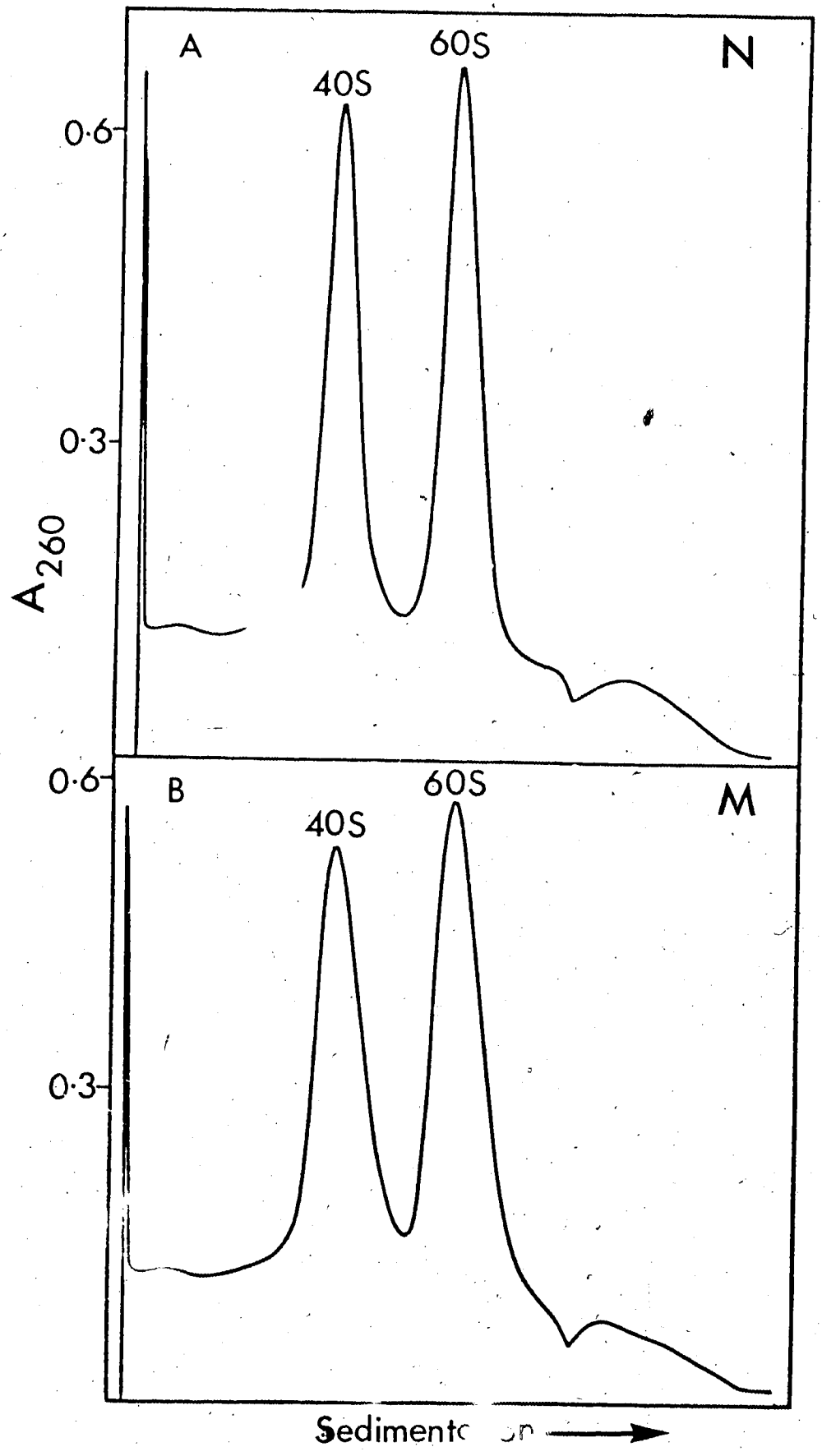
Reassociation of Ribosomal Subunits

For reassociation studies the ribosomal subunits collected as shown in Figure 2 were used. In addition to the purity tests already referred to, the 40S and 60S ribosomal subunits were mixed in a ratio of 1:1 A_{260} units in dissociation buffer and centrifuged in sucrose gradient in dissociation buffer. As seen in Figure 5 the subunits of both the normal and mutant remained intact and there was no reassociation. On the other hand when purified large and small ribosomal subunits isolated from normal and mutant barley embryos were mixed in a ratio of 1:1 A_{260} units in reassociation buffer they reassociated readily to form stable 80S monosomes. Figure 6A shows the reassociation of the

FIG. 5. Sucrose gradient centrifugation of 40S and 60S subunits of ribosomes from normal and mutant Gateway barley embryos.

Five A_{260} units of each of the subunits collected as shown in Figure 2 were mixed (1:1) in dissociation buffer and incubated for 20 min at room temperature. Two A_{260} units were analysed on a 5-20% (w/v) linear sucrose gradient in dissociation buffer by centrifugation at 26,000 rpm for 4.25 hr at 25°C in a Spinco SW 27 rotor.

A- Normal; B- Mutant.


A
60S
N
40S
0·6
0·3
A_{260}
0·6
B
60S
M
40S
0·3
Sedimentation

ribosomal subunits of normal embryos and Figure 6B indicates the re-association of the ribosomal subunits of the mutant. It can be seen as well that ribosomal subunits of normal and mutant barley were able to recombine to form hybrid 80S monosomes (Fig. 6C and D). Both the homologous and heterologous combination formed monosomes having a sedimentation value of 80S as judged by sucrose density gradient (Fig. 6). Since almost all the material of the gradients was in the 80S component it seems that the ribosomal subunits were almost completely used in formation of the monosomes.

The phenylalanine incorporation ability of the reconstituted ribosomes will be presented in the Appendix.

The present results are in agreement with the previous findings by App *et al.* (1971) who found that ribosomal subunits from rice embryos could be separated and reassociated to reform active ribosomes in a medium containing 10 mM Mg^{2+} and 60 mM KCl. The inability to reassociate the wheat embryo ribosomal subunits by Wolfe and Kay (1967) could be explained on the basis that they dissociated the ribosomes without Mg^{2+} or very low Mg^{2+} concentrations whereas it has been found that reasonable concentrations of Mg^{2+} are necessary in the dissociation media to obtain active subunits (Cammarano *et al.*, 1972).

Separation of Total Ribosomes into 70S and 80S Monosomes

To compare the chloroplast and cytoplasmic ribosomal proteins of normal and mutant barley seedlings it was important to obtain pure preparations of chloroplast and cytoplasmic ribosomes. Chloroplast ribosomes have been obtained from several plant sources by isolating purified chloroplasts and subsequently extracting the ribosomes from them

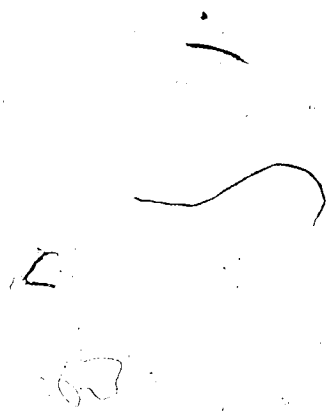


FIG. 6. Reassociation of cytoplasmic ribosomal subunits from embryos of Gateway barley and its mutant.

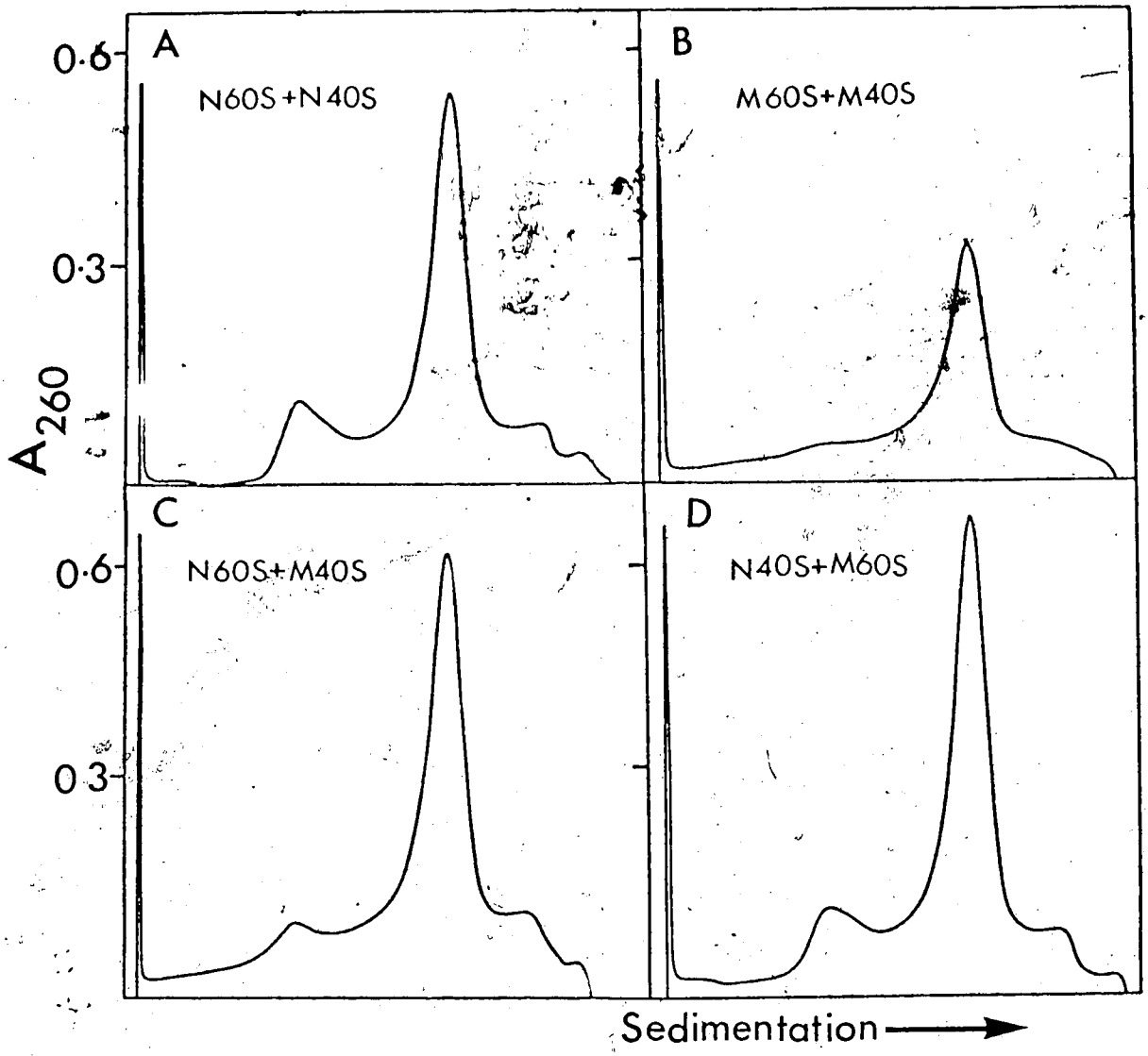
Five A_{260} units of each of the subunits collected as shown in Figure 2 were mixed (1:1) in reassociation buffer and incubated for 20 min at room temperature. Two A_{260} units were analysed on a 5-20% (w/v) linear sucrose gradient in reassociation buffer by centrifuging at 26,000 rpm for 4.25 hr at 25°C in a Spinco SW 27 rotor.

A- Normal 60S + Normal 40S.

B- Mutant 60S + Mutant 40S.

C- Normal 60S + Mutant 40S.

D- Normal 40S + Mutant 60S.


A
N60S+N40S
B
M60S+M40S
C
N60S+M40S
D
N40S+M60S
0·6
0·3
0·6
0 3
A260
Sedimentation

(Hadziyev and Zalik, 1969; Odintsove and Yurina, 1969; Vasconcelos and Bogorad, 1971; Schwartzbach *et al.*, 1974). In preliminary studies a few attempts were made to isolate pure chloroplast ribosomes by isolating chloroplasts from barley seedlings but the procedure did not give satisfactory results. The yield of chloroplast ribosomes from barley seedlings was very low and the preparation contained more 80S than 70S ribosomes. Rawson and Stutz (1969) and Jones *et al.* (1972) also encountered difficulty in obtaining good yields of chloroplast monosomes free of cytoplasmic ribosomes from *Euglena gracilis* and wheat seedlings respectively. Ultimately it was decided to separate chloroplast (70S) ribosomes from total leaf ribosome preparations. The barley leaves were ground in buffer containing 4% Triton X-100 as described in the methods. The detergents, sodium deoxycholate and Triton X-100, have been used to release ribosomes from membranes. Since it has been reported that sodium deoxycholate induces dissociation of chloroplast ribosomes (Scott *et al.*, 1970; Munns, 1972), Triton X-100 was used in this study. Total ribosome yield was about 1400 and 1000 A_{260} units per 80 g of normal and mutant barley seedlings, respectively. Thus normal seedlings gave approximately 40% higher yield of total ribosomes than the mutant.

Figure 7 illustrates the separation of the total leaf ribosomes. Only two peaks which were not especially well separated were obtained. However, by collecting these as indicated in the figure clean fractions of chloroplast and cytoplasmic ribosomes were recovered (Fig. 7A and B). Using these methods with a maximum loading of 150 A_{260} units per tube the results were reproducible but 5 independent preparations were required to provide enough chloroplast ribosomes for further studies.

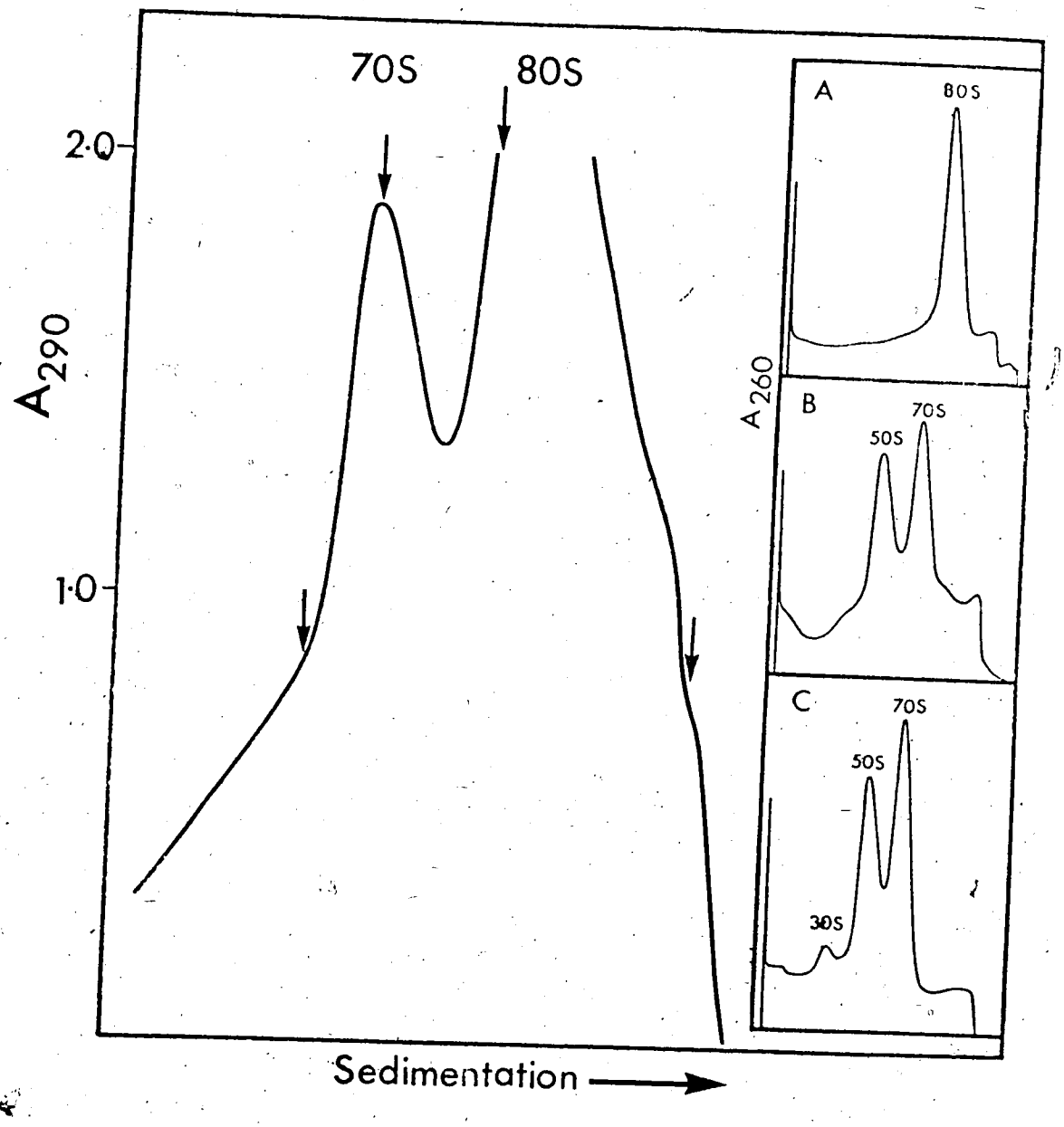
FIG. 7. Sucrose gradient separation of chloroplast (70S) and cytoplasmic (80S) ribosomes from leaves of 6-day old Gateway barley seedlings.

Ribosomal material resuspended in buffer IV containing 150 A_{260} units (300 µl) were layered on a 7-37% (w/v) linear sucrose gradient in the same buffer. After centrifugation at 22,000 rpm for 16 hr at 4°C the portions of 70S and 80S ribosomes indicated by arrows were collected and pooled. The ribosomes in each fraction were pelleted by centrifugation in a Spinco Ti 60 rotor at 55,000 rpm for 10 hr at 4°C. Two A_{260} units of each ribosomal fraction and 2 A_{260} units of *E. coli* ribosomes were applied to 5-20% (w/v) linear sucrose gradients in buffer III and centrifuged at 26,000 rpm for 4.25 hr at 4°C. The eluants were monitored at 260 nm.

A- Cytoplasmic ribosomal fraction.

B- Chloroplast ribosomal fraction.

C- *E. coli*.


70S
80S
2·0
1·0
A290
Sedimentation
A260
A
80S
B
70S
50S
C
70S
50S
30S

When more than 150 A_{260} units were loaded the separation was poor.

The total ribosomal preparation yielded approximately 20% 70S ribosomes and 80% 80S ribosomes. Similar yields of 70S and 80S ribosomes were reported by Jones *et al.* (1973), from wheat leaves using zonal centrifugation. Chua *et al.* (1973) lso separated the total ribosomes from *Chlamydomonas reinhardi* into 70S and 80S monosomes by zonal centrifugation. They (Chua *et al.*, 1973) obtained good separation at 22500 rpm for 14 hr, but at a speed of 48000 rpm for 5 hr under the same ionic conditions the 70S but not the 80S ribosomes dissociated almost completely. From their results they suggested that chloroplast ribosomes are more pressure sensitive than the cytoplasmic ribosomes. In this study it is evident that 70S chloroplast ribosomes and the 70S ribosomes of *E. coli* were not as stable as the cytoplasmic ribosomes. Upon recentrifugation, 70S ribosomes showed considerable dissociation (Fig. 7B and C).

Purity Analysis of the Separated Monosomes

i. Sucrose gradient analysis

When the cytoplasmic and chloroplast ribosomal fractions indicated in Figure 7 were analysed on sucrose density gradients a single peak was obtained for the cytoplasmic (80S) ribosomes without any contamination by 70S ribosomes (Fig. 7A). The chloroplast 70S ribosomes were free of 80S contamination but the chloroplast ribosomes partially dissociated into 50S and 30S subunits during centrifugation (Fig. 7B). To further assess the purity of these ribosomal fractions their RNA's were extracted and electrophoresed.

As indicated by Figure 3C and D the cytoplasmic and chloroplast ribosomal fractions each yielded two distinct RNA components in PAGE. Those of the 80S ribosomes were heavier than the corresponding RNA fractions from the 70S ribosomes and their respective S values were taken as 25S and 18S and 23S and 16S. From the electrophoretic pattern, it is apparent that the RNA's were intact and there was no cross contamination of either fractions.

ii. Sedimentation coefficients

The sedimentation coefficients of the two ribosomal fractions were determined by analytical ultracentrifugation. As shown in Figure 8A the cytoplasmic ribosomal fraction gave a sedimentation coefficient of 82S and was almost devoid of 70S contamination. The chloroplast fraction gave a sedimentation coefficient of 66S (Fig. 8B) and was almost devoid of 80S contamination. The sedimentation coefficients here are in agreement with those obtained for chloroplast and cytoplasmic ribosomes of various plants (Clark *et al.*, 1964; Boardman *et al.*, 1965, 1966; Sager and Hamilton, 1967; Stutz and Noll, 1967; Hoober and Blobel, 1969; Hadziyev *et al.*, 1969; Gualerzi and Cammerano, 1969).

Ribosomal Proteins

Two-Dimensional Polyacrylamide Gel Electrophoresis of Ribosomal Proteins from Barley Embryos

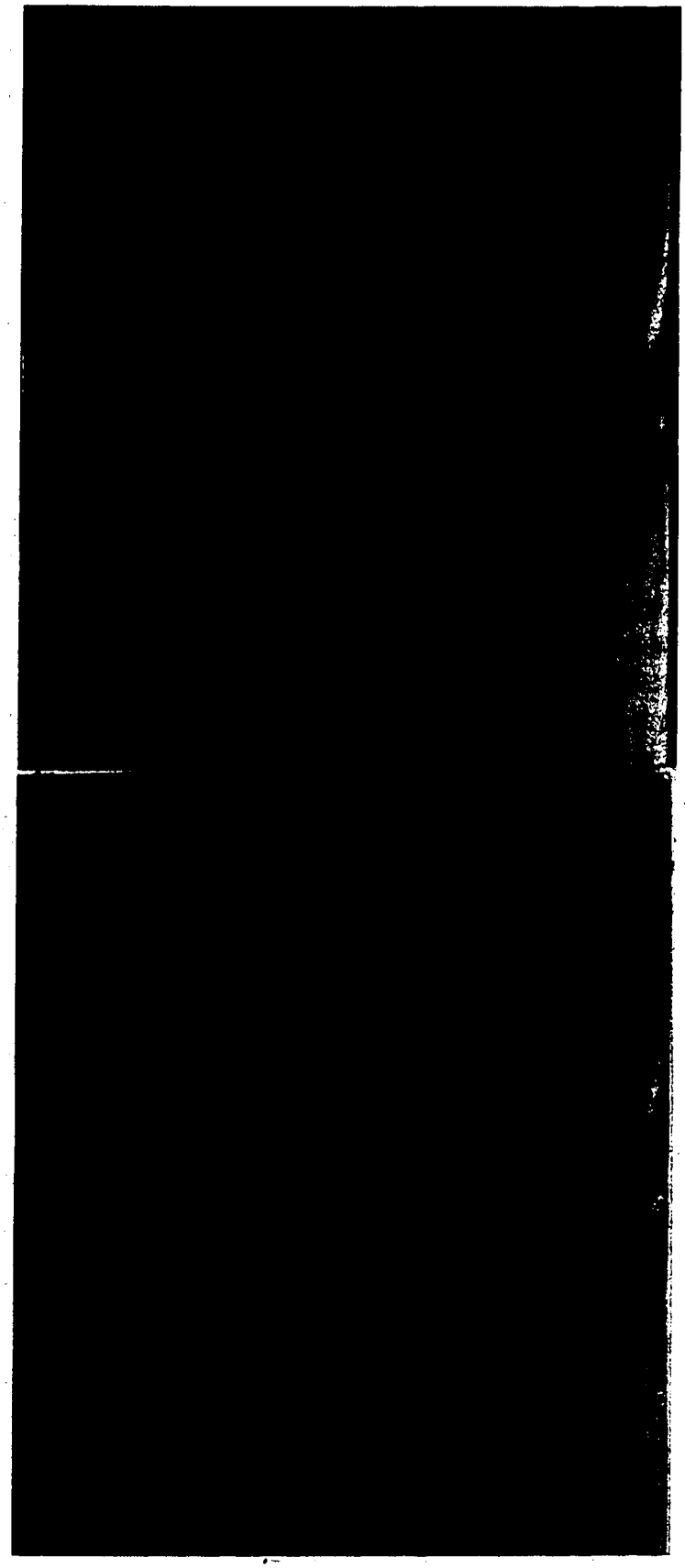
At least three separate ribosomal preparations were made for each of the normal and mutant barley embryos. Separate electrophoretic runs were carried out for each preparation by two-dimensional PAGE. The protein patterns of the individual runs were similar and were therefore

FIG. 8. Analytical ultracentrifugation pattern of chloroplast and cytoplasmic ribosomes from leaves of 6-day old Gateway barley seedlings.

The ribosomal fractions collected as indicated in Figure 7 were pelleted, resuspended in buffer III and centrifuged. Pictures were taken 12 min after reaching a speed of 39,460 rpm at 20°C. The direction of the run was from left to right.

A- Cytoplasmic ribosomes.

B- Chloroplast ribosomes.



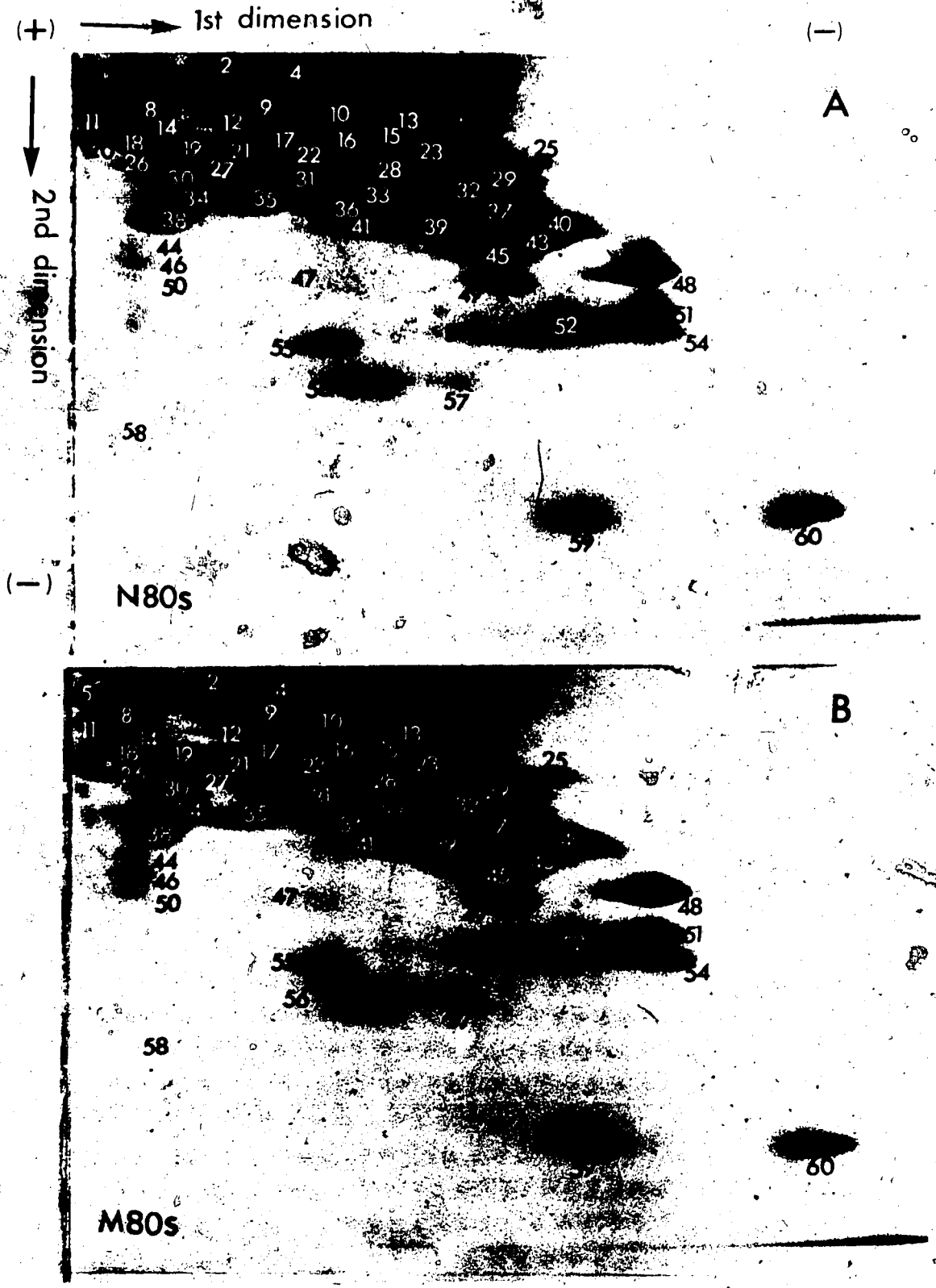
used to compare the ribosomal proteins of the normal and mutant on the basis of their mobilities. To help in making the comparisons the gels were photographed and a grid was placed over the photographs to count the spots (Nagabhushan *et al.*, 1974). Each of the stained spots was assigned a number depending upon the mobility of the individual protein in the second-dimension (Kaltschmidt and Wittmann, 1970b). Although a number of protein spots which stained on the gels were faint or barely detectable in the photographs they were clearly visible when the gels were placed on a light box. Moreover, when twice the usual amount of ribosomal protein was electrophoresed these spots were distinct.

There have been a number of reports (Jones *et al.*, 1972; Sherton and Wool, 1972; Gualerzi *et al.*, 1974; Hanna and Godin, 1975) which pointed out that different ribosomal proteins may show different intensities upon staining. Thus even the faint spots were regarded as indicating the presence of a ribosomal protein. However, these spots might represent ribosomal proteins which are present in only part of the total population of ribosomes.

As seen from Figure 9 the 80S protein pattern of normal (A) and mutant (B) were similar. Both resolved into 60 basic ribosomal proteins. The majority of the ribosomal proteins of the normal and mutant were strongly stained with no apparent differences between the two lines. However, for the less strongly stained proteins the mutant had sharper bands for numbers 1, 7, 25, 50, and 58 than did the normal. Because of the large numbers of proteins in the monosomes and inadequate separation of them even by two-dimensional PAGE a more detailed comparison was made by electrophoresing the proteins of their respective subunits.

FIG. 9. Two-dimensional gel electrophoretograms of proteins from the cytoplasmic ribosomes from embryos of Gateway barley (A) and its mutant (B).

Samples of 50 µg of ribosomal proteins were applied to 4% disc gels and electrophoresed with buffer containing 6 M urea, 0.15 M boric acid, 6.5 mM Na_2EDTA and 0.12 M Trizma, pH 8.6, for 15 hr at 5 mA per gel at 4°C. After the first-dimensional run the gel was equilibrated in 6 M urea in acetate buffer pH 4.6 and placed on the 18% gel slab. Electrophoresis in the second-dimension using 0.018 M glycine-acetate buffer pH 4.6 was for 18 hr at 120 V with current limited to 220 mA at 4°C. Electrophoresis in both dimensions was from anode to cathode. Details of the procedure and for staining with coomassie are given in the Materials and Methods.


(+)
1st dimension
(−)
2nd dimension
A
N80s
B
M80s
(−)

Two-Dimensional PAGE of Proteins from Ribosomal Subunits of Barley Embryos

i. Proteins of small subunits

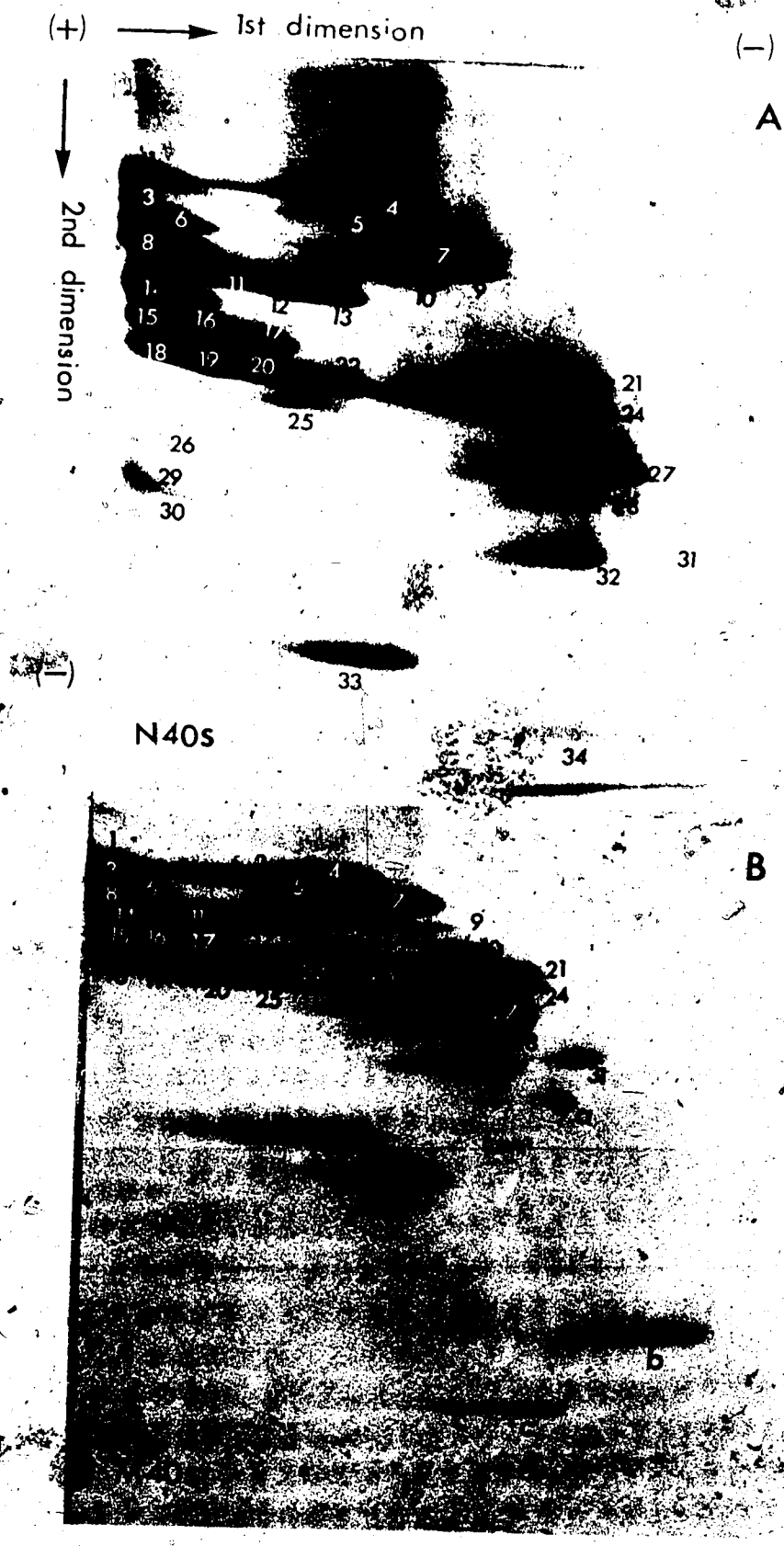
The protein patterns of the 40S subunit of the normal and mutant are shown in Figure 10. Although the conditions of electrophoresis were standardized as much as possible in terms of gel concentration, amount of protein applied, electrophoresis buffer, the distance the marker dyes moved, the duration of the run in each dimension, the current applied and the temperature, it can be seen that the patterns of the proteins of the 40S ribosomal subunits of the normal and mutant were different. For example proteins number 7 and 27 which are likely the same proteins in both lines migrated differently especially in the second-dimension. There is no obvious explanation for this observation, but it was consistent in the three independent runs.

Both the normal and mutant contained 34 basic proteins but even allowing for the difference in patter some of the proteins of the normal subunit are absent from the mutant, i.e., 26, 29, 30. Another difference between the two is the appearance of bands a, b and c only in the mutant. In addition band 31 which is faint in the normal is distinct in the mutant.

Proteins of the small subunits of both the normal and mutant appeared as bands upon electrophoresis. Since very few bands were observed when the proteins of the monosomes, which should have contained all of the proteins of the subunit, were electrophoresed it is probable that formation of bands may have been due to overloading. Sherton and Wool (1972) reported band formation upon electrophoresing ribosomal

FIG. 10. Two-dimensional gel electrophoretograms of the proteins of the 40S subunit of cytoplasmic ribosomes from embryos of Gateway barley (A) and its mutant (B).

Samples of 500 µg of protein of the ribosomal subunits were applied to the 4% disc gels and electrophoresis in the 1st and 2nd dimension was performed as described in Figure 9.


(+)
1st dimension
(−)
A
2nd dimension
3
6
8
4
5
7
1
11
12
13
15
16
17
18
19
20
21
24
25
26
29
30
27
31
32
33
(−)
N40s
34
B
9
21
24
25
b

subunit proteins of rat liver.

ii. Proteins of the large subunits

The two-dimensional polyacrylamide gel electrophoretic patterns of the 60S subunits of normal and mutant barley are shown in Figure 11. As seen from Figure 11 the protein patterns of the two lines differed. As with the smaller subunit the proteins of the mutant 60S migrated less than those of the normal subunit in the second-dimension. Both the normal and mutant contained 41 basic proteins of which a few were lightly stained. In the case of the proteins designated as 39 and 41 the spots were intense in the normal but were faint in the mutant. The three proteins 4, 5 and 13 which were present in the normal 60S subunit were absent from the mutant while the mutant 60S subunit showed three additional protein spots a, b and c which were absent from the normal. All of these differences in the protein patterns of the two lines were reproducible.

Regarding the additional protein spots obtained for both the small and large subunits of the mutant it should be noted that even if bands b and c had been present in the subunits of the normal they might have run off the gels because of the higher mobility of the proteins of the normal in the second-dimension.

In comparing the protein patterns of the small and large subunits (Fig. 10 and 11), it can be seen that the proteins of the 40S differ considerably from proteins of their respective 60S subunit in number, mobility and band formation. These differences were observed for the subunits of both the normal and the mutant.

There were several spots with identical electrophoretic mobilities

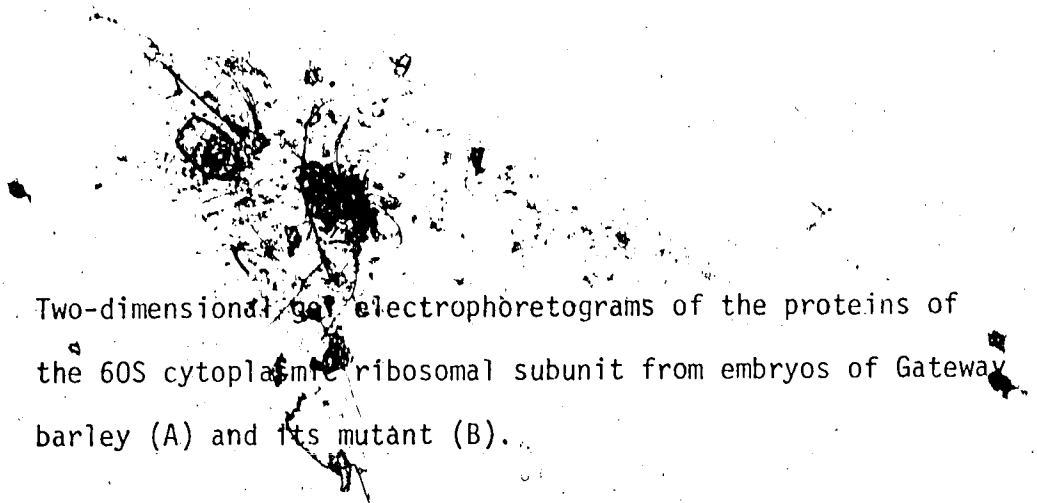
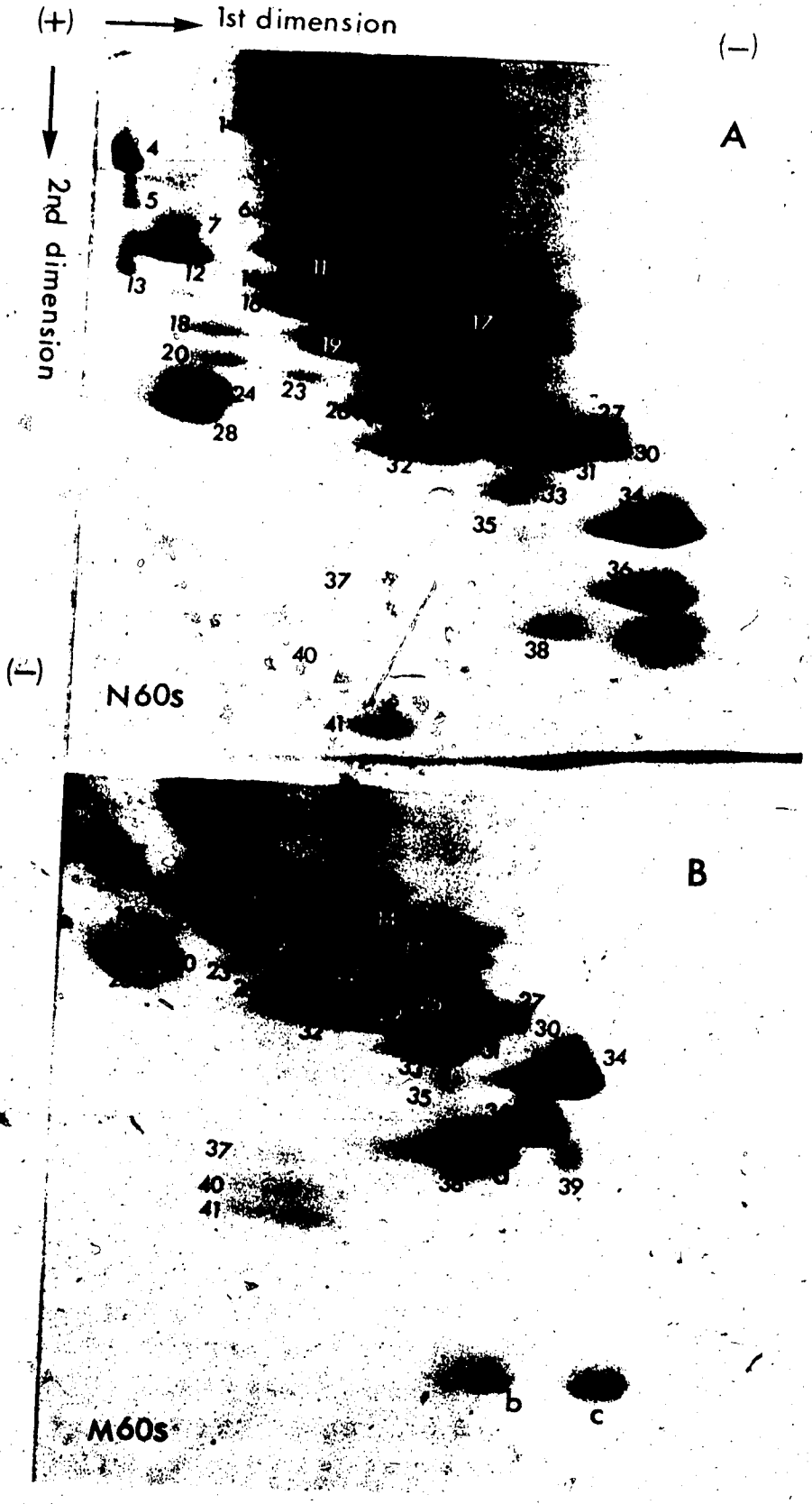


FIG. 11. Two-dimensional gel electrophoretograms of the proteins of the 60S cytoplasmic ribosomal subunit from embryos of Gateway barley (A) and its mutant (B).

Samples of 500 μg of protein of the ribosomal subunits were applied to the 4% disc gels and electrophoresis in the 1st and 2nd dimension was performed as described in Figure 9.


(+)
1st dimension
(−)
2nd dimension
A
N60s
(−)
B
M60s
b
c

in the small and large subunits but it is difficult to decide whether these represent identical proteins occurring in both the subunits or whether they represent protein with identical migration. The summation of the basic ribosomal proteins of both small and large subunits gave 75 proteins while the monosomes had only 60 protein spots for both the normal and the mutant. This may have been due to poor resolution of the high number of proteins present in the monomeric ribosomes or to identical mobilities of proteins from the two subunits.

The ribosomal subunits were also analyzed for their acidic proteins. The proteins of each of the ribosomal subunits were electrophoresed by one-dimensional PAGE at pH 8.6 and run from the cathode to the anode. The results are shown in Figures 12A and 13. As seen from these figures 4 acidic proteins were present in each of the ribosomal subunits of the embryos from both the normal and mutant. Thus the isoelectric points of these proteins were lower than pH 8.6. In addition to these bands there was protein staining material at the top of the gels. The mobility of the proteins in replicate runs was reproducible.

Considering the acidic and basic cytoplasmic ribosomal subunit proteins of the embryos of both lines there were altogether 38 proteins present in the small subunit, 34 of which migrated cationically (Fig. 10) and four anionically (Fig. 12A). There were 45 proteins in the large subunit, 41 of which migrated cationically (Fig. 11) and 4 anionically (Fig. 12A).

There is no reported two-dimensional PAGE study of the ribosomal proteins isolated from the ribosomes of dry embryos. The only studies of this kind for higher plants have involved ribosomes isolated from

Fig. 12. One-dimensional polyacrylamide gel electrophoresis of acidic proteins of ribosomes and ribosomal subunits of Gateway barley.

Samples of 150 μg of ribosomal proteins were applied to 4% disc gels and electrophoresed with buffer containing 6 M urea, 0.15 M boric acid, 6.5 mM Na_2EDTA and 0.12 M Trizma, pH 8.6, for 10 hr at 5 mA per gel at 4°C. The procedure for electrophoresis and staining with coomassie are given in Materials and Methods.

A- Acidic proteins of ribosomal subunits from barley embryos.

Normal- N:60S, 40S; Mutant- M:60S, 40S.

B- Acidic ribosomal proteins of chloroplast and cytoplasmic ribosomes from leaves of barley seedlings.

Normal- N:80S, 70S; Mutant- M:80S, 70S.

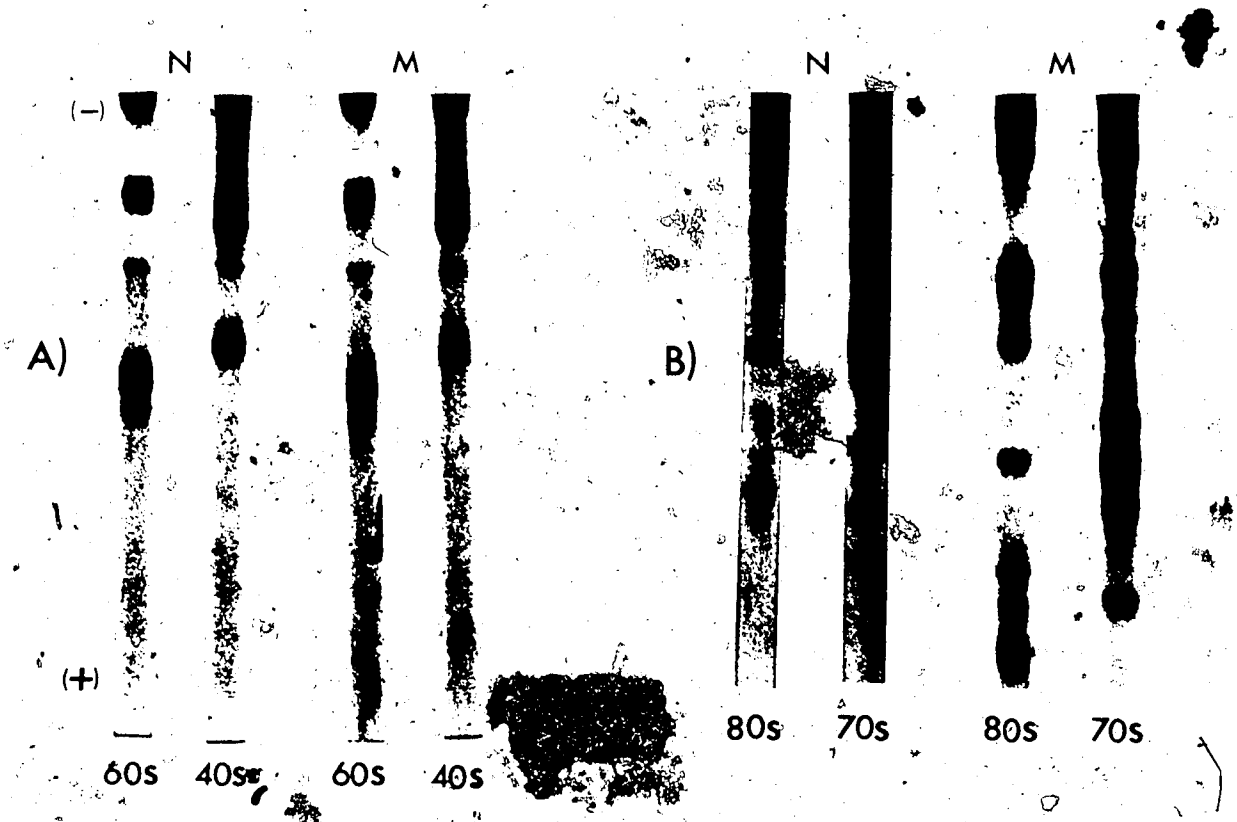
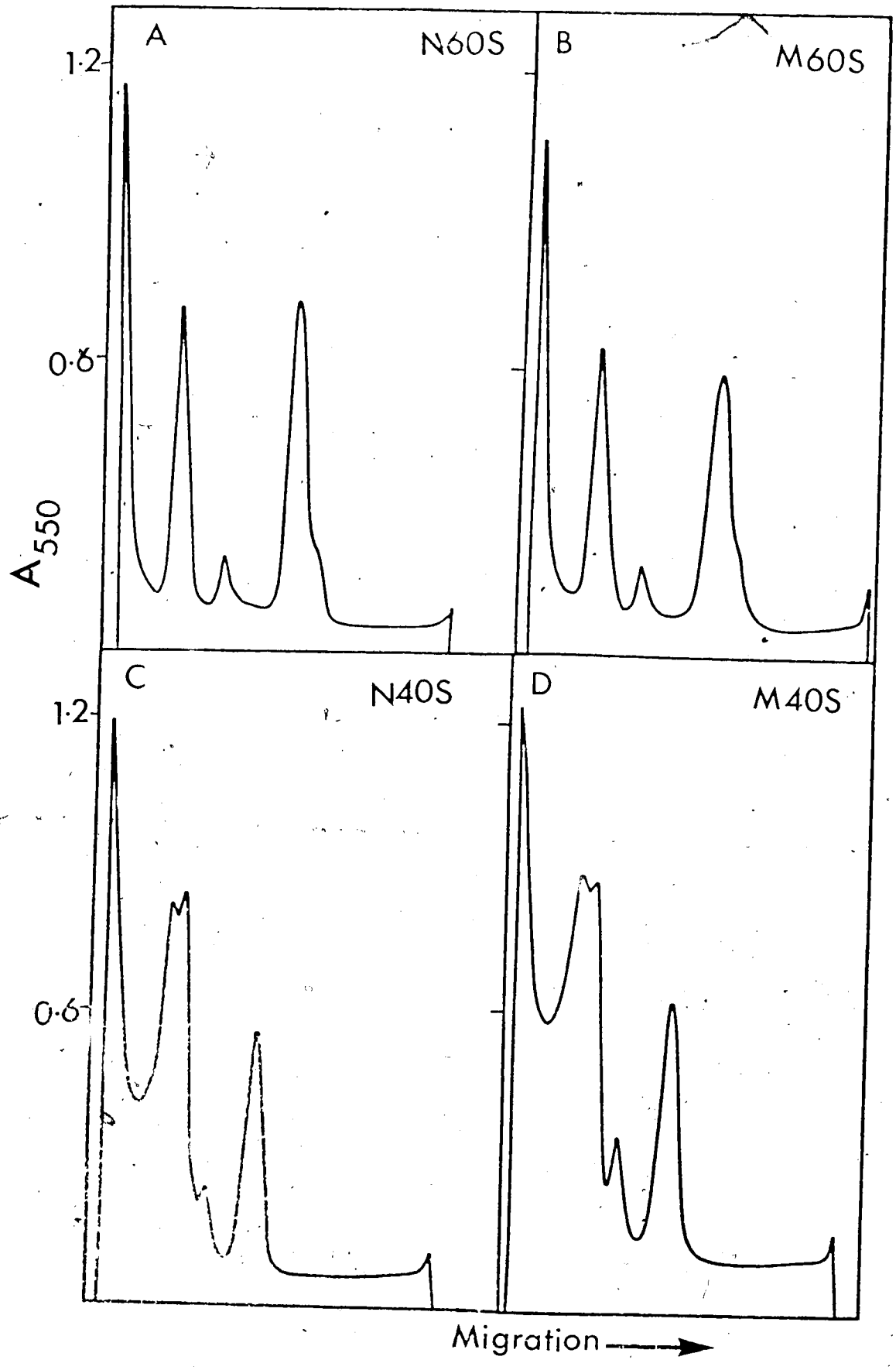

N
M
(−)
A)
(+)
60s
40s
60s
40s
N
M
B)
80s
70s
80s
70s

FIG. 13. Densitometric tracings of gels of acidic proteins of ribosomal subunits of barley embryos.

The stained gels shown in Figure 12A were scanned at 550 nm.

A- Normal 60S; B- Mutant 60S.

C- Normal 40S; D- Mutant 40S.


A
N60S
B
M60S
C
N40S
D
M40S
1·2
0·6
1·2
0·6
A_{550}
Migration

imbibed seeds or from leaves. Since different isolates and slight differences in the electrophoresis procedure may result in large differences in the pattern of ribosomal proteins by two-dimensional PAGE, there is no basis for making direct comparisons of my results with previous studies. For example Nagabhushan *et al.* (1974) reported 44 cytoplasmic ribosomal proteins from 4.5-day old leaves of Gateway barley, 31 of which were basic and 13 were acidic. The lower number of ribosomal proteins obtained by them might have been due to the lower Mg^{2+} concentration in the acetate used for the extraction of ribosomal proteins since a high level of magnesium ions (100 mM) has been found to increase the yield of ribosomal proteins (Hardy *et al.*, 1969).

Two-Dimensional PAGE of Chloroplast and Cytoplasmic Ribosomal Proteins Isolated from Leaves of 6-Day Old Barley Seedlings

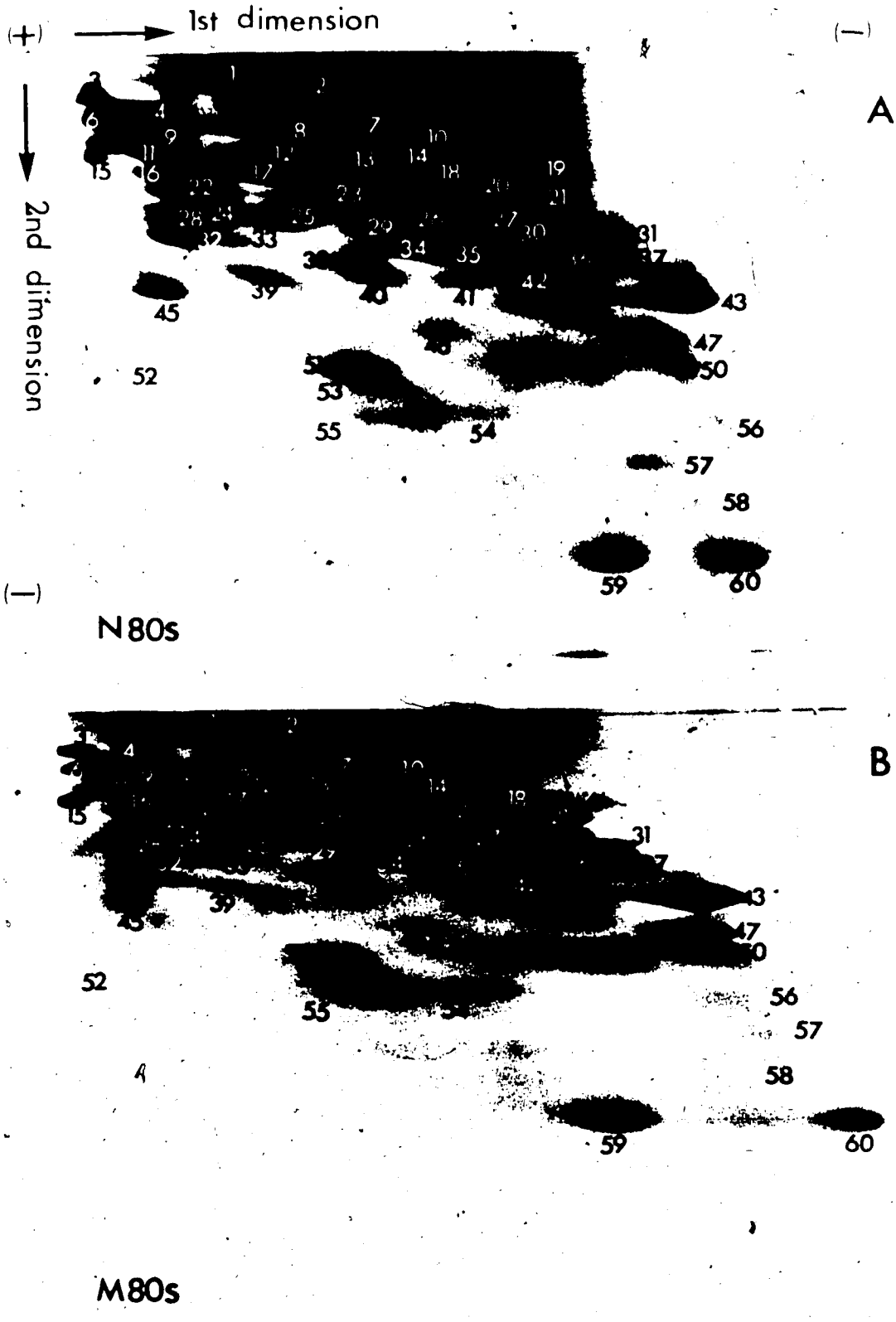
To study the chloroplast and cytoplasmic ribosomal proteins at least three separate chloroplast and cytoplasmic ribosomal preparations were made for each of the normal and the mutant seedlings as mentioned earlier. Separate electrophoretic runs were carried out for each preparation by two-dimensional PAGE.

The two-dimensional electrophoretic protein patterns of 80S normal and mutant seedlings are shown in Figure 14. Both the normal and the mutant had 60 basic proteins. Except for some minor differences in mobility and intensity of spots these protein patterns were remarkably similar and reproducible.

A comparison of the patterns of the basic proteins of cytoplasmic ribosomes from barley seedlings (Fig. 14) and barley embryos (Fig. 9) shows that there is a similarity in their protein patterns and they

FIG. 14. Two-dimensional gel electrophoretograms of the proteins from cytoplasmic ribosomes from the leaves of Gateway barley (A) and its mutant (B).

Samples of 500 µg of protein of the cytoplasmic ribosomes were applied to the 4% disc gels and electrophoresis in the 1st and 2nd dimension was performed as described in Figure 9.


(+)
1st dimension
(−)
2nd dimension
A
(−)
N80s
B
M80s

contain the same number of proteins. On the basis of the shape of the spots, the intensity and their position on the slab many of the proteins are probably the same in both the embryo and seedling material as well as both lines. For example spots 48 and 51 of the embryo (Fig. 9) are probably the same as spots 43 and 47 of the seedlings (Fig. 14).

As seen from Figure 15 the protein pattern and number of proteins in the 70S ribosomes of both lines were similar. There were minor differences in the mobility and intensity of some proteins. Both lines had 53 basic proteins.

To analyze the acidic proteins of the 70S and 80S leaf ribosomes the proteins were electrophoresed by one-dimensional PAGE and run from the cathode to the anode. The cytoplasmic ribosomes of the normal had 9 acidic proteins while the mutant had 8 (Figures 12B and 16A and B). Four of the proteins of the mutant migrated differently than those of the normal. The chloroplast ribosomes of the normal and mutant seedlings had 10 and 9 acidic proteins, respectively. Again some of the acidic proteins of the mutant migrated differently than those of the normal. In all of these one-dimensional runs a considerable amount of protein staining material remained at the top of the gel.

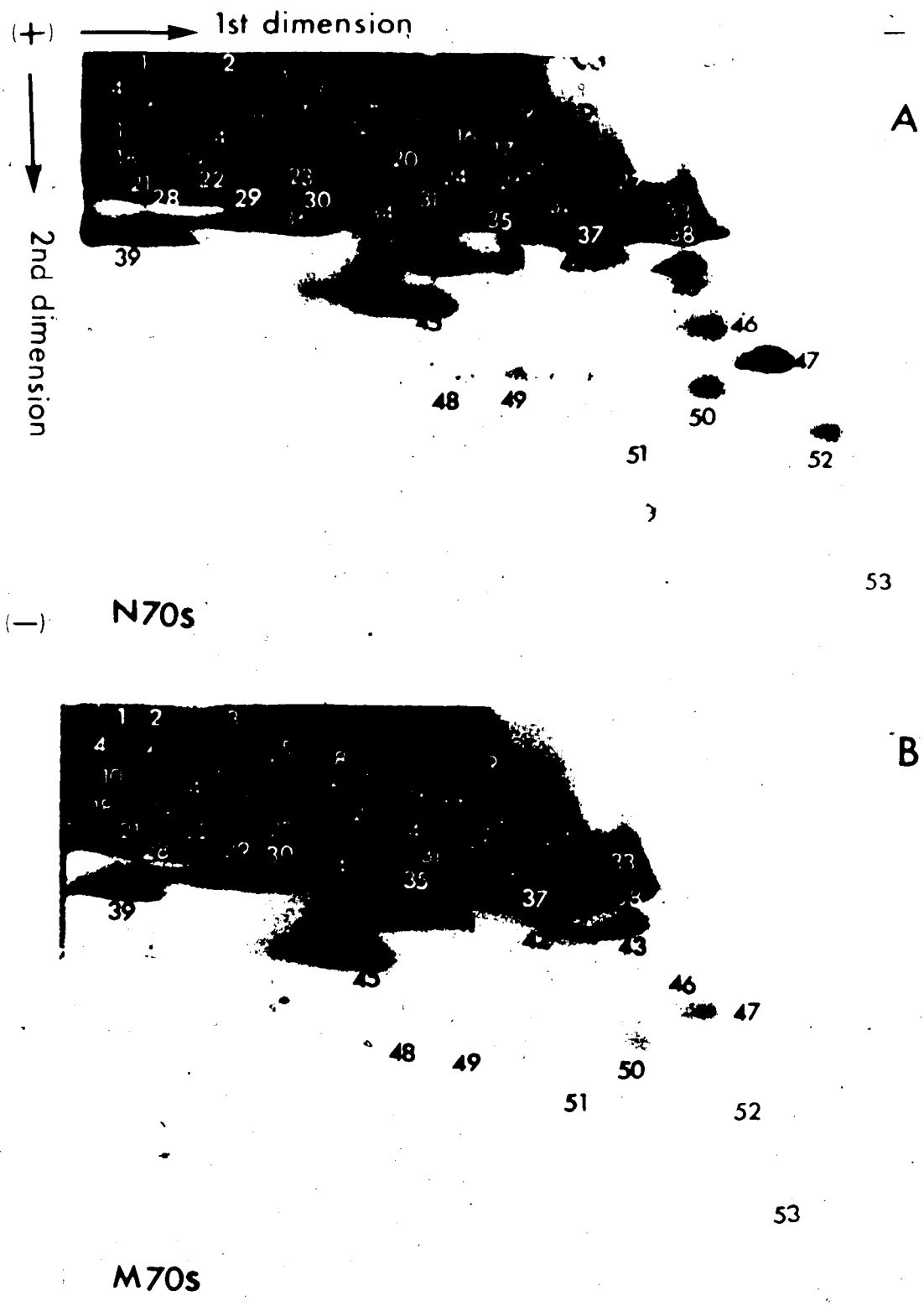
Considering the cytoplasmic ribosomal proteins of the seedlings there were altogether 69 proteins present in the normal 80S ribosomes of which 60 were basic proteins (Fig. 14A) and 9 were acidic proteins (Fig. 16A). The mutant 80S contained a total of 68 proteins, 60 of which were basic (Fig. 14B) and 8 were acidic (Fig. 16B). A total of 63 proteins were present in the chloroplast ribosomes of normal barley seedlings, 53 of which were basic (Fig. 15A) and 10 were acidic (Fig. 16B). The proteins of the chloroplast ribosomes of the mutant

contained a total of 62 proteins, 53 of which were basic (Fig. 15B) and 9 were acidic (Fig. 16B)

As already mentioned, the patterns of the basic proteins of the cytoplasmic ribosomes isolated from the embryos or the leaves were essentially the same and were similar for both lines. Likewise the electrophoretic patterns of the basic proteins from chloroplast ribosomes of both lines were alike. On the other hand, the pattern and number of basic proteins in the chloroplast ribosomes was quite different from those of the cytoplasmic ribosomes and was consistent for inter- as well as intra-line comparison.

FIG. 15. Two-dimensional gel electrophoretograms of the protein from chloroplast ribosomes of the Gateway barley (A) and its mutant (B).

Samples of 500 µg of proteins of the chloroplast ribosomes were applied to the 4" disc gells and electrophoresis in the 1st and 2nd dimension was performed as described in Figure 9.

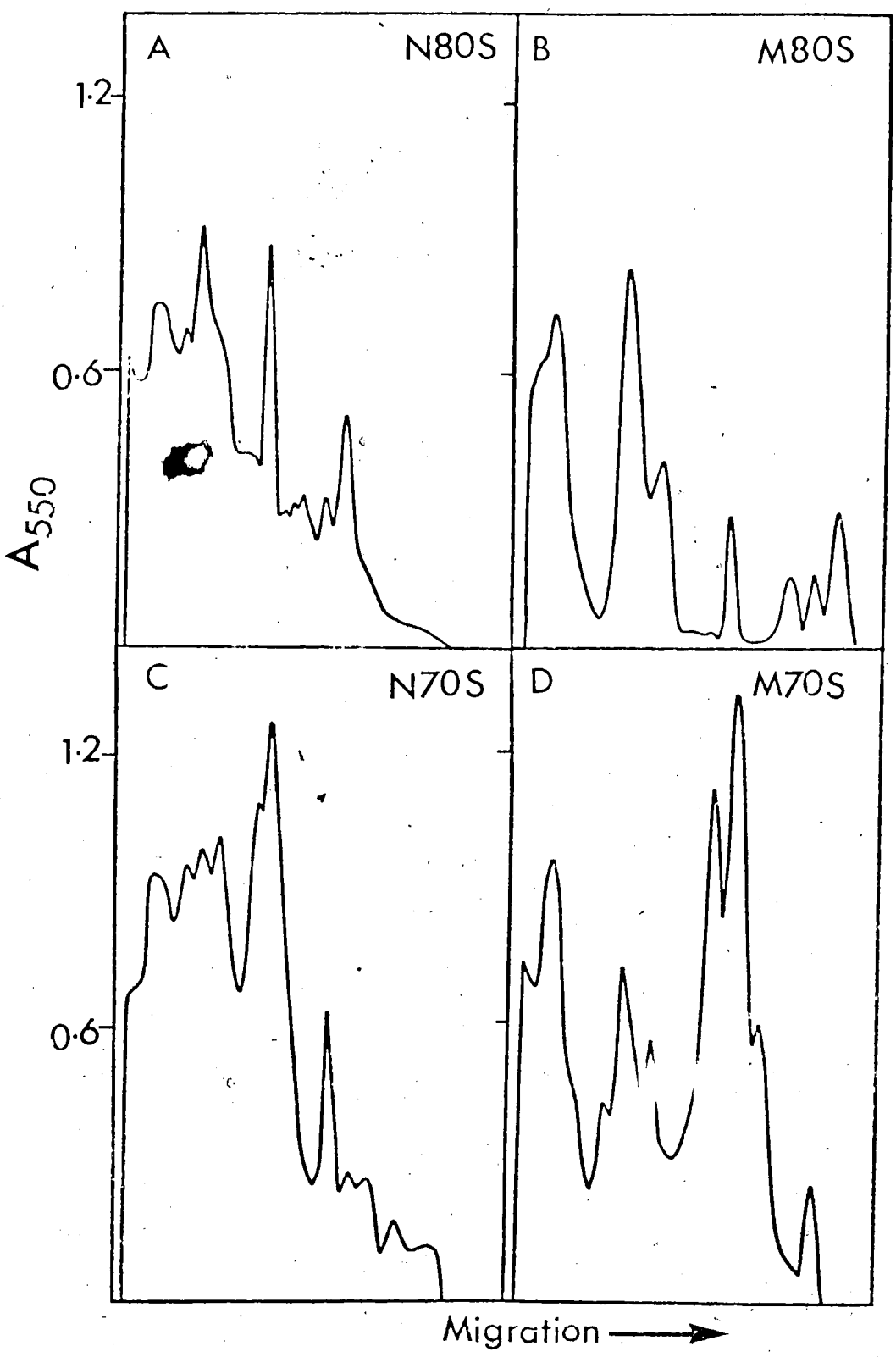

(+)
1st dimension
2nd dimension
A
1
2
28
29
30
35
37
38
39
46
47
48
49
50
51
52
53
(—)
N70s
B
1
2
4
30
33
35
37
39
43
45
46
47
48
49
50
51
52
53
M70s

16. 16. Densitometric tracings of gels of acidic proteins of chloroplast (70S) and cytoplasmic (80S) ribosomes from barley seedlings.

The stained gels shown in Figure 128 were scanned at 550 nm.

A- Normal; B- Mutant.

C- Normal; D- Mutant.


A
N80S
B
M80S
C
N70S
D
M70S
1·2
0·6
1·2
0·6
A550
Migration

GENERAL DISCUSSION AND CONCLUSIONS

As mentioned previously, on a quantitative basis dry embryos of Gateway barley gave a slightly higher yield of ribosomes than the embryos of the virescens mutant and also the fresh leaves of 6-day old seedlings of the normal yielded about 40% more total ribosomes (70S and 80S) than the mutant. While this is in accord with the previous unpublished results from this laboratory on the yield of ribosomes from the two lines, it cannot be presumed to be the cause nor central to the symptoms manifested by the mutant. Rather, at early stages in development the mutant has fewer ribosomes along with its other deficiencies like a low level of pigments, poorly developed plastids and lack of some of the soluble and lamellar proteins (Jhamb and Zalik, 1973, 1975). On the other hand since this mutant is the result of a single gene nuclear mutation (Stephansen and Zalik, 1971) a quantitative difference between the ribosomes of the normal and mutant, especially between their cytoplasmic ribosomes might indicate a close link with the mutation. There is a number of documented cases of gene mutations being related to the alteration or absence of specific ribosomal proteins (Funatsu and Wittmann, 1972; Davidson *et al.*, 1974; Brügger and Boschetti, 1975). Consequently, this study was devoted primarily to a comparison of ribosomal proteins of the two lines by PAGE. Ribosomes were isolated from dry embryos and leaves of 6-day old seedlings as at this stage it was possible to get sufficient ribosomal yield for the studies of

ribosomal proteins of both the lines.

The ribosomal structural proteins of *E. coli* have been identified on the basis of two-dimensional PAGE and their designation has been verified by other criteria including the reconstitution of active ribosomal subunits from individual protein and RNA components. The ribosomes of higher organisms have thus far not been characterized to this level. Therefore, in this study it was necessary to assume that by employing a consistent parallel procedure, only ribosomal structural proteins were being compared. Furthermore, since many ribosomal proteins of *E. coli* have been found in less than one copy per particle (Kurland *et al.*, 1969; Weber, 1972, Kjeldgaard and Gausing, 1974); the mere occurrence of a spot at similar mobilities in both lines, regardless of intensity, was interpreted as presence of that polypeptide.

Making these major assumptions, the major findings of the study can be summarized as follows. By two-dimensional PAGE the proteins of 80S monosomes isolated from either dry embryos or seedling leaves of both lines gave similar patterns and all showed 60 basic proteins. Thus these rather complicated protein patterns for the monosomes showed no difference between the normal and the mutant. However, when the cytoplasmic ribosomes isolated from the dry embryos were dissociated and the proteins of the subunits compared, differences between the normal and mutant were detected. For the large (60S) subunits of the mutant, the basic proteins migrated less than those of the normal and the mutant lacked proteins 4, 5, and 13 but had three additional spots a, b and c (Fig. 11). The protein patterns for the small (40S) subunits also differed. The mutant lacked proteins 26, 29 and 30 and had

three additional spots a, b and c (Fig. 10).

It is difficult to explain the loss of three proteins in each of the ribosomal subunits of the mutant. As this mutant is a result of a single gene nuclear mutation, only one protein would be expected to show alteration. However, the organization of different proteins in the ribosomes is very complicated and it has been shown that both the assembly process and the functional structure of the ribosomes are controlled by precise interactions between ribosomal proteins and their binding sites on the ribosomal RNA (Nomura *et al.*, 1969). Therefore, it may be possible that alteration in one protein due to change in amino acid sequence or a secondary modification of the polypeptide structure might result in the alteration or absence of the other proteins. Regarding the additional proteins found in the mutant these may be weakly bound to the normal ribosomal subunits and thus washed off during the preparation procedure, but in the mutant where a structural change has possibly occurred elsewhere in the subunit, the affinity of these proteins might be enhanced and thus could remain attached to the particles. It is difficult to say whether they are true ribosomal proteins or not. Another possibility is that these additional proteins might be the same proteins which were found missing but due to alteration they migrated differently.

Since these differences were not evident from the protein patterns of the monosomes, the possibility cannot be ruled out that during the dissociation procedure, alterations occurred in a few related proteins of the two subunits and accounted for the differences in mobility. Despite these differences between the respective subunits, both homologous and heterologous subunits of two lines were able to reassociate when

mixed in a ratio of 1:1 A_{260} units (Fig. 6) to form monosomes. As could be seen from Table II poly (U)-directed polyphenylalanine synthesis was equivalent for all the reassociated monosomes. On the basis of these results it can be concluded that the ribosomal subunits that were analysed contained a complement of proteins sufficient to support incorporation activity and the loss of several proteins in the mutant subunits did not affect this activity adversely. However, since *in vitro* polyphenylalanine synthesis is not the same as protein synthesis *in vivo*, there is still the possibility that ribosomes of the mutant or a portion of its ribosomal population may be defective in the synthesis of a part protein.

The other major differences between the normal and mutant were the differences in number of bands and mobility of acidic proteins derived from leaf cytoplasmic (80S) and chloroplast (70S) ribosomes (Fig. 12B and 16). It is probable that among the ribosomal proteins, the acidic proteins are primarily affected by the nuclear mutation. Since this part of the study was done at the end of the investigation period the observed differences in the acidic proteins indicated the need for further research. It is suggested that additional studies with the acidic proteins using two-dimensional PAGE would be useful in obtaining further information.

In addition, while the gel electrophoresis technique can identify differences between proteins, it cannot distinguish differences in primary amino acid sequence from differences due to secondary modification of identical sequence. The utilization of immunological methods could help in making this distinction and thus would be valuable in estimating the structural homologies between the ribosomal proteins of Gateway barley and its virescens mutant.

LITERATURE CITED

1. ADELMAN, M.R., D.D. SABATINI, and G. BLOBEL. 1973. Ribosome-membrane interaction. Non-destructive disassembly of rat liver rough microsomes into ribosomal and membranous components. J. Cell. Biol. 56:206-229.

2. ALLENDE, J.E. and M. BRAVO. 1966. Amino acid incorporation and aminoacyl transfer in a wheat embryo system. J. Biol. Chem. 241:5813-5818.

3. AMELUNXEN. F. and E. SPIESS. 1971. Untersuchungen zur structur der ribosomen. Ein beitrag zur konformation der 80S ribosomen von *Pisum sativum*. Cytobiologie 4:293-306.

4. APP, A.A. 1969. Involvement of aminoacyl-tRNA transfer factors in polyphenylalanine synthesis by rice embryo ribosomes. Plant Physiol. 44:1132-1138.

5. APP, A.A. and M.M. GEROSA. 1966. A soluble fraction requirement in the transfer reaction of protein synthesis by rice embryo ribosomes. Plant Physiol. 41:1420-1424.

6. APP, A.A., M.G. BULIS, and W.J. McCARTHY. 1971. Dissociation of ribosomes and seed germination. Plant Physiol. 47:81-86.

7. AVADHANI, N.G. and D.E. BUETOW. 1972. Isolation of active polyribosomes from the cytoplasm mitochondria and chloroplasts of *Euglena gracilis*. Biochem. J. 128:353-365.

8. BAR-NUN, S. and I. OHAD. 1977. Presence of polypeptides of cytoplasmic and chloroplastic origin in isolated photoactive preparations of photosystems I and II in *Chlamydomonas reinhardi* Y-I. Plant Physiol. 59:161-166.

9. BAYLEY, S.T. 1964. Physical studies on ribosomes from pea seedlings. J. Mol. Biol. 8:231-238.

10. BICKLE, T.A. and R.R. TRAUT. 1971. Differences in size and number of 80S and 70S ribosomal proteins by dodecyl-sulphate gel electrophoresis. J. Biol. Chem. 246:6828-6834.

11. BICKLE, T.A. and R.R. TRAUT. 1974. Determination of ribosomal protein molecular weight distribution from stained dodecyl-sulphate gels. Methods Enzymol. 30:545-553.

12. BIELKA, H. and H. WELFLE. 1968. Characterization of ribosomal proteins from different tissues and species of animals by electrophoresis on polyacrylamide gel. Molec. Gen. Genet. 102:128-131.

13. BLOBEL, G. and V.R. POTTER. 1967. Studies on free and membrane-bound ribosomes in rat liver. II. Interaction of ribosomes and membranes. J. Mol. Biol. 26:293-301.

14. BOARDMAN, N.K. 1966. Ribosome composition and chloroplast development in *Phaseolus vulgaris*. Exptl. Cell. Res. 43:474-482.

15. BOARDMAN, N.K., R.I.B. FRANCKI, and S.G. WILDMAN. 1965. Protein synthesis by cell-free extracts from tobacco leaves. II. Association of activity with chloroplast ribosomes. Biochemistry 4:872-876.

16. BOARDMAN, N.K., R.I.B. FRANCKI, and S.G. WILDMAN. 1966. Protein synthesis by cell-free extracts of tobacco leaves. III. Comparison of the physical properties and protein synthesizing activities of 70S chloroplast and 80S cytoplasmic ribosomes. J. Mol. Biol. 17:470-489.

17. BORST, P. 1972. Mitochondrial nucleic acids. Ann. Rev. Biochem. 41:333-376.

18. BORST, P. and L.A. GRIVELL. 1971. Mitrochondrial ribosomes. FEBS Lett. 13:73-87.

19. BOULTER, D. 1970. Protein synthesis in plants. Ann. Rev. Plant Physiol. 21:91-114.

20. BOULTER, D., R.J. ELLIS, and A. YARWOOD. 1972. Biochemistry of protein synthesis in plants. Biol. Rev. 47:113-175.

21. BOURQUE, D.P., J.E. BOYNTON, and N.W. GILLHAM. 1971. Studies on the structure and cellular location of various ribosomes and ribosomal RNA species in the green alga *Chlamydomonas reinhardi*. J. Cell Sci. 8:153-183.

22. BOURQUE, D.P. and S.G. WILDMAN. 1970. Evidence that nuclear genes code for several chloroplast ribosomal proteins. Biochem. Biophys. Res. Commun. 103:89-90.

23. BRAWERMAN, G. 1963. The isolation of [illegible] species of ribosomes associated with chloroplast deve[illegible] in *Euglena gracilis*. Biochim. Biophys. Acta [illegible]:3

24. BREEN, M.D., E.I. WHITEHEAD, and D.G. KENE[illegible]. Requirements for extraction of polysomes from barley [illegible] Plant Physiol. 49:733-739.

25. BRÜGGER, M. and A. BOSCHETTI. 1975. Two-dimensional gel electrophoresis of ribosomal proteins from streptomycin-sensitive and streptomycin-resistant mutants of *Chlamydomonas reinhardi*. Eur. J. Biochem. 58:603-610.

26. BRUSKOV, V.I. and N.A. KISELEV. 1968. Electron microscope investigation of the structure of cytoplasmic ribosomes of bean leaves. J. Mol. Biol. 38:443-445.

27. BRUSKOV, V.I. and N.A. ODINTSOVA. 1968. Comparative electron microscopic studies of chloroplast and cytoplasmic ribosomes. J. Mol. Biol. 32:471-473.

28. CAMMARANO, P., A. ROMEO, M. GENTILE, A. FELSANI, and C. GUALERZI. 1972a. Size heterogeneity of the large ribosomal subunits and conservation of the small subunits in eukaryotic evolution. Biochim. Biophys. Acta 281:597-624.

29. CAMMARANO, P., A. FELSANI, M. GENTILE, C. GUALERZI, A. ROMEO, and G. WOLF. 1972b. Formation of active hybrid 80S particles from subunits of pea seedlings and mammalian liver ribosomes. Biochim. Biophys. Acta 281:625-642.

30. CHAO, F.C. 1957. Dissociation of macromolecular ribonucleoprotein of yeast. Arch. Biochem. Biophys. 70:426-431.

31. CHAO, F.A. and H.K. SCHACHMAN. 1956. The isolation and characterization of macromolecular ribonucleoprotein from yeast. Arch. Biochem. Biophys. 61:220-230.

32. CHATTERJEE, S.K., M. KAZEMIE, and H. MATTHAEI. 1973. Separation of the ribosomal proteins by two-dimensional electrophoresis. Hoppe-Seyler's Z. Physiol. Chem. Bd. 354:481-486.

33. CHEN, J.L. and WILDMAN, S.G. 1970. "Free" and membrane-bound ribosomes and nature of products formed by isolated tobacco chloroplasts incubated for protein synthesis. Biochim. Biophys. Acta 209:207-219.

34. CHUA, N., G. BLOBEL, and P. SIEKEVITZ. 1973. Isolation of cytoplasmic and chloroplast ribosomes and their dissociation into active subunits from *Chlamydomonas reinhardtii*. J. Cell. Biol. 37:798-814.

35. CHUA, N., G. BLOBEL, P. SIEKVITZ, and G.E. PALADE. 1973. Attachment of chloroplast polysomes to thylakoid membranes in *Chlamydomonas reinhardtii*. Proc. Nat. Acad. Sci. USA 70: 1554-1558.

36. CLARK, M.F., R.E.F. MATTHEWS, and R.K. RALPH. 1964. Ribosomes and polyribosomes in *Brassica pekinensis*. Biochim. Biophys. Acta 91:289-304.

37. CLICK, R.E. and D.P. HACKETT. 1966. The isolation of ribonucleic acid from plant, bacterial or animal cells. Biochim. Biophys. Acta 129:79-84.

38. CLICK, R.E. and B.L. TINT. 1967. Comparative sedimentation rates of Plant, Bacterial and Animal ribosomal RNA. J. Mol. Biol. 25:111-122.

39. CRAVEN, G.R., P. VOYNOW, S.J.S. HARDY, and C.G. KURLAND. 1969. Ribosomal proteins of *E. coli*. II. Chemical and physical characterization of 30S proteins. Biochemistry 8:2906-2915.

40. DALLNER, G., P. SIEKEVITZ, and G.E. PALADE. 1966. Biogenesis of endoplasmic reticulum membranes. II. Synthesis of constitutive microsomal enzymes in developing rat hepatocyte. J. Cell. Biol. 30:97-117.

41. DAVIDSON, J.N., M.R. HANSON, and L. BOGORAD. 1974. An altered chloroplast ribosomal protein in ery-M1 mutants of *Chlamydomonas reinhardi*. Molec. Gen. Genet. 132:119-129.

42. de GROOT, N., A. PANET, and Y. LAPIDOT. 1967. Some biological properties of acetyl- and formylphenylalanyl-sRNA. Biochem. Biophys. Res. Commun. 26:447-453.

43. DZIONARA, M., E. KALTSCHMIDT, and H.G. WITTMANN. 1970. Ribosomal proteins, XIII. Molecular weights of isolated ribosomal proteins of *Escherichia coli*. Proc. Nat. Acad. Sci. USA 67:1909-1913.

44. EISENSTADT, J.M. and G. BRAWERMAN. 1964. The protein-synthesizing systems from the cytoplasm and the chloroplast of *Euglena gracilis*. J. Mol. Biol. 10:392-402.

45. EYTAN, G. and I. OHAD. 1970. Biogenesis of chloroplast membranes. VI. Cooperation between cytoplasmic and chloroplast ribosomes in the synthesis of photosynthetic lamellar proteins during the greening process in a mutant of *Chlamydomonas reinhardi*. J. Biol. Chem. 247:122-129.

46. FABER, A.J. and T. TAMAOKI. 1976. Preparation of active runoff 80S ribosomes and their subunits from mouse liver. Can. J. Biochem. 54:1034-1039.

47. FALK, H. 1969. Rough thylakoids: Polysomes attached to chloroplast membranes. J. Cell. Biol. 92:582-587.

48. FREYSSINET, G., and J.A. SCHIFF. 1974. The chloroplast and cytoplasmic ribosomes of *Euglena*. II. Characterization of ribosomal proteins. Plant Physiol. 53:543-554.

49. FUNATSU, G. and H.G. WITTMANN. 1972. Ribosomal Proteins. XXXIII. Location of amino acid replacements in protein S12 from *Escherichia coli* mutants resistant to streptomycin. J. Mol. Biol. 68:547-550.

50. FUNATSU, G., W. PULS, E. SCHILTZ, J. REINBOLT, and H.G. WITTMANN. 1972. Ribosomal proteins. XXXI. Comparative studies on altered proteins S4 of six *Escherichia coli* revertants from streptomycin dependence. Molec. Gen. Genet. 115:131-139.

51. GILLHAM, N.W. 1969. Uniparental inheritance in *Chlamydomonas reinhardi*. Am. Nat. 103:355-388.

52. GLICK, M.C. and L. WARREN. 1969. Membranes of animal cells, III. Amino acid incorporation by isolated surface membranes. Proc. Nat. Acad. Sci. USA 63:563-570.

53. GLITZ, D.G. and C.A. DEKKER. 1963. The purification of properties of ribonucleic acid from wheat germ. Biochemistry 2:1185-1192.

54. GOODENOUGH, U.W. and R.P. LEVINE. 1970. Chloroplast structure and function in ac-20, a mutant strains of *Chlamydomonas reinhardi*. III. Chloroplast ribosomes and membrane organization. J. Cell. Biol. 44:547-562.

55. GOULD, H.J. 1970. Proteins of rabbit reticulocyte ribosomal subunits. Nature 227:1145-1147.

56. GUALERZI, C. and P. CAMMARANO. 1969. Comparative electrophoretic studies on the protein of chloroplasts and cytoplasmic ribosomes of spinach leaves. Biochim. Biophys. Acta 190:170-186.

57. GUALERZI, C. and P. CAMMARANO. 1970. Species specificity of ribosomal proteins from chloroplast and cytoplasmic ribosomes of higher plants. Electrophoretic studies. Biochim. Biophys. Acta 199:205-213.

58. GUALERZI, C., H.G. JANDA, H. PASSOW, and G. STÖFFLER. 1974. Studies on the protein moiety of plant ribosomes. Enumeration of the proteins of the ribosomal subunits and determination of the degree of evolutionary conservation by electrophoretic and immunological methods. J. Mol. Biol. 249:3347-3355.

59. GULYAS, A. AND B. PARTHIER. 1971. Pea seedling cell-free polypeptide synthesis. Functional characteristics of ribosome and supernatant fractions. Biochem. Physiol Pflanzen. 162:60-74.

60. GUMILEVSKAYA, N.A., L.V. CHUMIKINA, E.B. KUVAEVA, and V.L. KRETOVICH. 1975. Isolation and characterization of subunits of 80S ribosomes of dry pea seeds. Biochemistry 40:148-156. (Eng. trans.).

61. GUNNING, B.E.S. and M.P. JAGOE. 1967. The prolamellar body. In: Biochemistry of chloroplasts. T.W. Goodwin, ed. Academic Press, New York and London. pp. 655-676.

62. HADZIYEV, D. and S. ZALIK. 1970. Amino acid incorporation by ribosomes and polyribosomes from wheat chloroplasts. Biochem. J. 116:111-124.

63. HADZIYEV, D., S.L. MEHTA, and S. ZALIK. 1968. Studies on the ribonucleic acid from wheat leaves and chloroplasts. Plant Physiol. 43:229-237.

64. HADZIYEV, D., S.L. MEHTA, and S. ZALIK. 1969. Nucleic acids and ribonucleases of wheat leaves and chloroplasts. Can. J. Biochem. 47:273-282.

65. HANNA, N. and C. GODIN. 1975. Comparison of the effect of various methods of dissociation on the protein composition of rat liver ribosomal subunits. Can. J. Biochem. 53:935-942.

66. HANSON, M.E., J.N. DAVIDSON, L.J. METS, and L. BOGORAD. 1974. Characterization of chloroplast and cytoplasmic ribosomal proteins of *Chlamydomonas reinhardi* by two-dimensional gel electrophoresis. Molec. Gen. Genet. 132:105-118.

67. HARDY, S.J.S., C.G. KURLAND, P. VOYNOW, and G. MORA. 1969. The ribosomal proteins of *Escherichia coli*. I. Purification of the 30S ribosomal proteins. Biochemistry 8:2897-2905.

68. HOOBER, J.K. 1970. Sites of synthesis of chloroplast membrane polypeptides in *Chlamydomonas reinhardi* Y-I.. J. Biol. Chem. 245:4327-4334.

69. HOOBER, J.K. 1972. A major polypeptide of chloroplast membranes of *Chlamydomonas reinhardi*. Evidence for synthesis in cytoplasm as a soluble component. J. Cell. Biol. 52:84-96.

70. HOOBER, J.K. and G. BLOBEL. 1969. Characterization of the chloroplastic and cytoplasmic ribosomes of *Chlamydomonas reinhardi*. J. Mol. Biol. 41:121-138.

71. HORAK, A. and S. ZALIK. 1975. Development of photoreductive activity in plastids of virescens mutant of barley. Can. J. Bot. 53:2399-2404.

72. HOWARD, G.A., J.A. TRAUGH, E.A. CROSER, and R.R. TRAUT. 1975. Ribosomal proteins from rabbit reticulocytes: Number and molecular weights of proteins from ribosomal subunits. J. Mol. Biol. 93:391-404.

73. ISHIGURO, J. 1976. Study on proteins from yeast cytoplasmic ribosomes by two-dimensional gel electrophoresis. Molec. Gen. Genet. 145:73-79.

74. JACKSON, A.O. and B.A. LARKINS. 1976. Influence of ionic strength, pH, and chelation of divalent metals on isolation of polyribosomes from tobacco leaves. Plant Physiol. 57:5-10.

75. JANDA, H.G. and H.G. WHITTMANN. 1968. Ribosomal Proteins. V. Comparison of protein patterns of 70S and 80S ribosomes from various plants by polyacrylamide gel electrophoresis. Molec. Gen. Genet. 103:238-243.

76. JHAMB, S. and S. ZALIK, 1973. Soluble and lamellar proteins in seedlings of barley and its virescens mutant in relation to chloroplast development. Can. J. Bot. 51:2147-2154.

77. JHAMB, S. and S. ZALIK. 1975. Plastid development in a virescens barley mutant and chloroplast microtubules. Can. J. Bot. 53: 2014-2025.

78. JOHNSON, V.G. 1968. Chloroplast ribosomes in *Chlamydomonas reinhardi* I. Their involvement in the structural organization of the chloroplast and in the synthesis of ribulose-1,5-diphosphate carboxylase. J. Cell. Biol. 39:68a.

79. JONES, B.L., N. NAGABHUSHAN, and S. ZALIK. 1972a. Isolation and partial characterization of chloroplast and cytoplasmic ribosomes and ribosomal subunits from wheat. Symp. Biol. Hung. 13:249-262.

80. JONES, B.L., N. NAGABHUSHAN, A. GULYAS, and S. ZALIK. 1972b. Two-dimensional acrylamide gel electrophoresis of wheat leaf cytoplasmic and chloroplast ribosomal proteins. FEBS Lett. 23:167-170.

81. JONES, B.L., N. NAGABHUSHAN, E.B. TUCKER, and S. ZALIK. 1973. Dissociation reassociation and phenylalanine incorporation by chloroplast and cytoplasmic wheat-leaf ribosomes. Can. J. Biochem. 51:686-693.

82. KALTSCHMIDT, E. and H.G. WITTMANN. 1969. Ribosomal proteins. VI. Preparative polyacrylamide gel electrophoresis as applied to the isolation of ribosomal proteins. Anal. Biochem. 30:132-141.

83. KALTSCHMIDT, E. and H.G. WITTMANN. 1970a. Ribosomal proteins. VII. Two-dimensional polyacrylamide gel electrophoresis for fingerprinting of ribosomal proteins. Anal. Biochem. 36:401-412.

84. KALTSCHMIDT, E. and WITTMANN. 1970b. Ribosomal proteins. XII. Number of proteins in small and large ribosomal subunits of *Escherichia coli* as determined by two-dimensional gel electrophoresis. Proc. Nat. Acad. Sci. USA 67:1276-1282.

85. KALTSCHMIDT, E., M. DZIONARA, D. DONNER, H.G. WITTMANN. 1967. Ribosomal proteins. I. Isolation, amino acid composition, molecular weights and peptide mapping of proteins from *E. coli* ribosomes. Molec. Gen. Genet. 100:364-373.

86. KALTSCHMIDT, E., M. DZIONARA, D. DONNER, H.G. WITTMANN. 1970. Ribosomal proteins. XVII. Comparative studies on ribosomal proteins of four strains of *Escherichia coli*. Molec. Gen. Genet. 109:303-308.

87. KAWASHIMA, N. and S.G. WILDMAN. 1972. Studies on fraction I proteins. Mode of inheritance of primary structure in relation to whether chloroplast or nuclear DNA contains the code for a chloroplast proteins. Biochim. Biophys. Acta 262:42-49.

88. KELLEMS, R.E. and R.A. BUTOW. 1962. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. I. Evidence for ribosome binding sites on yeast mitochondria. J. Biol. Chem 247: 8043-8050.

89. KELLEMS, R.E. and R.A. BUTOW. 1974. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. III. Changes in the amount of ribosomes in response to changes in metabolic state. J. Biol. Chem. 249:3304-3310.

90. KELLEMS, R.E., V.F. ALLISON, and R.A. BUTOW. 1974. Cytoplasmic type 80S ribosomes associated with yeast mitochondria. II. Evidence for the association of cytoplasmic ribosomes with the outer mitochondrial membrane *in situ*. J. Biol. Chem. 249:3297-3303.

91. KING, H.W.S., H.J. GOULD, and J.J. SHEARMAN. 1971. Molecular weight distribution of proteins in rabbit reticulocyte ribosomal proteins. J. Mol. Biol. 61:143-156.

92. KJELDGAARD, N.O. and K. GAUSING. 1974. Regulation of Biosynthesis of Ribosomes. In: Ribosomes. M. Nomura, A. Tissières and P. Lengyel, eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 333-369.

93. KREMBEL, J. and D. APIRION. 1968. Changes in ribosomal proteins associated with mutants in a locus that affects *Escherichia coli*. J. Mol. Biol. 33:363-368.

94. KURIYAMA, Y. and D.J.L. LUCK. 1973. Membrane-associated ribosomes in mitochondria of *Neurospora crassa*. J. Cell. Biol. 59:776-784.

95. KURLAND, C.G. 1971. The proteins of the bacterial ribosomes. In: Protein Synthesis - I. E.H. McConkey, ed. Marcel Dekker, Inc. New York. pp. 179-223.

96. KURLAND, C.G. 1972. Structure and function of the bacterial ribosome. Ann. Rev. Biochem. 41:377-408.

97. KURLAND, C.G., P. VOYNOW, S.J.S. HARDY, L. RANDALL, and L. LUTTER. 1969. Physical and functional heterogeneity of *E. coli* ribosomes. Cold Spring Harbor Symp. quant. Biol. 34:17-24.

98. LAMFRTSSON , A.G. 1972. The ribosomal proteins of [illegible]. Molec. Gen. Genet. 118:215-222.

99. LARKINS, B.A. and E. DAVIES. 1973. Polyribosomes from peas. III. Stimulation of polysome degradation by exogenous and endogenous calcium. Plant Physiol. 52:655-659.

100. LARKINS, B.A. and E. DAVIES. 1975. Polyribosomes from peas. V. An attempt to characterize the total free and membrane-bound polysomal population. Plant Physiol. 55:749-756.

101. LEE, S.G. and W.R. EVANS. 1971. Hybrid ribosome formation from [illegible] and chloroplast ribosome subunits. Science 173:241-242.

102. LIM, L. and D.H. ADAMS. 1967. Microsomal components in relation to amino acid incorporation by preparations from the developing rat brain. Biochemical J. 104:229-238.

103. LIN, C.Y. and J.L. KEY. 1971. Dissociation of N_2 gas-induced monomeric ribosomes and functioning of the derived subunits in protein synthesis in peas. Plant Physiol. 48:547-552.

104. LIN, C.Y., R.L. TRAVIS S.Y. CHIA, and J.L. KEY. 1973. Protein synthesis by 80S ribosomes during plant development. Phytochem. 12:515-522.

105. LOENING, U.E. 1968. Molecular weights of ribosomal RNA in relation to evolution. J. Mol. Biol. 38:355-365.

106. LOENING, U.E. 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J. 113:131-138.

107. LOENING, U.E. and J. INGLE. 1967. Diversity of RNA components in green plant tissue. Nature 215:363-367.

108. LOMFROM, H. and E.R. GLOWACKI. 1962. Controlled dissociation of rabbit reticulocyte ribosomes and its effect on hemoglobin synthesis. J. Mol. Biol. 5:97-108.

109. LOW, K.B., I.G. WOOL, and T.E. MARTIN. 1969. Skeletal muscle ribosomal proteins: General characteristics and effect of diabetes. Biochim. Biophys. Acta 194:190-202.

110. LOWRY, O.H , N.J. ROSENBROUGH, A.L. FARR, and R.J. RANDALL. 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193:265-275.

111. LYTTLETON, J.W. 1960. Nucleoproteins of white clover. Biochem. J. 74:82-90.

112. LYTTLETON, J.W. 1962. Isolation of ribosomes from spinach chloroplasts. Exptl. Cell. Res. 26:312-317.

113. LYTTLETON, J.W. 1968. Protein constituents of plant ribosomes. Biochim. Biophys. Acta 154:145-149.

114. MACLACHLAN, S. and S. ZALIK. 1963. Plastid structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of barley. Can. J. Bot. 41:1053-1063.

115. MANGIAROTTI, G. and D. SCHLESSINGER. 1966. Polyribosome metabolism in *Escherichia coli*. I. Extraction of polysomes and ribosomal subunits from fragile, growing *Escherichia coli*. J. Mol. Biol. 20:123-143.

116. MANGIAROTTI, G. and D. SCHLESSINGER. 1967. Polyribosome metabolism in *E. coli*. II. Formation and lifetime of messenger RNA molecules, ribosomal subunit couples and polyribosomes. J. Mol. Biol. 29:395-418.

117. MARCUS, A. and J. FEELEY. 1964. Activation of protein synthesis in the imbibition phase of seed germination. Proc. Nat. Acad. Sci. USA 51:1075-1079.

118. MARCUS, A. and J. FEELEY. 1965. Protein synthesis in imbibed seeds. II. Polysome formation during imbibition. J. Biol. Chem. 240:1675-1680.

119. MARCUS, A. and J. FEELEY. 1966. Ribosome activation and polysome formation *in vitro*: requirement for ATP. Proc. Nat. Acad. Sci. USA 56:1770-1777.

120. MARGULIES, M.M. and A. MICHAELS. 1974. Ribosomes bound to chloroplast membranes in *Chlamydomonas reinhardtii*. J. Cell. Biol. 60:65-77.

121. MARGULIES, M.M. and A. MICHAELS. 1975. Free and membrane-bound chloro[illegible] polyribosomes in *Chlamydomonas reinhardtii*. Bi[illegible]chim[illegible]hys. Acta 402:297-308.

122. MARGULI[illegible].M. [illegible].L. TIFFANY, and A. MICHAELS. 1975. Vectorial disci[illegible] of nascent polypeptides attached to chloroplast thylakoid membranes. Biochim. Biophys. Res. Commun. 64:735-739.

123. MARTIN, T.E. and I.G. WOOL. 1968. Formation of active hybrids from subunits of muscle ribosomes from normal and diabetic rats. Proc. Nat. Acad. Sci. USA 60:569-574.

124. MARTIN, T.E. and I.G. WOOL. 1969. Active hybrid 80S particles formed from subunits of rat, rabbit and protozoan (*Tetrahymena pyriformus*) ribosomes. J. Mol. Biol. 43:151-161.

125. MARTIN, T.E., F.S. ROLLESTON, R.B. LOW, and I.G. WOOL. 1969. Dissociation and reassociation of skeletal muscle ribosomes. J. Mol. Biol. 43:135-149.

126. MARTIN, T.E., J.N. BICKNELL, and A. KUMAR. 1970. Hybrid 80S monomers formed from subunits of ribosomes from protozoa, fungi, plants and mammals. Biochem. Genet. 4:603-615.

127. MARTINI, O.H. and H.J. GOULD. 1971. Enumeration of rabbit reticulocyte ribosomal proteins. J. Mol. Biol. 63: 403-405.

128. MEHTA, S.L., D. HADZIYEV, and S. ZALIK. 1969. Amino acid incorporation by cytoplasmic polysomes from wheat leaves. Biochim. Biophys. Acta 195:515-522.

129. MENDIOLA, L.R., A. KOVACS, and C.A. PRICE. 1970. Separation and partial characterization of chloroplast and cytoplasmic ribosomes from *Euglena gracilis*. Plant and Cell. Physiol. 11:335-344.

130. METS, L. and L. BOGORAD. 1971. Mendelian and uniparental alterations in erythromycin binding by plastid ribosomes. Science 174:707-709.

131. METS, L. and L. BOGORAD. 1972. Altered chloroplast ribosomal proteins associated with erythromycin resistant mutants in two genetic systems of *Chlamydomonas reinhardi*. Proc. Nat. Acad. Sci. USA 69:3779-3783.

132. MICHAELS, A. and M.M. MARGULIES. 1975. Amino acid incorporation into protein by ribosomes bound to chloroplast thylalcoid membranes: Formation of discrete products. Biochim. Biophys. Acta 390:352-362.

133. MILLER, A., U. KARLSSON, and N.K. BOARDMAN. 1966. Electron microscopy of ribosomes isolated from tobacco leaves. J. Mol. Biol. 17:487-489.

134. MILLER, R.A. 1965. Physiological and biochemical studies of a mutant from Gateway barley. Ph.D. Thesis, University of Alberta, Canada.

135. MIRAULT, M.E. and K. SCHERRER. 1971. Isolation of preribosomes from HeLa cells and their characterization by electrophoresis on uniform and exponential-gradient-polyacrylamide gels. Eur. J. Biochem. 23:372-386.

136. MOORE, P.B., R.R. TRAUT, H. NOLLER, P. PEARSON, and H. DELIUS. 1968. Ribosomal proteins of *Escherichia coli*. II. Proteins from the 30S subunit. J. Mol. Biol. 31:441-461.

137. MORA, G., D. DONNER, P. THAMMANA, L. LUTTER, and C.G. KURLAND. 1971. Purification and characterization of 50S ribosomal proteins of *Escherichia coli*. Molec. Gen. Genet. 112:229-242.

138. MUNNS, R.E. 1972. Synthesis of RNA during chloroplast development in *Euglena gracilis*. Ph.D. thesis. University of Sydney, Australia. (cited in reference 162).

139. MURTHY, M.R.V. 1972. Free and membrane-bound ribosomes of rat cerebral cortex. Protein synthesis *in vivo* and *in vitro*. J. Biol. Chem. 247:1936-1943.

140. NAGABHUSHAN, N., A. GULYAS, and S. ZALIK. 1974. Comparison of plant cytoplasmic ribosomal proteins by two-dimensional polyacrylamide gel electrophoresis. Plant Physiol. 53: 516-518.

141. NOMURA, M. 1973. Assembly of bacterial ribosomes. Science 179:864-873.

142. NOMURA, M., A. TISSIÈRES, and P. LENGYEL. 1974. eds. "Ribosomes" Cold Spring Harbor Lab., Cold Spring Harbor, New York.

143. NOMURA, M., S. MIZUSHIMA, M. OZAKI, P. TRAUB, and C.V. LOWRY. 1969. Structure and function of ribosomes and their molecular components. Cold Spring Harbor Symp. quant. Biol. 34:49-61.

144. ODINTSOVA, M.S. and N.P. YURINA. 1969. Ribosome proteins of chloroplasts and cytoplasm, as investigated by polyacrylamide gel electrophoresis. Biochemistry 34:533-538. (Eng. trans.).

145. ODINTSOVA, M.S., V.I. BRUSKOV, and E.V. GOLUBEVA. 1967. Comparative study of ribosomes of chloroplasts and cytoplasm of certain plant species. Biochemistry 32:867-878. (Eng. trans.).

146. OPIK, H. 1968. Development of cotyledon cell structure in ripening *Phaseolus vulgaris* seeds. J. Expt. Bot. 19:64-76.

147. PAYNE, P.I. and D. BOULTER. 1969. Free and membrane-bound ribosomes of the cotyledons of *Vicia faba*. Planta 84: 263-271.

148. PEETERS, B., L. VANDUFFEL, A. DEPUYDT, and W. ROMBAUTS. 1973. The number and size of the proteins in the subunits of human placental ribosomes. FEBS Lett. 36:217-221.

149. PETERMANN, M.L. 1964. The physical and chemical properties of ribosomes. American Elsevier Publ. Co., New York.

150. PHILLIPS, L.A., B. HOTHAM-IGLEWSKI, and R.M. FRANKLIN. 1969. Polyribosomes of *Escherichia coli*. Effects of monovalent cation on the distribution of polysomes, ribosomes and ribosomal subunits. J. Mol. Biol. 40:279-288.

151. PRATT, H. and R.A. COX. 1973. Proteins from biologically active ribosomal subparticles of *Xenopus laevis*. Biochim. Biophys. Acta 310:188-204.

152. RAMAGOPAL, S. and T.C. HSIAO. 1973. Polyribosomes from maize leaves. Isolation at high pH and amino acid incorporation. Biochim. Biophys. Acta 299:460-467.

153. RAWSON, J.R. and E. STUTZ. 1969. Isolation and characterization of *Euglena gracilis* cytoplasmic and chloroplast ribosomes and their ribosomal RNA components. Biochim. Biophys. Acta 190:368-380.

154. REDMAN, C.M. 1967. Studies on the transfer of incomplete polypeptide chains across rat liver microsomal membranes *in vitro*. J. Biol. Chem. 242:761-768.

155. REDMAN, C.M. and D.D. SABATINI. 1966. Vectorial discharge of peptides released by puromycin from attached ribosomes. Proc. Nat. Acad. Sci. USA 56:608-615.

156. SAGER, R. and M.G. HAMILTON. 1967. Cytoplasmic and chloroplast ribosomes of *Chlamydomonas* ultracentrifugal characterization. Science 157:709-711.

157. SANE, P.V. and S. ZALIK. 1970. Metabolism of acetate-2-^{14}C, glycine-2-^{14}C, leucine-U-^{14}C, and effect of δ-aminolevulinic acid on chlorophyll synthesis in Gateway barley and its mutant. Can. J. Bot. 48:1171-1178.

158. SCHLANGER, G. and R. SAGER. 1974. Localization of five antibiotic resistances at the subunit level in chloroplast ribosomes of *Chlamydomonas*. Proc. Nat. Acad. Sci. USA 71:1715-1719.

159. SCHLANGER, G., R. SAGER, and Z. RAMANIS. 1972. Mutation of a cytoplasmic gene in *Chlamydomonas* alters chloroplast ribosome function. Proc. Nat. Acad. Sci. USA 69:3551-3555.

160. SCHLESSINGER, D. and F. GROS. 1963. Structure and properties of active ribosomes of *Escherichia coli*. J. Mol. Biol. 7:350-359.

161. SCHLESSINGER, D., G. MANGIAROTTI, and D. APIRION. 1967. The formation and stabilization of 30S and 50S ribosome couples in *Escherichia coli*. Proc. Nat. Acad. Sci. USA 58:1782-1789.

162. SCHWARTZBACH, S.D., G. FREYSSINET, and J.A. SCHIFF. 1974. The chloroplast and cytoplasmic ribosomes of *Euglena*. 1. Stability of chloroplast ribosomes prepared by an improved procedure. Plant Physiol. 53:533-542.

163. SCOTT, S.N., R. MUNNS, and R.M. SMILLIE. 1970. Chloroplast and cytoplasmic ribosomes in *Euglena gracilis*. FEBS Lett. 10:149-152.

164. SHERMAN, M.I. 1972. The role of ribosomal conformation in protein biosynthesis. Eur. J. Biochem. 25:291-300.

165. SHERTON, C.C. and I.G. WOOL. 1972. Determination of the number of proteins in liver ribosomes and ribosomal subunits by two-dimensional polyacrylamide gel electrophoresis. J. Biol. Chem. 247:4460-4467.

166. SHERTON, C.C. and I.G. WOOL. 1974. A comparison of the proteins of rat skeletal muscle and liver ribosomes by two-dimensional polyacrylamide gel electrophoresis. J. Biol. Chem. 249: 2253-2267.

167. SISSAKIAN, N.M., I.I. FILIPPOVICH, E.N. SVETAILO, and K.A. ALIYEV. 1965. On the protein synthesizing system of chloroplasts. Biochim. Biophys. Acta 95:474-485.

168. SMILLIE, R.M., N.S. SCOTT, D. GRAHAM, A.M. GRIEVE, and T.F. TOBIN. 1968. Formation and function of chloroplast ribosomes in *Euglena*. Plant Physiol. 43;s-6.

169. SPENCER, D. and P.R. WHITFELD. 1966. The nature of the ribonucleic acid of isolated chloroplasts Arch. Biochem. Biophys. 117:337-346.

170. SPIESS, H. and C.G. ARNOLD. 1975. Ribosomal proteins in plastids of a mendelian and non-mendelian streptomycin-resistant mutant of *Chlamydomonas reinhardi* determined by two-dimensional gel electrophoresis. Arch. Microbiol. 103:89-90.

171. SPIRIN, A.S. 1961. The "temperature effect" and macromolecular structure of high polymer ribonucleic acids of various origin. Biochemistry 26:454-463. (Eng. trans.).

172. SPIRIN, A.S. and L.P. GAVRICOVA. 1969. The Ribosomes. Molecular Biology, Biochemistry and Biophysics - 4. A. Kleinzeller, G.F. Springer, and H.G. Wittmann, eds. Springer-Verlag, New York, Inc.

173. STEPHANSEN, K. and S. ZALIK. 1971. Inheritance and qualitative analysis of pigments in a barley mutant. Can. J. Bot. 49:49-51.

174. STUTZ, E. and J.R. RAWSON. 1968. The subunits and structural ribonucleic acids of Jensen sarcoma ribosomes. Biochim. Biophys. Acta 114:264-276.

175. SUN, T.T., T.A. BICKLE, and R.R. TRAUT. 1972. Similarity in the size and number of ribosomal proteins from different prokaryotes. J. Bacteriol. 111:474-480.

176. TASHIRO, Y. and P. SIEKEVITZ. 1965. Ultracentrifuge studies on the dissociation of hepatic ribosomes. J. Mol. Biol. 11: 149-165.

177. THOMAS, H. 1973. Gel electrophoresis of ribosomal components from seeds of *Pisum sativum* L. Exptl. Cell. Res. 77:298-302

178. TISSIÈRES, A., D. SCHLESSINGER, and F. GROS. 1960. Amino acid incorporation into proteins by *Escherichia coli* ribosomes. Proc. Nat. Acad. Sci. USA 46:1450-1462.

179. TRAUT, R.R., H. DELIUS, C. AHMAD-ZADEK, P. PEARSON, and A. TISSIÈRES. 1969. Ribosomal proteins of *E. coli*. Stoichiometry and implications for ribosome structure. Cold Spring Harbor Symp. quant. Biol. 34:25-38.

180. TS'O, P.O.P. and J. VINOGRAD. 1961. Studies of ribosomes from reticulocytes. Biochim. Biophys. Acta 49:113-129.

181. TS'O, P.O.P., J. BONNER, and J. VINOGRAD. 1958. Structure and properties of microsomal nucleoprotein particles from pea seedlings. Biochim. Biophys. Acta 30:570-582.

182. TUCKER, E.B. and S. ZALIK. 1973. ^{14}C-phenylalanine incorporation by wheat seedling cytoplasmic ribosomes. Can. J. Biochem. 51:699-700.

183. VAN DER ZEIJST, A.M., A.J. KOOL, and H.P.J. BLOEMERS. 1972. Isolation of active ribosomal subunits from yeast. Eur. J. Biochem. 30:15-25.

184. VAN HOLDE, K.E. and W.E. HILL. 1974. General physical properties of ribosomes. In: Ribosomes. M. Nomura, A. Tissères and P. Lengyel, eds. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., USA.

185. VAN KAMMEN, A. 1967. Characteristics of *in vitro* amino acid incorporation by the ribosomal fraction from tobacco leaves. Arch. Biochem. Biophys. 118:517-524.

186. VANYUSHIN, B.F. and D.B. DUNN. 1967. The dissociation of 80S ribosomes with polyvinyl sulphate. Biochim. Biophys. Acta 134:91-105.

187. VASCONCELOS, A.C.L. and L. BOGORAD. 1971. Proteins of cytoplasmic chloroplast and mitrochondrial ribosomes of some plants. Biochim. Biophys. Acta 228:492-502.

188. VON DER DECKEN, A., P. ASHBY, D. McREAVY, and P.N. CAMPBELL. 1970. The reversible dissociation and activity of ribosomal subunits of liver and skeletal muscle from rats. Biochem. J. 120:815-819.

189. WEBER, H.J. 1972. Stoichiometric measurements of 30S and 50S ribosomal proteins from *Escherichia coli*. Molec. Gen. Genet. 119:233-248.

190. WELFLE, H. 1971. Studies on proteins of animal ribosomes. XI. A simple method of two-dimensional polyacrylamide gel electrophoresis of ribosomal proteins of rat liver. Acta Biol. Med. Germ. 27:547-551.

191. WELFLE, H., J. STAHL, and H. BIELKA. 1971. Studies on proteins of animal ribosomes. VIII. Two-dimensional polyacrylamide gel electrophoresis of ribosomal proteins of rat liver. Biochim. Biophs. Acta 243:416-419.

192. WELFLE, H., J. STAHL, and H. BIELKA. 1972. Studies on proteins of animal ribosomes. XIII. Enumeration of ribosomal proteins of rat liver. FEBS Lett. 26:228-232.

193. WELLBURN, A.R., P.H. QUAIL, and B.E.S. GUNNING. 1977. Examination of ribosome-like particles in isolated prolamellar bodies. Planta 134:45-52.

194. WHITTLER, E.D., D.E. BUSHNELL, and V.R. POTTER. 1968. RNA associated with the outer membrane of rat liver nuclei. Biochim. Biophys. Acta 161:41-50.

195. WITTMANN, H.G. and G. STÖFFLER. 1972. Structure and function of bacterial ribosomal proteins. In: The mechanism of protein synthesis and its regulation. L. Bosch, ed. North Holand Publ., Amsterdam, Netherlands. pp. 285-351.

196. WITTMANN, H.G., G. STÖFFLER, I. HINDENNACH, C.G. KURLAND, L. RANDALL-HAZELBAUR, E.A. BIRGE, M. NOMURA, E. KALTSCHMIDT, S. MIZUSHIMA, R. TRAUT, and T.A. BICKLE. 1971. Correlation of 30S ribosomal proteins of *Escherichia coli* isolated in different laboratories. Molec. Gen. Genet. 111:327-333.

197. WOLFE, F.H. and C.M. KAY. 1967. Physiological, chemical and biological studies on wheat embryo ribosomes. Biochemistry 6:2853-2860.

198. ZALIK, S. and B.L. JONES. 1973. Protein biosynthesis. Ann. Rev. Plant Physiol. 24:47-68.

APPENDIX

Phenylalanine Incorporation by Cytoplasmic Ribosomal Subunits

As shown in Table II, in the present study neither 40S nor 60S subunits individually could support active *in vitro* poly (U)-directed incorporation of phenylalanine. Both subunits were required for polyphenylalanine synthesis. The individual ribosomal subunits were added directly in various combinations to the incubation mixture, or various combinations of subunits were first reassociated and the recominants or hybrids were added to the incubation mixture. When the four possible combinations of the subunits were assayed, the activity of the previously reassociated subunits was slightly higher than that for the corresponding subunits added directly to the incubation mixture.

Recombination of the subunits (60S:40S) in a ratio of 1:1 and 2:1 (A_{260}) yielded monosomes that were equally active in the poly (U)-directed polyphenylalanine synthesis. The activity of the recombinants was found to be about 50% of the activity of the undissociated monosomes. To determine whether ribosomal subunits from normal and mutant ribosomes differed in activity, polyphenylalanine synthesis by hybrids containing normal and mutant subunits was studied. A comparison of the activities of different possible hybrids (N60S + M40S and N40S + M60S) gave almost the same incorporation of ^{14}C-phenylalanine as homologous combinations (N60S + N40S and M60S + M40S). Thus the subunits used in these studies contained intact RNA and a sufficient complement of proteins to reassociate into monosomes which could support active polyphenylalanine synthesis.

[1]Ribosomes were resuspended in the resuspension buffer containing 20% glycerol and isolated subunits were resuspended in resuspension buffer containing 20% glycerol and 40 mM KCl. All ribosomal preparations were stored in liquid nitrogen at a concentration of $10A_{260}$ units/ml.

[2]Components in the reaction mixture were at the following concentrations in a final volume of 0.5 ml: 40 mM tris-HCl, pH 8.4; 12 mM $MgCl_2$; 70 mM KCl; 9 mM DTT; 0.5 mM GTP; 1 mM ATP; 8 mM creatine-phosphate; 20 µg/ml creatine phosphokinase; 0.7 µci/ml, ^{14}C-phenylalanine (sp. act. 486 mci/mmol); 240 µg/ml poly (U); 60 µg/ml brewer's yeast tRNA; 0.3 A_{260} units/ml ribosomes; 600 µg/ml dialysed post-ribosomal supernatant protein. After incubation for 60 min at 30°C, 10 µl aliquots were collected on Whatmann 3 MM filter paper discs (21 mm dia) and hot trichloroacetic acid-insoluble radioactivity was measured.

[3]Separate subunits added directly to the incubation mixture.

[4]Previously reassociated subunits added to the incubation mixture.

TABLE II. Phenylalanine incorporation by cytoplasmic ribosomes and combinations of the ribosomal subunits from embryos of Gateway barley and its mutant

Ribosomes or ribosomal subunits added[1]	Ratio	^{14}C-Phenylalanine incorporated (cpm)/10λ incubation mixture[2]
N60S		64
N40S		166
M60S		85
M40S		149
N60S+N40S	(1:1)[3]	799
N60S+M40S	(1:1)	786
M60S+N40S	(1:1)	791
M60S+M40S	(1:1)	812
N60S+N40S	(2:1)[3]	728
N60S+M40S	(2:1)	704
M60S+N40S	(2:1)	722
M60S+M40S	(2:1)	718
[N60S+N40S]	(1:1)[4]	932
[N60S+M40S]	(1:1)	934
[M60S+N40S]	(1:1)	916
[M60S+M40S]	(1:1)	931
N80S		1734
M80S		1762

Data in the table represent the average of at least two independent experiments with three sample replicates.