1991

The characterization of nuclear mutants which are affected in chloroplast psbD gene expression at the post-transcriptional level in Chlamydomonas

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THE CHARACTERIZATION OF NUCLEAR MUTANTS WHICH ARE AFFECTED IN CHLOROPLAST psbD GENE EXPRESSION AT THE POST-TRANSCRIPTIONAL LEVEL IN *CHLAMYDOMONAS*

by

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A Thesis

Presented to the Graduate Committee

of Lehigh University

in Candidacy for the Degree of

Master of Science

in Molecular Biology

Lehigh University
September, 1991

Approved and recommended for acceptance as a thesis in partial fulfillment of the requirements for the degree of Master of Science.

9/20/91
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ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my advisor Dr. Michael Kuchka for the guidance and support he has given me while I have conducted research in his laboratory. I greatly appreciate his genuine interest in both my research project and in the advancement of my career at Lehigh University. I know I have learned a great deal from his scientific expertise and professional integrity.

I am eternally grateful to my parents Oliver and Shou-be Wu for their unrelenting encouragement, understanding, and love throughout my life. My success as a graduate student would not have been possible without the many opportunities they have unconditionally provided for me.

I would also like to thank Dr. Vassie Ware and Dr. Jeff Sands for their involvement in my graduate studies as my thesis committee members. I appreciate the valuable time, patience, and support they have offered me. The countless contributions of my colleagues and friends Arati Khanna-Gupta, Maureen Donahue, and Kent Fung are also acknowledged with my utmost appreciation.

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

DNA	deoxyribonucleic acid
PSII	photosystem II
NADP	nicotinamide adenine dinucleotide phosphate
ATP	adenosine triphosphate
mRNA	messenger ribonucleic acid
RUBISCO	ribulose-1,5-bisphosphate carboxylase/oxygenase
RNA	ribonucleic acid
DEPC	diethyl pyrocarbonate
SSC	sodium citrate, sodium chloride
SDS	sodium dodecyl sulfate
PCR	polymerase chain reaction
A_{260}	absorbance 260 nanometers
EDTA	ethylenediaminetetraacetic acid
PD	parental ditype
NPD	nonparental ditype
T	tetratype
bp	base pairs

ABSTRACT

In order to investigate the role of the nucleus during chloroplast biogenesis, nuclear mutants which are affected in the expression of the chloroplast gene psbD in Chlamydomonas reinhardtii have been characterized and compared. The four nuclear mutants of this study, nac1-11, nac1-18, ac-115, and 6.2u are affected at the translational and/or post-translational level of psbD gene expression. Northern analysis has indicated that the psbD message is present in all mutant strains. Protein pulse-labeling experiments have demonstrated that the D2 protein which is encoded by the psbD gene is absent, suggesting the protein is either not actively synthesized or is rapidly degraded in the mutant strains of interest. The NAC1 gene, which is mutated in both nac1-11 and nac1-18, has been mapped to chromosome IX of the C. reinhardtii genome. Additionally, it has been determined that the NAC1 and AC-115 loci are unlinked. Therefore, at least two distinct nuclear factors participate in the expression of psbD at the post-transcriptional level. Dot Blot analysis was conducted on ribosomal and thylakoid membrane extracts of wild-type, nac1-11, ac-115, and 6.2u in order to distinguish the precise post-transcriptional step(s) which are affected in these mutant strains. While the psbD transcript was associated with polysomes and to a lesser extent with monosomes in wild-type and nac1-11, the message was found to be almost exclusively associated with polysomes in 6.2u. This

may indicate a block in a late step of translation in the 6.2u strain. In the ac-115 strain, the psbD transcript was preferentially associated with monosomes. Dot blot analysis of thylakoid membranes, the site of D2 protein synthesis, indicated that psbD message association with thylakoids was reduced in ac-115 as compared to wild-type, nac1-11, and 6.2u. This result, taken collectively with psbD transcript association primarily with monosomes in ac-115, suggests D2 expression is affected at an early step of translation in the ac-115 strain. Thus, it appears that the nuclear encoded products which are affected in nac1-11, ac-115, and 6.2u may be involved at distinct steps of D2 translation and/or protein degradation.

INTRODUCTION

A fundamental difference between prokaryotic and eukaryotic organisms is the presence of distinct organelles within eukaryotic cells. Organelles are specialized compartments inside the cell which perform biological functions vital for the organism. The energy transducing organelles include the mitochondria, the site of oxidative metabolism, and the chloroplast, which is responsible for photosynthesis. While all eukaryotes contain mitochondria, only green algae and higher plants possess chloroplasts. Both mitochondria and chloroplasts are semi-autonomous organelles. They contain their own DNA which is distinct from the nuclear genome and they are able to perform basic biological processes including transcription and translation. Yet, they depend on the cell nucleus for structural proteins and other factors which are essential for organellar gene expresssion. The coordinated interaction which exists between the nucleus and each specialized compartment is not well understood. In this study, nuclear mutants affected in the synthesis of a chloroplast encoded protein are characterized to help define the role of the nucleus in chloroplast gene expression.

Photosynthetic mutants of Chlamydomonas reinhardtii as a model system

The unicellular green alga Chlamydomonas reinhardtii is

readily utilized to study nuclear control of chloroplast development primarily due to its excellent genetics, the relative ease by which relevant mutants can be screened, and the characterization of polypeptides and genes of interest. Like higher plants, C. reinhardtii is capable of photosynthesis, the process by which radiant energy provided by the sun is converted into biochemical energy in the form of reduced NADP and ATP which is eventually used to synthesize carbohydrate. Without plants, life on earth would not exist as it does today. The light reactions of photosynthesis require the collaboration of many multisubunit protein-pigment complexes which together form the photosynthetic apparatus. These highly structured complexes are embedded within chloroplast thylakoid membranes and are composed of proteins which are encoded by chloroplast and nuclear genes (Homann, 1988). Photosystem II (PSII) is one such complex essential for photosynthetic electron transport. PSII consists of a reaction center, a water-splitting apparatus, and a light-harvesting antenna complex (Chua and Bennoun, 1975). PSII generates a charge polarization across the thylakoid membrane which sustains the energy needed to make ATP. PSII is also responsible for the evolution of molecular oxygen due to its water:plastoquinone oxidoreductase activity. The core of PSII contains eight major proteins which are chloroplast encoded. The three peripheral oxygen-evolution enhancer proteins as well as the polypeptides of the light-harvesting complex are

nuclear encoded and must be imported into the plastid during chloroplast biogenesis (Rochaix and Erickson, 1988). Additionally, many PSII nuclear mutants have been isolated which do not contain primary lesions in PSII structural genes, rather they are affected in nuclear encoded products involved in the expression of specific chloroplast encoded PSII components (Jensen et al., 1986; Kuchka et al., 1988; Rochaix et al., 1989, Gamble and Mullet, 1989). Therefore, the synthesis and assembly of PSII requires an elegant coordination between nuclear and chloroplast gene expression.

The electron-transfer reactions orchestrated by PSII occur within the reaction center of the complex. The major structural components of the reaction center are the polypeptides D1, D2, P5, P6, and two smaller proteins which bind cytochrome b_{559} as shown in figure 1 (Reviewed in Rochaix, 1987). D1 and D2 are encoded by chloroplast genes psbA and psbD respectively (Erickson et al., 1984; Erickson et al., 1985). They are transmembrane proteins which are situated at the heart of the PSII reaction center (Rochaix, 1987). Together they ensure the proper structure of the photosystem by aligning the PSII electron carriers in an extremely precise array (Reviewed in Mattoo et al., 1989). It has been previously demonstrated that the absence of a single PSII core protein results in the increased turnover of associated protein subunits. As a result, functional PSII complexes are not present in mutants which fail to synthesize one individual

core protein and ultimately the process of photosynthesis is inhibited (de Vitry et al., 1989). Thus, the exact stoichiometric amounts of each core protein are required to form stable PSII complexes.

The study of nuclear mutants deficient in PSII activity has implicated a number of nuclear genes which participate at many levels of chloroplast gene expression including transcription, translation, and protein turnover. Recent evidence indicates that for each chloroplast gene which has been extensively studied, at least one nuclear encoded factor exists which is required for the expression of this gene (Rochaix et al., 1989). Limitations in chloroplast genome size may mandate this complex interplay which prevails between the nucleus and the chloroplast. Nuclear mutants which exhibit defective PSII activity due to a block at the post-transcriptional level of D2 production were characterized in this study to address nuclear participation in chloroplast gene expression.

Four nuclear mutants have been identified as strains which are affected in D2 synthesis at the translational and/or post-translational level in C. reinhardtii. The collection of mutants include nac1-11 (Kuchka et al., 1988), nac1-18 (Kuchka et al., 1988), ac-115, (Levine and Goodenough, 1970; M. Kuchka, personal communication) and 6.2u (L. Sieburth, personal communication). These PSII deficient strains are easily maintained within the laboratory due their ability to proliferate in media which contains acetate as a carbon

source. Of these mutant strains, nac1-11 and nac1-18 are allelic (Kuchka et al., 1988). Alternatively, complementation analysis has implied that the mutation of strain ac-115 lies outside the NAC1 gene (Kuchka et al., 1988). To elucidate the genetic linkage between these mutants, intercrosses were performed estimating the minimum number of nuclear factors which are involved in D2 synthesis at the post-transcriptional level. Moreover, tetrad analysis of meiotic products was used to map these nuclear genes to specific C. reinhardtii chromosomes.

The mechanism by which nuclear factors act to promote D2 synthesis is also an area of interest in our laboratory. As mentioned previously; nac1-11, nac1-18, ac-115, and 6.2u are all mutants which are affected in the translation and/or turnover of D2. The chloroplast psbD message which encodes D2 accumulates in the mutant strains nac1-11, nac1-18, and ac-115 based on Northern analysis (Kuchka et al., 1988; M. Kuchka, personal communication). Additionally, the psbD transcript isolated from the mutant strains is equivalent in size to the wild-type message. These results imply that psbD transcription and message stability are not affected in these strains. In the present study, Northern analysis was performed on mutant strains nac1-11, nac1-18, ac-115, and 6.2u to confirm the presence of the psbD message and to compare relative psbD mRNA levels. Immunoblot analysis of nac1-11 and nac1-18 mutants indicated a pleiotropic decrease of other PSII

polypeptides which are associated with D2 in the thylakoid membrane (Kuchka et al., 1988). However, protein pulse-labeling experiments show that D2 is the only chloroplast encoded PSII protein which is either not actively translated or is rapidly degraded in the mutant strains of interest (Kuchka et al., 1988). The absence of D2 is thought to prohibit proper PSII formation and results in the increased turnover of unassembled polypeptides P5, P6, and D1. Although these experiments are helpful in localizing the general step at which D2 production is blocked, they are unable to pinpoint the exact translational and/ or post-translational event which is impeded. Experiments conducted in this study identify more specifically the mechanism involving certain nuclear factors in D2 synthesis.

The D2 protein of PSII is synthesized on ribosomes which exist within the interior of the chloroplast (Herrin and Michaels, 1985). The ribosomes of chloroplasts are thought to resemble and behave much like their prokaryotic counterparts based on extensive sequence homology between chloroplastic ribosomal components and constituents of the bacterial ribosome. Moreover, antibiotic sensitivity of the chloroplast ribosome is identical to that of the prokaryotic ribosome (Reviewed in Bonham-Smith and Bourque, 1988). Yet, the mechanisms of transcript association with ribosomes, translation initiation and elongation are not well understood in the chloroplast. It is believed that translation of the D2

protein occurs on polysomes which are physically attached to the membrane via the hydrophobic amino-terminus of the nascent chain (Herrin et al., 1981). The association of chloroplast ribosomes to the thylakoid membrane is thought to be analogous to polysomes bound to the exterior of the endoplasmic reticulum membrane in eukaryotic cells. The nuclear encoded products which are mutated in nac1-11, nac1-18, ac-115, and 6.2u may be involved in various steps of D2 synthesis. These include but are not limited to psbD transcript association with ribosomes, translation initiation, translocation of the nascent D2 polypeptide across the thylakoid membrane, translation elongation or termination, and post-translational protein degradation. Examples of gene regulation at many of these steps is documented in eukaryotes and prokaryotes indicating post-transcriptional control of gene expression is a common level of regulation in a wide variety of species (Lindahl and Zengal, 1986; Rouault et al., 1988; McCarthy and Gualerzi, 1990; Mathews, 1986).

Translational and post-translational control of chloroplast gene expression

Translational and post-translational regulation of chloroplast gene expression appears to be an important level of control in alga and higher plants. Many chloroplast encoded proteins undergo rapid and dramatic changes in synthesis irrespective of their mRNA levels in the organelle.

Therefore, plants must be able to regulate the post-transcriptional expression of plastid encoded proteins in a temporal as well as spatial manner based on environmental cues and intrinsic signals which relate to the needs of the organism. Obviously, sunlight is a significant extrinsic stimulus which participates in plant development by affecting both nuclear and chloroplast gene expression. There are many examples of light dependent post-transcriptional control of chloroplast gene expression. In the case of higher plants including mustard (Link, 1984), pea (Sasaki et al., 1987) maize (Rodermel and Bogorad, 1985) and barley (Klein and Mullet, 1987), chloroplast maturation is dependent on the presence of light. Dark grown barley seedlings contain plastids which lack photosynthetic activity due to the absence of chlorophyll and chlorophyll-binding proteins which are components of photosystem I (Klein et al., 1988) and II (Eichacker et al., 1990). In the dark, transcripts encoding chlorophyll-binding proteins are present but their corresponding proteins do not accumulate, suggesting post-transcriptional control of the expression of these products. Mullet and coworkers showed that illumination of etiolated (immature) seedlings induced a recruitment of psbA transcripts which encode the D1 protein onto chloroplast ribosomes (Klein et al., 1988). The amount of a specific mRNA associated with translationally active polysomes greatly influences the translational efficiency of a given transcript and may serve

as a control site for gene expression. In dark grown plants of Amaranthus hypochondriacus, ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) subunit messages rbcL and rbcS are associated with inactive monosomes and/or ribosomal subunits (Berry et al., 1990). These data imply that translation initiation of rbcL and rbcS transcripts are inhibited in the dark. Therefore, translation initiation appears to be a light-regulated level of chloroplast gene expression.

Translation elongation is a common stage of gene expression which is also mediated by light. When light grown amaranth plants are transferred to total darkness, both rbcS and rbcL mRNA remained bound to polysomes but the messages were not translated in vivo (Berry et al., 1988). It is apparent that under these conditions, RUBISCO gene expression is exercised at the translation elongation step. Similar studies in Lemna gibba also indicated that light dependent regulation exists at the level of translation elongation (Slovin and Tobin, 1982). The light-regulated translation of several chloroplast transcripts has also been observed in many other plant species including Volvox (Kirk and Kirk, 1985), pea (Inamine et al., 1985), and barley (Klein and Mullet, 1986). In barley, the synthesis of two photosystem I chlorophyll-a-apoproteins is arrested on membrane bound polysomes at the level of translation elongation in dark grown plants (Klein et al., 1988). It is believed that these chlorophyll binding proteins

are unable to insert co-translationally into the thylakoid membrane in the absence of chlorophyll. In studies in which PSII protein accumulation was examined in dark grown barley, Mullet showed that chlorophyll stabilizes proteins D1 and P5 during translation elongation (Mullet et al., 1990). The results of these experiments implicate two distinct post-transcriptional levels of plastid gene expression which are modulated by light. It is conceivable that light induced nuclear encoded factors may be involved in the regulation of certain proteins at these steps. A nuclear mutant of barley (virdis-115) which lacks PSII activity has been characterized as a mutation which affects the translation and stability of two PSII proteins D1 and P5 (Gamble and Mullet, 1989). Although the precise step of translation blockage in this mutant has not been established, transcript recruitment and premature translation termination may be possible candidates. It is apparent that translational regulation cannot be ignored as a primary control site for the expression of several chloroplast proteins.

Light induced translational stimulation of chloroplast encoded proteins has also been observed in Chlamydomonas reinhardtii. In contrast to higher plants, chloroplast maturation in C. reinhardtii is not dependent on light. Studies performed on the y-1 mutant of C. reinhardtii which is dependent on light for plastid development, demonstrated light induced synthesis of both D1 and D2 at the level of

translation (Malnoe et al., 1988). While psbA and psbD mRNA transcription was unaffected by illumination, the rate of D1 and D2 synthesis increased dramatically in dark grown plants which were subjected to light, suggesting gene expression of psbA and psbD encoded proteins is primarily controlled at the level of translation. These studies demonstrated that translation of D1 and D2 is stimulated by light and that the stimulatory effect depends on the developmental stage of the chloroplast (Malnoe et al., 1988). Since the synthesis of chlorophyll is not light dependent in C. reinhardtii, nuclear encoded protein products may act in a manner similar to chlorophyll in barley and serve as stabilizing factors of PSII proteins.

Several nuclear mutants of C. reinhardtii have been isolated which are affected in the translational and/or post-translational expression of PSII proteins. Nuclear mutants F34 and F64 are unable to synthesize and stably accumulate the P6 protein, even though the psbC message which codes for P6 is stable (Rochaix et al., 1989). A chloroplast suppressor of the F34 strain has been isolated which maps to the middle of the 5'- untranslated region of psbC. This region is capable of forming a stem-loop structure which might serve as a target site for the binding of a nuclear factor involved in P6 translation. Two other nuclear mutants have been isolated which affect the translation of P5 and D1 (Jensen et al., 1986; M. Kuchka, personal communication). In one mutant D1

mRNA translation was completely arrested while P5 translation was partially arrested. In the other mutant, the opposite affect was evident. P5 translation was completely blocked while the translation of the D1 protein was markedly decreased. Jensen and coworkers hypothesized that the synthesis of these two proteins are cooperatively regulated post-translationally via protein interactions within the thylakoid membrane (Jensen et al., 1986). It is evident that translational and post-translational control of chloroplast protein synthesis is an important level of regulation in Chlamydomonas and that nuclear encoded products play a crucial role in these processes.

Nuclear gene products involved in mitochondrial gene expression

In the yeast Saccharomyces cerevisiae, many nuclear gene encoded products are found to affect mitochondrial gene expression at the level of protein translation. In addition to nuclear genes which code for general proteins involved in the translation of mitochondrial proteins, many mitochondrial mRNAs require message-specific activators which are nuclear encoded (Reviewed in Fox, 1986). Well studied examples include the expression of subunit III of cytochrome c oxidase (coxIII) and cytochrome b apoprotein (cob).

The mitochondrial oxi2 gene codes for coxIII. The synthesis of the coxIII polypeptide appears to rely on at

least three distinct nuclear encoded proteins which promote the translation of coxIII. Three recessive mutants affected in nuclear genes PET494 (Costanzo and Fox, 1986), PET54 (Costanzo et al., 1989), and PET122 (Haffter et al., 1990) are deficient in the coxIII protein, although the oxi2 mRNA is present. Genetic studies conducted in the laboratory of Thomas Fox indicate both pet494 and pet54 nuclear mutants are suppressed by mitochondrial DNA rearrangements that replace the normal 5'-untranslated leader sequence of the oxi2 message with other mitochondrial gene 5'- leader sequences. These data imply that both PET54 and PET494 encode products which may bind to the 5' end of the oxi2 mRNA and initiate translation. The third nuclear gene which encodes a product involved in coxIII synthesis is PET122. Nuclear mutants in the PET122 gene are suppressed by mutations in two unlinked nuclear genes PET123 and MRP1 (Haffter et al., 1990). Both PET123 and MRP1 code for components of the small subunit of mitochondrial ribosomes. These results suggest that PET122 promotes the translation of the oxi2 mRNA by facilitating transcript association with the small subunit of mitochondrial ribosomes.

The expression of cytochrome b apoprotein (cob), a mitochondrial gene encoded protein, appears to be dependent on at least two nuclear genes, CBP6 (Dieckmann and Tzagoloff, 1985) and MK2 (Roedel et al., 1985). Mutations in these genes result in no accumulation of cob protein although cob

transcripts are present (Fox, 1986). Restriction mapping of CBP6 and MK2 cloned genes demonstrate these genes are unlinked (Fox, 1986). MK2 mutants are suppressible by mitochondrial gene rearrangements at the 5'- untranslated leader sequence of cob (Roedel et al., 1985). This data in conjunction with similar results noted in coxIII synthesis suggest the expression of certain mitochondrial encoded proteins may be primarily controlled at the level of translation initiation and that the 5'-untranslated leader sequences may be recognized by certain nuclear factors which promote the translation of specific mRNA species.

Translational modulation of bacteriophage gene expression

Gene regulation at the level of translation is not merely confined to organellar encoded proteins. Rather it appears to be an important step of control for a multitude of organisms ranging from phage infected bacteria (Wulczyn and Kahmann, 1991) to the South African toad Xenopus laevis (Fu et al., 1991). The role of mRNA secondary structure on translation initiation has been well documented in prokaryotic organisms (reviewed in Stormo, 1986). Recently, experiments conducted on bacteriophage Mu infected Escherichia coli have demonstrated the specific translational stimulation of the phage Mom protein (Wulczyn and Kahmann, 1991). mom gene expression is highly regulated, its synthesis is modulated at both transcriptional and translational levels. Efficient

translation of Mom requires the presence of another Mu encoded protein denoted Com. The Com protein serves as a translational activator of mom mRNA. Using a gel retardation assay and RNase T1 and dimethylsulfate footprinting, Wulczyn and Kuhmann (1991) were able to identify Com as an RNA-binding protein which associates with the 5'-untranslated leader sequence of mom mRNA in a sequence specific manner to promote mom translation. Furthermore, Wulczyn and Kuhmann (1991) propose Com binds to a translation activating sequence which in the absence of bound Com is capable of forming an inhibitor stem-loop structure which "masks" the ribosome binding site (Shine-Dalgarno sequence) and the translation initiation codon. The binding of the Com protein to this region is thought to destabilize the stem-loop structure and release the Shine-Dalgarno sequence allowing the mom transcript to associate with the 30S subunit of the ribosome and undergo translation. Nuclear encoded products of C. reinhardtii analyzed in this study may also serve as RNA binding proteins which activate the translation of specific chloroplast encoded proteins by a similar mechanism.

Characterization of nuclear mutants affected in the expression of the D2 protein at the translational and/or post-translational level

The major goals of this study are as follows: 1) To gain a better understanding of the role of the nucleus in

chloroplast gene expression, 2) To strengthen our knowledge of post-transcriptional control of gene expression. There is precedence for translational and /or post-translational control of gene expression in many diverse species. In addition, many nuclear mutants have been isolated which affect the expression of organellar encoded proteins. The use of nuclear mutants in Chlamydomonas reinhardtii which affect the post-transcriptional expression of a specific chloroplast encoded protein serves as an excellent model system to attain both of these goals. A genetic, biochemical, and molecular approach has been pursued in order to characterize four nuclear mutants nac1-11, nac1-18, ac-115, and 6.2u which are unable to synthesize the D2 protein of PSII due to a post-transcriptional block in expression. Experiments have been conducted to compare these four nuclear mutants with respect to psbD mRNA levels and the synthesis of the D2 protein. Through genetic studies, a minimum number of nuclear gene encoded factors involved in D2 translational and/ or post-translational events has been established. Additionally, the NAC1 locus has been mapped to a C. reinhardtii chromosome. Finally, experiments have been performed in order to determine whether the psbD transcript is associated with ribosomes in mutant strains, confronting the question of whether psbD mRNA recruitment to the protein synthesis machinery is affected. Moreover, psbD transcript association with specific subpopulations of ribosomes (monosomes versus polysomes) has

been addressed. psbD message association with monosome populations alone may indicate a block in translation initiation. Thylakoid membranes of mutant strains were also isolated to elucidate whether the psbD message is associated with thylakoid membrane bound ribosomes. Since the D2 protein is inserted co-translationally into the thylakoid membrane, the failure of the psbD transcript to associate with thylakoid membranes may suggest the inability of the nascent D2 polypeptide to translocate across the thylakoid membrane. The results generated in these studies further identify the precise stage(s) at which specific nuclear encoded products act to control the expression of the D2 protein in Chlamydomonas reinhardtii.

MATERIALS AND METHODS

1) Cell maintenance, culture conditions, and mutants

Wild-type (WTCC125) and PSII deficient mutant strains nac1-11, nac1-18, ac-115 and 6.2u of C. reinhardtii were maintained on minimal plates containing Sager and Granick media (Harris, 1989) supplemented with sodium acetate (22mM final concentration) at 23°C under dim light. Multiply-marked C. reinhardtii strain CC-1735 was kindly provided by Dr. E. Harris (Chlamydomonas Genetics Center, Duke University) and maintained on minimal media at 23°C under continuous fluorescent light. Asynchronous liquid cultures of wild-type and PSII mutants were grown under continuous illumination at room temperature in the medium of Sager and Granick to a cell density of approximately 1 x 10^6 cells/mL for RNA extraction, protein pulse-labeling, ribosome purification, and thylakoid membrane isolation experiments.

2) Genetic analysis

PSII deficient mutants nac1-11 or nac1-18 and ac-115, were intercrossed in a pairwise fashion to determine genetic linkage. A double mutant with lesions in the NAC1 and AC-115 genes was generated from nac1-18 x ac-115 intercrosses. To map genetically the NAC1 gene, nac1-11 and nac1-18 strains were crossed with C. reinhardtii strain CC-1735 which contains genetic markers on chromosomes I (methionine sulfoximine resistance), III (nitrate sensitivity), IV (pyrithiamine resistance), VIII (neamine resistance), IX (streptomycin

resistance), and XII - XIII (paralyzed flagella) (E. Harris, personal communication). Standard genetic protocols of C. reinhardtii were followed for matings, tetrad dissection, and meiotic product analysis (Levine and Ebersold, 1960). Scoring of meiotic products was performed on minimal plates and on minimal plates supplemented with one of the following compounds; sodium acetate, methionine sulfoximine, pyrithiamine, neamine, or streptomycin sulfate to final concentrations specified by Harris, (1989). Paralyzed flagella were scored visually through a phase-contrast microscope under 400X magnification power.

3) RNA extraction, electrophoresis, and Northern analysis

Total RNA was phenol:chloroform:isoamyl alcohol (25:25:1) extracted from exponentially grown C. reinhardtii cells from WTCC125, nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant strains using a "miniprep" procedure. The RNA was recovered by ethanol precipitation, washed with 70% ethanol and allowed to air dry. Dried pellets were resuspended in 25ul of 0.01% DEPC treated distilled water (dH_2O) and stored at -20°C.

Five micrograms of each RNA sample was electrophoretically separated on a denaturing 6% formaldehyde, 1.2% agarose gel (Lehrach et al., 1977) and transferred onto nitrocellulose filter paper by capillary blot procedure. The filter was baked at 80°C for 2 hours under vacuum. The filter was then incubated in a prehybridization solution consisting of 50%

formamide, 5X SSC, 0.5% non-fat milk, and 0.1% SDS for 1-2 hours at 42°C (Kuchka et al., 1988). After prehybridization, a nick-translated radioactively-labeled psbD gene-specific probe derived from the R3 plasmid (Erickson et al., 1984) was added to the prehybridization solution and the filter was allowed to incubate in this mixture overnight at 42°C. The filter was washed two times at room temperature with 2X SSC, 0.1% SDS for 15 minutes each, followed by two additional room temperature washes with 1X SSC, 0.1% SDS for 15 minutes each. The washed filter was then exposed to autoradiography.

4) Pulse-labeling of proteins with [^{14}C]acetate

Ten milliliters of exponentially grown C. reinhardtii cells from WTCC125, nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant were treated with cycloheximide (10ug/mL final concentration), an inhibitor of cytoplasmic protein synthesis, and labeled with 25 uCi of [^{14}C] acetate for 10 minutes as described in Mayfield et al. (1987). Protein samples were prepared according to Mayfield et al. (1987) and electrophorectically separated on 7.5% to 15% SDS-polyacrylamide gradient gels (Chua, 1980). Gels were Coomassie stained, destained, treated with Enlighting solution (Dupont NEN) , dried, and exposed to autoradiography.

5) Amplification of psbD probe by polymerase chain reaction

To ensure psbD message specificity for dot blot hybridizations, a 1041 base pair internal fragment of the psbD coding region was amplified using the polymerase chain

reaction (PCR) (Mullis et al., 1986). Oligonucleotide primers which flank the psbD gene were produced by a DNA synthesizer (Biosearch) using a phosphoramidite synthesis program. The primers 5'-TCCGGGACATATCAAGAGAAACG-3' (amino acid positions 5 to 13, Erickson et al., 1984) and 5'-GCGTTACCACGTGGTAATACTTC-3' (amino acid positions 343 to 353, Erickson et al., 1984) were purified by chromatography through a Sephadex G-10 column (Sambrook et al., 1989) and used in the PCR reaction mixture. DNA amplification was conducted in a DNA thermal cycler (Perkin Elmer Cetus) using 1ug of SmaI digested pH3 plasmid which contains the psbD gene (Erickson et al., 1984) as template DNA. Remaining components of the reaction mixture were added as specified by the manufacturer (Perkin Elmer Cetus). One complete cycle of the PCR program utilized included 1 minute at 94°C to denature the double stranded DNA, 1 minute at 46°C to allow the DNA primers to anneal with the template DNA, and 2 minutes at 72°C for DNA extension by Taq polymerase. Fifty cycles were programmed to amplify the psbD fragment.

Amplified DNA was purified by Clean-a-gene protocol (Andes Scientific) and was used as a template for the synthesis of a psbD gene-specific probe by nick translation.

6) Polysome isolation, fractionation and dot blot analysis

Crude ribosome isolation from WTCC125, nac1-11, ac-115, and 6.2u strains was performed according to Mishkind and Schmidt, (1983) with slight modifications. A 2.5 L culture of log

phase cells was treated for 15 minutes with chloramphenicol, an inhibitor of chloroplast protein synthesis, to a final concentration of 100ug/ml. The cells were harvested by centrifugation and resuspended in 25mM Tris-acetate (pH 8.4), 2.5mM Magnesium acetate, 50mM Potassium acetate, 1mM Dithiotheritol (DTT), and 1mg/mL Heparin as a ribonuclease inhibitor. The cells were broken by rapid decompression in a Yeda pressure cell at 1600-2000 psi for two 5 minute periods. Cell debris was removed by centrifugation at 36,000g (SW41 rotor, 17000 rpm) for 15 minutes at 4°C. Ribosomes were pelleted by centrifugation through a 2M sucrose cushion prepared in extraction buffer at 70,000g (SW27 rotor, 23000 rpm) for 4 hours at 4°C. The resulting pellet was resuspended in 200 ul of dH_2O and either treated with 10 units of RQ1 DNase and 20 units of RNasin for 30 minutes or subjected to fractionation on a sucrose gradient.

Separation of crude polysome extracts into ribosome subpopulations (polysomes and monosomes) was conducted by applying approximately 50 A_{260} units of the crude ribosome extract on linear 11.5 mL sucrose gradients (10-30% weight/volume) formed above a 0.5 mL cushion of 60% (weight/volume) sucrose. The sucrose solutions were prepared in acetate buffer as described above. Following centrifugation of the linear gradients at 90,000g (SW41, 27000 rpm) for 1.5 hours at 4°C, 0.5 mL gradient fractions were collected. The fractions were measured manually by

spectrophotometer (Beckman DU-64) to determine their A_{260} reading and to generate a polysome profile. The RNA from each fraction was extracted by phenol:chloroform:isoamyl alcohol as previously described. Equal amounts of extracted RNA from DNase treated crude ribosomal extracts and ribosome subpopulation fractions was applied onto nitrocellulose filter paper according to the dot blot procedure of Sambrook et al. (1989), using a dot blot manifold (Bio-rad). The filter was baked, prehybridized, hybridized with the PCR generated psbD probe, washed, and exposed to autoradiography as previously described.

7) Thylakoid membrane isolation and dot blot analysis

Rough thylakoid membrane isolation procedure was based on the protocol of Herrin and Michaels, (1985). Exponentially grown C. reinhardtii cells from WTCC125, nac1-11, ac-115, and 6.2u strains were harvested by centrifugation and washed with 15 mLs of Buffer I (0.3M Sucrose, 25mM Hepes-HCl, pH 7.5, 1mM $MgCl_2$). Washed cells were again pelleted and resuspended in 15 mL of Buffer I. The resuspended cells were broken in a Yeda pressure cell as previously described and subjected to centrifugation at 2,000g (Beckman GPR centrifuge, 3750 rpm) for 10 minutes at 4°C. The pellet was recovered and resuspended in 25mL of Buffer II (0.3M Sucrose, 5mM Hepes-HCl, pH 7.5, 10 mM EDTA, pH 7.5) and centrifuged at 50,000g (SW27, 20000 rpm) for 10 minutes at 4°C to remove soluble cell debris. This step was repeated once. The resulting pellet

containing cell membranes was resuspended in Buffer III (1.8M Sucrose, 5mM Hepes-HCl, pH 7.5, 10mM EDTA, pH 7.5) to a final volume of 5 mL. The 5 mL sample was overlaid with 2 mL of Buffer IV (1.3M Sucrose, 5mM Hepes-HCl, pH 7.5, 10mM EDTA, pH 7.5) and 5 mL of Buffer V (0.5M Sucrose, 5mM Hepes-HCl, pH 7.5) forming a discontinuous sucrose gradient in a ultracentrifuge tube. The tube was centrifuged at 150,000g (SW41, 35000 rpm) for 1 hour at 4°C. Following centrifugation, the 1.3M Sucrose fraction containing thylakoid membranes was collected and diluted with 3 volumes of Buffer VI (5mM Hepes-HCl, 10mM EDTA, pH 7.5). The diluted sample was centrifuged at 50,000g (SW27, 20000 rpm) for 10 minutes at 4°C and the resulting pellet was resuspended in 0.4 mL of Buffer V. Isolated rough thylakoid membranes were RQ1 DNase and RNasin treated and associated RNA was phenol:chloroform:isoamyl alcohol extracted as previously described. Equal amounts of extracted RNA from thylakoid membranes was applied onto nitrocellulose filter paper by dot blot procedure as described earlier. The filter was baked, prehybridized, hybridized with PCR amplified psbD gene-specific probe, washed, and exposed to autoradiography as previously described.

RESULTS

Genetic analysis of PSII deficient strains

To elucidate whether nuclear mutants are affected in the same or distinct gene(s), pairwise intercrosses between PSII deficient mutants were conducted and their meiotic segregants were subjected to tetrad analysis. The ac-115, mating type + (mt+) strain mated at high efficiency with the nac1-18, mating type - (mt-) strain resulting in excellent zygote germination and viability of tetrad products. Complete tetrad segregants were scored for their acetate-requiring phenotype. Intercrosses between acetate-requiring nuclear mutants generate tetrad products which fall into three categories: 1) parental ditype (PD) tetrads exhibit 4:0 acetate requiring to wild-type phenotype, 2) nonparental (NPD) tetrads have a ratio of 2:2 and, 3) tetratypes (T) display a 3:1 ratio. Typically, closely linked loci generate a tetrad distribution of NPD = 0, and PD > T (Harris, 1989). Alternatively, unlinked loci characteristically produce a tetrad distribution of PD = NPD, and T > PD (Harris, 1989). From 32 complete tetrad segregants of the nac1-18, mt- x ac-115, mt+ intercross, the tetrad ratio (PD:NPD:T) was 8:7:17 which strongly suggests the NAC1 and AC-115 genes are unlinked. These data correspond well with complementation studies conducted between these two strains by Kuchka et al., (1988), which suggested that these strains were mutated in distinct complementation groups. Therefore, at least two distinct nuclear encoded factors, the NAC1 and AC-

115 gene products, are involved in the expression of the chloroplast gene psbD at the translational and/or post-translational level. Crosses attempted with the 6.2u mutant strain were unsuccessful. As a result, possible genetic linkage between 6.2u and nac1-11, nac1-18 or ac-115 could not be determined.

In order to map the NAC1 gene to a C. reinhardtii chromosome, the nac1-18, mt- strain was mated with the multiply-marked strain CC-1735, mt+ which contains genetic markers on chromosomes I, III, IV, VIII, IX, XII, and XIII. The CC-1735, mt+ x nac1-18, mt- tetrad segregants were scored for acetate requirement, methionine sulfoximine resistance, nitrate sensitivity, pyrithiamine resistance, neamine resistance, streptomycin resistance, and paralyzed flagella. When scored for acetate requirement and streptomycin resistance, a tetrad ratio of 21:0:7 resulted indicating tight genetic linkage between the streptomycin marker (sr-1) on chromosome IX and the NAC1 gene (Figure 2). Map distance between linked loci was determined using the equation:

$$\text{Map distance} = [0.5(T + 6NPD) / (PD + NPD + T)] \times 100.$$

The calculated map distance between sr-1 and the NAC1 gene is 12.5 map units. The CC-1735 strain also contains a paralyzed flagella marker which is located near the centromere of chromosome XII. This marker was used to discern whether or not the NAC1 gene is centromere linked. Two unlinked loci which are both centromere linked generate tetrad distributions

of T < PD or NPD, and PD = NPD (Harris, 1989). Tetrad analysis of CC-1735, mt+ x nac1-18, mt- produced a ratio of 4:2:7 when scored for acetate requirement and paralyzed flagella. This result suggests the NAC1 gene is not centromere linked, thus the NAC1 gene is positioned approximately 12.5 map units to the left of sr-1 as shown in figure 2. The AC-115 gene has been previously mapped to chromosome I (Levine and Goodenough, 1970).

<u>The psbD message is present in nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant</u>

To verify the presence and to compare the relative amounts of the psbD transcript in the mutant strains of interest, total cellular RNA was isolated and subjected to Northern analysis. Equal amounts of cellular RNA from WTCC125, nac1-11, nac1-18, ac-115, 6.2u. and the nac1-18/ac-115 double mutant was separated on a denaturing agarose gel, blotted onto nitrocellulose filter paper, and hybridized with a nick-translated, psbD gene-specific probe. The psbD message is present in all the strains tested as shown in figure 3. Relative amounts of the psbD message varied slightly between strains. psbD mRNA abundance in the nac1-11 strain was approximately equivalent to the wild-type strain (Figure 3, lanes 1 and 2). However, psbD message levels in nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant appear to be somewhat elevated as compared to the wild-type strain (Figure 3, lanes 1, 3, 4, 5, and 6). These results indicate that psbD

transcription is not blocked and that psbD message stability is not affected in the mutant strains of interest.

The D2 protein is not actively synthesized or is rapidly degraded in nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant

To determine whether the five mutants at hand are affected in the synthesis of the D2 protein at the translational and/or post-translational level, chloroplast protein synthesis pulse-labeling experiments were conducted. Carbon starved mutant cells from WTCC125, nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant were treated with 10 ug/mL cycloheximide, an inhibitor of cytoplasmic protein synthesis, prior to a 10 minute pulse with ^{14}C-acetate. As a result, the radioactive label is incorporated into proteins synthesized by the chloroplast. Protein samples from pulse-labeled cells were fractionated by electrophoresis on 7.5 -15 % gradient SDS-polyacrylamide gels (Chua, 1980). The D1 and D2 proteins were identified based on their characteristic migration pattern through the gradient gel and their approximate molecular weight of 32,000 Daltons (M. Kuchka, personal communication) as shown in figure 4. Autoradiography of the gel indicated that the D2 protein is absent in nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant. This suggests that D2 is either not actively synthesized or is rapidly degraded in all the mutant strains assayed. In addition, the D1 protein band in the mutant strains also

appeared to be fainter than the D1 protein band in the wild-type lane (Figure 4), indicating the synthesis and/or turnover of the D1 protein may also be affected. The synthesis of several unidentified chloroplast proteins also appears to be reduced in the mutant strains when compared to wild-type.

Association of psbD mRNA with ribosomes in WTCC125, nac1-11, ac-115, and 6.2u

To determine whether or not psbD message recruitment to ribosomes is affected in mutants strains nac1-11, ac115, and 6.2u, ribosomal extracts from each of these strains as well as wild-type were purified through a 2M sucrose cushion. Equal amounts of extracted RNA from these preparations were applied to nitrocellulose filter paper by dot blot procedure. The filter was hybridized with a PCR generated psbD specific probe. Figure 5 shows the PCR amplified product which contains 1041 base pairs of the psbD coding region. The hybridized dot blot filter revealed the psbD transcript is associated with wild-type, nac1-11, ac-115, and 6.2u ribosomal extracts (Figure 6). However, relative amounts of psbD message associated with the ribosomal preparations appear to vary. The extent of psbD transcript association with nac1-11 chloroplast ribosomes appeared to be equal to that of the wild-type strain (Figure 6, samples a & b). In contrast, ac-115 and 6.2u hybridization intensities were approximately three to four-fold higher than that of wild-type (Figure 6, samples a, c, & d). These results demonstrate that psbD mRNA

recruitment to ribosomes is not blocked in nac1-11, ac-115, and 6.2u.

Polysome profiles and psbD message association with ribosomal subpopulations

Ribosomal extracts from WTCC125, nac1-11, ac-115, and 6.2u were fractionated on 10-30 % sucrose gradients in order to separate monosomes from polysomes. Polyribosomes, due to their large size, migrate to the bottom of the gradient during ultracentrifugation. Alternatively, monosomes remain near the top of the gradient. Polysome profiles measured at A_{260} of WTCC125, nac1-11, ac-115, and 6.2u are shown in Figures 7a, 8a, 9a, and 10a. Based on the sedimentation coefficient equation (see appendix), theoretical calculations estimated that 70S monsomes were collected in fractions 15 and/or 16 of the sucrose gradients. Futhermore, 50S ribosomal subunits were expected in fractions 17 and\or 18, and 30S ribosomal subunits in fractions 19 and/or 20. The profiles from each strain generally resemble one another. Each profile contains one large polysome peak in fraction 1. Berry and co-workers have reported that a similar crude ribosome purification protocol which they utilized enriched for polysomes (Berry et al., 1988). Larger sized polysomes are more likely to migrate through a 2M sucrose cushion as compared to monosomes and ribosomal subunits. Enrichment of polysomes at this step of ribosome purification may account for the pronounced polysome peak in fraction 1.

To determine which subpopulation of ribosomes the psbD message is associated with in WTCC125, nac1-11, ac-115, and 6.2u, dot blot analysis was performed on the sucrose gradient fractions. In the wild-type strain, psbD message was associated with all fractions collected (Figure 7b). psbD mRNA association in wild-type appeared to decrease slightly at the top of the gradient where ribosomal subunits are located. In the nac1-11 strain, psbD transcript association with fractionated ribosomes resembled that of the wild-type strain. The last three fractions of the nac1-11 gradient showed weaker hybridization intensities relative to the other fractions tested (Figure 8b). The ac-115 strain demonstrated a contrasting result, the psbD message associated primarily with 30S ribosomal subunits as shown in figure 9b. This suggests psbD translation initiation or very early steps of elongation might be affected in ac-115. In the 6.2u strain, the psbD message was found to be almost exclusively associated with polysomal fractions as shown in figure 10b. Dot blot analysis of ribosomal fractions indicated psbD message association with ribosomal subpopulations differs between mutant strains nac1-11, ac-115, and 6.2u which suggests each mutant might be affected at a distinct step of psbD message translation or protein turnover.

Thylakoid membrane isolation and dot blot analysis

From isolated thylakoid membranes RNA was extracted and subjected to dot blot analysis with a psbD gene-specific probe

in order to determine whether the psbD message associates with thylakoid membranes in WTCC125, nac1-11, ac-115, and 6.2u. Since the D2 protein is a trans-thylakoid membrane protein, it is thought to be translated on ribosomes which are attached to the thylakoid membrane (rough thylakoids) (Herrin et al., 1981). Dot blot analysis indicated that the psbD transcript was associated with thylakoid membranes in wild-type and in each mutant strain (Figure 11). However, the hybridization intensity of the ac-115 strain was estimated to be four to five-times lighter than that of wild-type, nac1-11, and 6.2u. These results suggest that psbD transcript association with thylakoids is less efficient in the ac-115 strain. Insertion of the nascent D2 polypeptide into the thylakoid membrane may be affected in ac-115. Alternatively, psbD message association with thylakoids appears to be normal in nac1-11 and 6.2u strains.

DISCUSSION

The photosynthetic apparatus of higher plants and green alga is composed of both nuclear and chloroplast encoded proteins. In addition, many chloroplast encoded polypeptides which are involved in the process of photosynthesis require nuclear encoded products for their synthesis. Therefore, the nucleus plays a pivotal role in chloroplast gene expression. In order to study the coordinated interaction which exists between the nuclear and chloroplast genomes, nuclear mutants which are blocked in the synthesis of the chloroplast encoded D2 protein of photosystem II have been analyzed in Chlamydomonas reinhardtii. The four nuclear mutants of this study, nac1-11, nac1-18, ac-115, and 6.2u are affected at the level of D2 translation and/or protein degradation. These mutant strains were compared with wild-type and with each other in order to examine nuclear participation at the post-transcriptional level of chloroplast gene expression.

Genetic analyses of PSII deficient mutants

Genetic analysis has revealed that at least two distinct factors, the nuclear encoded products of the NAC1 and AC-115 genes, are required for the translational and/or post-translational expression of the D2 protein. Previously, another nuclear mutant nac2-26, had been isolated which is unable to synthesize the D2 protein due to its inability to accumulate the psbD transcript (Kuchka et al., 1989). Thus,

a minimum of three products originating from the nucleus are involved at distinct stages of psbD gene expression. Similar studies have identified several other nuclear mutants which are specifically affected in D1, P5, or P6 expression at the levels of mRNA stability (Sieburth et al., 1991), translation, or protein turnover (Jensen et al., 1986); (Rochaix et al., 1989). Taken collectively, the results generated from past studies and the data reported here further supports the notion that the expression of chloroplast encoded PSII proteins requires the participation many nuclear encoded products. Therefore, the chloroplast must rely heavily on the nucleus for the synthesis of its own genes.

The NAC1 gene has been positioned onto linkage group IX of the C. reinhardtii nuclear genome. The genetic map of the NAC1 and AC-115 loci may serve as preliminary data for the physical mapping of these nuclear genes using restriction fragment length polymorphism (RFLP) analysis (Ranum et al., 1988). The recent development of a feasible method of Chlamydomonas transformation (Kindle, 1990), may provide an alternative method of locating the NAC1 and AC-115 genes from a C. reinhardtii genomic library. The identification of the NAC1 and AC-115 genes would greatly facilitate the characterization of nuclear factors which are involved in the expression of a chloroplast gene at the post-transcriptional level.

The isolation of photosynthetically competent revertants of

nac1-11, nac1-18, ac-115, and 6.2u which contain second site suppressor mutations, may provide a genetic method of identifying other gene products involved in D2 expression. This approach was successfully utilized to recognize various nuclear encoded products involved in the translation of oxi2 mRNA in yeast (Haffter et al., 1990). In contrast, if a pseudorevertant is isolated which contains a chloroplast suppressor mutation within the psbD gene, cis-acting elements which are involved in the post-transcriptional expression of the D2 protein may be defined. This strategy was used to identify a cis-acting element in the 5'-untranslated region of the psbC gene which may serve as a target site for a nuclear factor which initiates P6 translation (Rochaix et al., 1989).

nac1-11, nac1-18, ac-115, and 6.2u are affected in the translational and/or post-translational synthesis of the D2 protein

Comparative studies have indicated that the psbD message is present in nac-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant, although psbD mRNA levels are not equivalent in each strain tested. psbD transcript levels appear to be slightly elevated in nac1-18, ac-115, 6.2u and the double mutant, while nac1-11 psbD mRNA levels are comparable to wild-type (Figure 3). Kuchka et al., (1988) previously observed a several-fold increase in psbD transcript levels in the nac1-11 and nac1-18 strains. The authors

suggested that the psbD message might be controlled by a feedback mechanism which would produce elevated psbD message levels due to a block in D2 synthesis (Kuchka et al., 1988). The data observed in this study does not fully support the feedback mechanism hypothesis since psbD mRNA abundance is not elevated in nac1-11. It remains unclear as to why psbD message levels are mildly elevated in nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant. Despite modest differences in psbD mRNA levels, the transcription and stability of the psbD message do not appear to be blocked in nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant.

The results of protein pulse-labeling experiments indicate that the mutant strains assayed are unable to synthesize the D2 protein (Figure 4). It is also possible that the D2 protein is translated in these strains, but it is degraded within a ten minute labeling period. This consideration is somewhat doubtful since the half-life of the D2 protein was estimated to be approximately one hour in PSII deficient mutant strains (Jensen et al., 1986). The synthesis of D1 also appears to be affected in nac1-11, nac1-18, ac-115, 6.2u. The intensity of the D1 protein band appeared to be reduced in the mutant strains when compared to wild-type. Erickson et al. (1986) have suggested that the D2 protein is involved in the expression of the D1 protein based on the characterization of FuD47, a chloroplast mutant which carries a structural gene

mutation in psbD. The decrease in D1 protein synthesis observed in protein pulse-labeling experiments conducted on PSII deficient mutants of this study supports the notion that the D2 protein may participate in the synthesis of the D1 polypeptide. Additionally, the translation of several unidentified chloroplast encoded proteins appeared to be diminished in the mutant strains assayed. These proteins may represent other PSII core proteins besides D1 and D2, or they may be auxiliary proteins whose role in PSII function and assembly are yet to be elucidated. In order to identify these proteins, immunoprecipitation experiments using specific antibodies to PSII polypeptides could be performed. These experiments would address the question of whether or not the synthesis of other PSII proteins is affected in nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant. Kuchka et al. (1988) have reported a pleiotropic decrease in P5, P6, D1, and D2 accumulation in nac1-11 and nac1-18 based on Western analysis. Furthermore, the authors demonstrated that the reduction of PSII core polypeptides was the result of post-translational protein degradation rather than a block in protein synthesis according to protein pulse-labeling experiments (Kuchka et al., 1988). Although the expression of several chloroplast encoded proteins may be reduced in the mutants at hand, the primary affect of mutations present in nac1-11, nac1-18, ac-115, 6.2u, and the nac1-18/ac-115 double mutant is the block in the translation of the D2 protein.

Identification of specific translational or post-translational events which may be affected in mutant strains nac1-11, nac1-18, ac-115, and 6.2u

Dot blot analyses of ribosomal and thylakoid membrane extracts were performed to elucidate the function of nuclear factors affected in nac1-11, ac-115, and 6.2u. Although data suggests these factors act at the post-transcriptional level of psbD expression, the precise role of these products is unclear. Dot blot analysis was conducted on crude ribosome preparations in order to determine whether or not the psbD message is properly recruited to chloroplast ribosomes in the mutant strains tested. Results from these experiments indicated that the psbD transcript is associated with ribosomes in nac1-11, ac-115, and 6.2u (Figure 6). Thus, the nuclear factors which are mutated in these strains do not appear to participate in guiding the psbD message to plastid ribosomes. Levels of psbD message association with ribosomes appeared to vary slightly between the mutant and wild-type strains. In both ac-115 and 6.2u, psbD transcript association with ribosomes was somewhat elevated as compared to the wild-type and nac1-11 strains. These results may suggest that the psbD message binds to ribosomes, but is not properly released after translation in ac-115 and 6.2u. A block in mRNA detachment from ribosomes may result in the observed increase in psbD message association in ac-115 and 6.2u.

Dot blot analysis of ribosomes fractionated into polysomes,

monosomes, and ribosomal subunits was performed in order to identify subpopulations of ribosomes with which the psbD message is associated in nac1-11, ac-115, and 6.2u. The psbD transcript was found to be associated with all fractions in the wild-type and nac1-11 strains, although psbD message levels decreased in fractions containing ribosomal subunits. The strong resemblance between ribosome fractionation dot blots of the wild-type and nac1-11 strains implies that psbD translation may not be affected in nac1-11 (Figures 7b & 8b). Rather, nac1-11 may be affected in D2 synthesis at the post-translational level. In the ac-115 strain, the psbD transcript was associated with all ribosomal fractions, but psbD message intensities were the greatest in 30S ribosomal subunit fractions (Figure 9b). An early step of translation may be blocked in ac-115, which results in the preferential psbD mRNA association with the small subunit of the ribosome. During translation initiation, the 30S ribosomal subunit must bind correctly to the mRNA so that the 50S large subunit may join the complex and begin elongation. Perhaps, translation initiation of the psbD transcript is unable to proceed without the AC-115 nuclear gene product. This factor may act as a psbD message-specific translational activator. Examples of mitochondrial message-specific translation activators is well documented in yeast (Fox, 1986). In addition, control of RUBISCO subunit expression has been demonstrated at the level of translation initiation in amaranth (Berry et al., 1990).

Alternatively, the psbD message may be translationally incompetent in ac-115 and may remain bound to the small ribosomal subunit due to the absence a nuclear factor which is responsible for a post-transcriptional modification of the psbD message which is required for translation. In vitro protein synthesis experiments using chloroplast extracts (Klein et al., 1988) could be conducted on ribosomal subunit and monosome fractions containing the psbD message to address the question of whether the psbD transcript is translationally competent outside the mutant cell. Ribosome fractionation studies could be performed on the nac1-18/ac-115 double mutant in order to compare psbD message association with ribosomal subpopulations in the double mutant with that of ac-115 and nac1-11. If the double mutant resembles the ac-115 strain and the psbD transcript is primarily associated with 30S ribosomal subunits, the notion that the AC-115 gene product participates prior to the NAC1 gene product during D2 translation would be supported. In the 6.2u strain, the psbD message is primarily associated with polysomes. psbD transcript intensities are extremely pronounced in polysome fractions while they are barely visible in monosome and ribosomal subunit fractions of 6.2u, suggesting a late step of psbD mRNA translation may be blocked (Figure 10b). RNA footprinting techniques which have been previously used to identify translational pause sites along an mRNA, could be pursued to determine whether psbD mRNA translation elongation or termination is inhibited (Schaefer

et al., 1989). Additional polysome association experiments should be conducted with other chloroplast messages which are not affected in the mutant strains, to ensure ribosome fractionation proceeded as expected. Since psbD mRNA association patterns with subpopulations of ribosomes vary noticeably between mutant strains nac1-11, ac-115, and 6.2u, the nuclear encoded factors which are affected in each of these strains may act at very distinct stages of D2 translation and/or protein turnover.

Dot blot analysis conducted on thylakoid membrane extracts was performed to determine whether or not the psbD message is associated with thylakoid membranes in the mutant strains of interest. The psbD message was found to be associated with thylakoid membranes in nac1-11, ac-115, and 6.2u, although the message intensity was greatly reduced in the ac-115 strain (Figure 11). This result suggests the translocation of the nascent D2 protein is inefficient in ac-115. Translocation of the D2 protein may require the presence of the nuclear factor encoded by the AC-115 gene. Alternatively, the AC-115 gene product may be involved in an early step of translation such as initiation, which occurs prior to the translocation of the nascent D2 polypeptide. Results generated from the nac1-11 and 6.2u strains indicated the psbD message is able to associate with thylakoid membranes. Therefore, the translational and/or post-translational step which is blocked in these two strains may follow psbD message association to

thylakoid membranes during translation. The thylakoid membranes of the nac1-18/ac-115 double mutant could also be assayed by dot blot analysis. If psbD message levels associated with thylakoid membranes are reduced in the double mutant, this would suggest that the role of the AC-115 gene product precedes that of the NAC1 gene product during D2 synthesis.

In summary, the results generated in this study suggest that the nuclear factors which are affected in nac1-11, ac-115, and 6.2u take part in different translational and/or post-translational events during D2 synthesis. A schematic model of the possible roles of the nuclear factors which are affected in nac1-11, nac1-18, ac-115, and 6.2u is depicted in figure 12. Since nac1-11 and nac1-18 are allelic, they are affected in the same gene. The NAC1 gene product may participate at the post-translational level of psbD expression since psbD message association studies conducted on crude ribosomes, fractionated ribosomes, and thylakoid membranes mimic the wild-type strain in every respect. The AC-115 gene product appears to act at an early stage of D2 translation since the psbD message is associated with primarily with 30S ribosomal subunits in fractionation experiments and psbD transcript association to thylakoids is reduced in the ac-115 mutant. Finally, the nuclear product affected in 6.2u may be involved in a late step of translation, since the psbD transcript is predominately associated with polysomes and it

is bound to thylakoid membranes to the extent equal to wild-type. Although much information has been gained from this study, further characterization of these nuclear mutants will greatly improve our understanding of nuclear control of chloroplast gene expression at the translational and/or post-translational level.

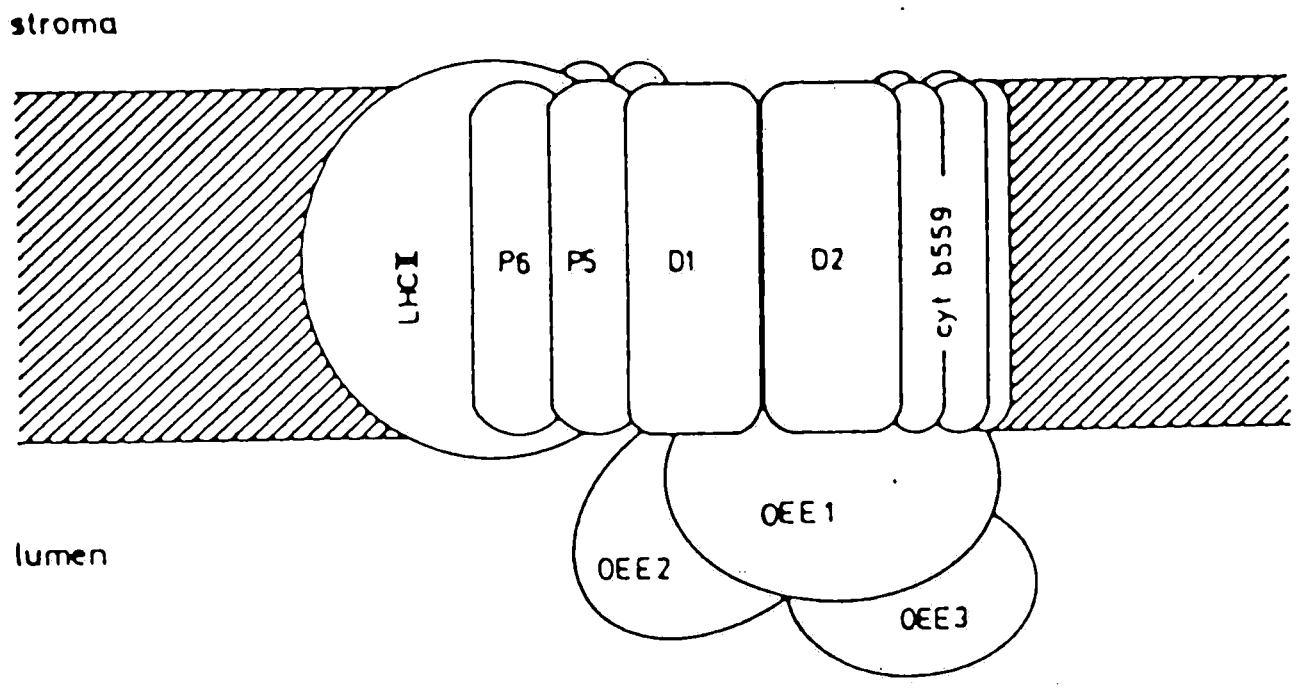


Figure 1. A schematic model of photosystem II in the thylakoid membrane of the chloroplast. PSII is a multi-subunit protein complex consisting of nuclear and chloroplast encoded polypeptides. D1, D2, P5, P6, and the two cytochrome b559 apoproteins are chloroplast encoded. The oxygen evolving enhancer proteins denoted OEE-1, -2, and -3, and the light harvesting complex (LHCII) are nuclear encoded. Figure taken from Kuchka et al., (1989).

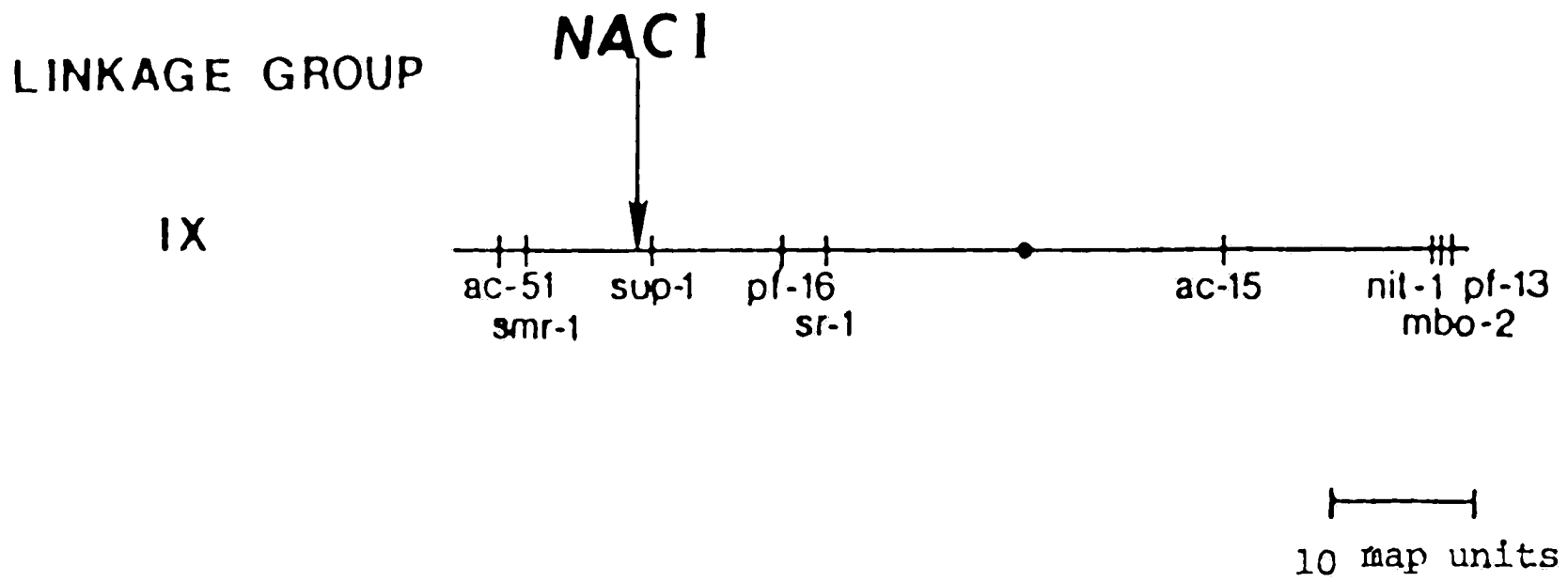


Figure 2. Genetic map of nuclear chromosome IX of Chlamydomonas reinhardtii. The NAC1 loci is positioned approximately 12.5 map units from the streptomycin marker (sr-1) on linkage group IX of the C. reinhardtii chromosome based on intercrosses between nac1-18 and the multiply-marked strain CC-1735. The AC-115 gene is located on chromosome I according to previous mapping studies reported by Levine and Goodenough, (1970). Figure taken from Harris et al., (1987).

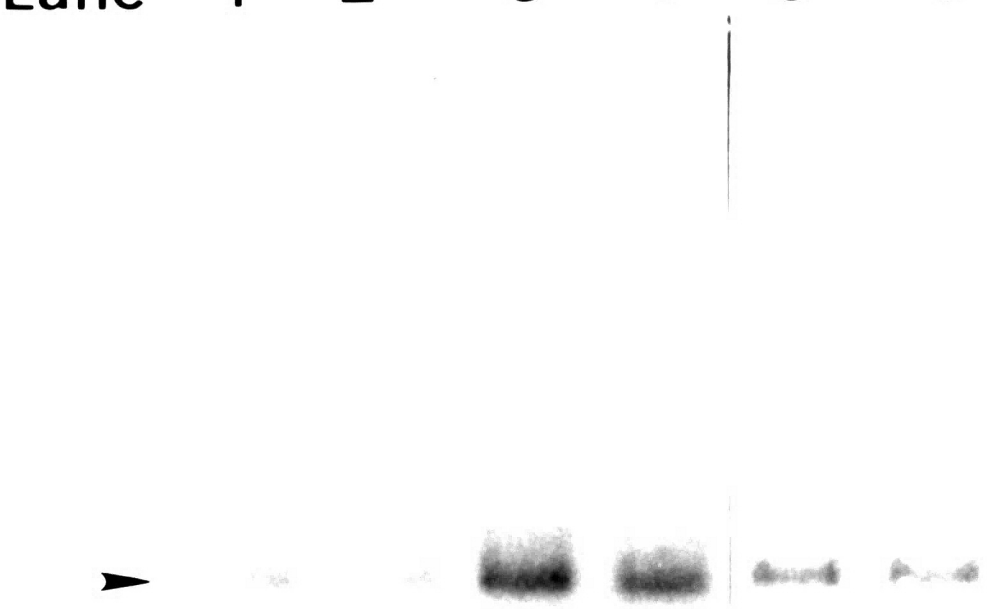


Figure 3. Northern analysis of total RNA from wild-type and PSII deficient mutants. Equal amounts of total cellular RNA were separated on a formaldehyde gel, blotted onto nitrocellulose filter paper, and hybridized with a psbD gene-specific probe. (lane 1 - wild-type, lane 2 - nac1-11, lane 3 - nac1-18, lane 4 - ac-115, lane 5 - 6.2u, lane 6 - nac1-18/ac-115 double mutant).

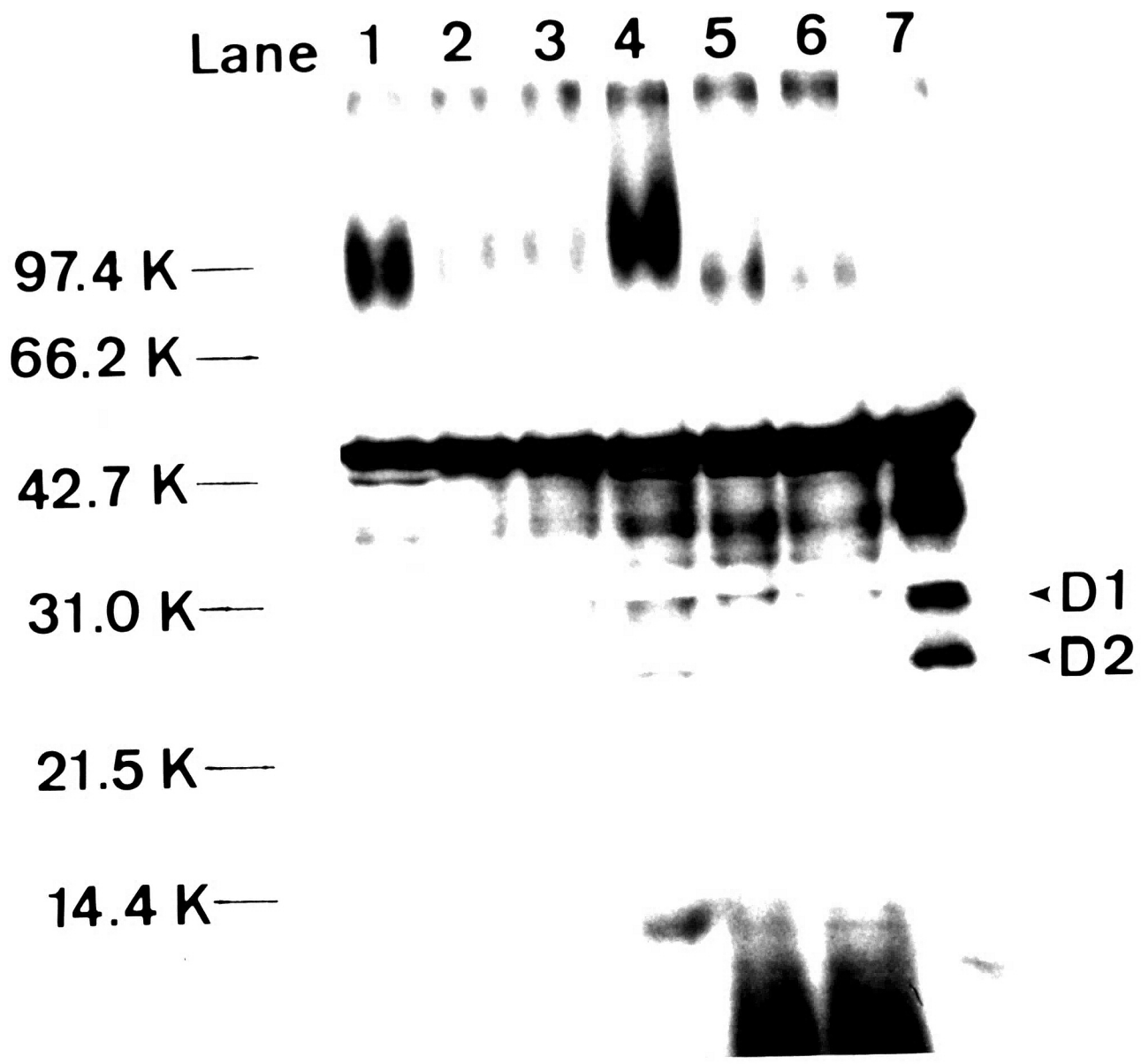


Figure 4. Autoradiograph of pulse-labeled total cell proteins separated by polyacrylamide gel electrophoresis. Cells were ^{14}C-acetate labeled for 10 minutes in the presence of 10 ug/mL cycloheximide, an inhibitor of cytoplasmic protein synthesis. Proteins were fractionated on a 7.5 - 15% polyacrylamide gel and subjected to autoradiography. (lane 1 - nac1-18/ac-115 double mutant, lane 2 -6.2u, lane 3 - ac-115, lane 4 - nac1-18 revertant R3, lane 5 - nac1-18, lane 6 - nac1-11 lane 7 - wild-type)

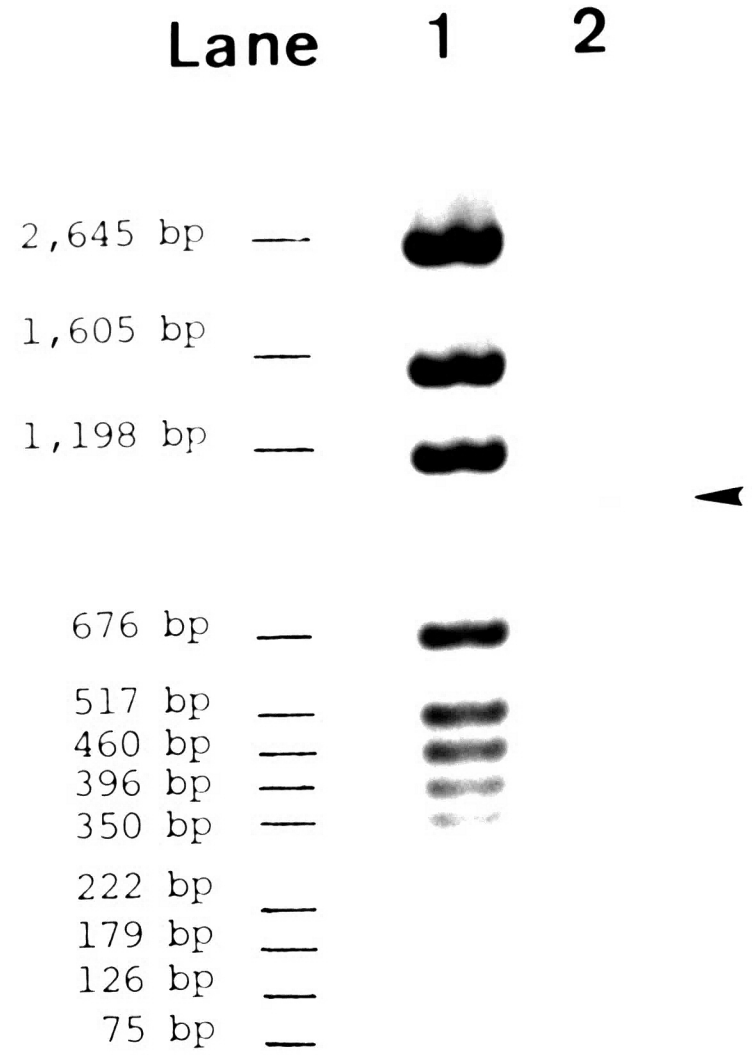
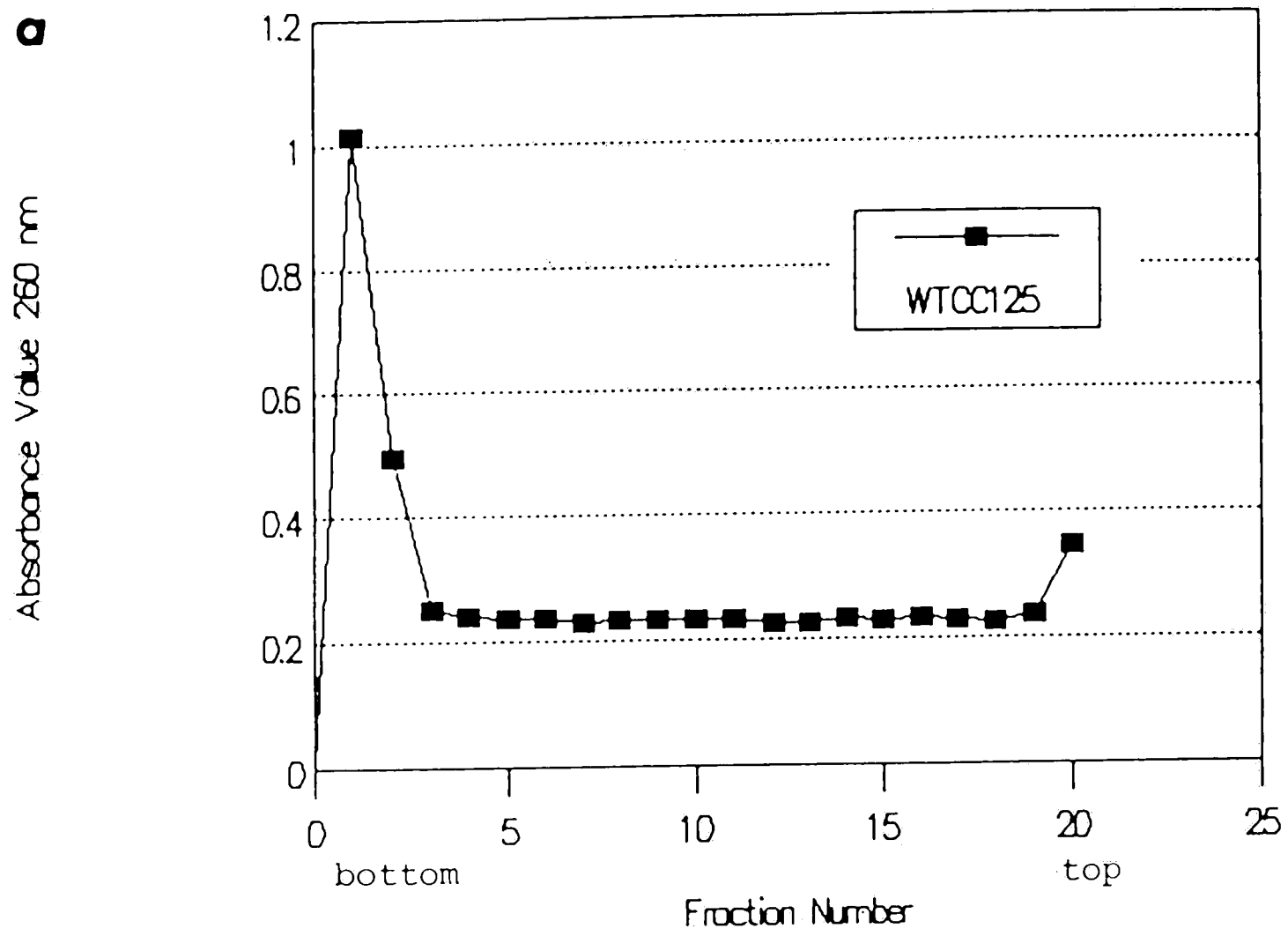


Figure 5. Photograph of an ethidium bromide stained 1.5% agarose gel containing the PCR amplified 1041 bp DNA fragment of the psbD gene. (lane 1 - pGem markers, lane 2 - amplified psbD DNA)

Figure 6. Dot blot analysis of RNA derived from total ribosome preparations. Crude ribosomes isolated from lysed cells were purified by 4 hour sedimentation through a 2M sucrose cushion at 48,000g. RNA from ribosome preparations was extracted following DNase treatment. Equal amounts of extracted RNA were dot blotted onto nitrocellulose paper and hybridized with a nick-translated psbD gene-specific probe. (sample a - wild-type, sample b - nac1-11, sample c - ac-115, sample d - 6.2u).



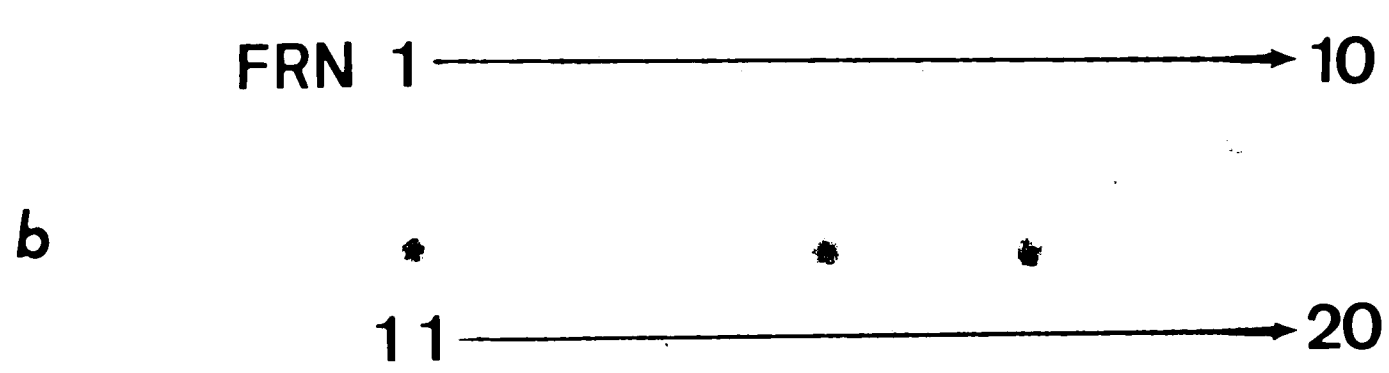


Figure 7. Polysome profile and dot blot analysis of wild-type ribosomes fractionated on a sucrose density gradient. Ribosomes isolated from wild-type cells were separated into polysomes and monosomes by centrifugation through a 10 - 30% sucrose gradient.

7a) A polysome profile was generated by measuring the A_{260} of each 0.5mL fraction collected from the gradient. Sedimentation is from right to left. Polysomes migrated to the bottom of the gradient due to their large size. Monosomes, being small in size, remained near the top of the gradient.

7b) Dot blot analysis of RNA extracted from ribosomal gradient fractions from wild-type. Equal amounts of extracted RNA from ribosomal fractions were applied onto nitrocelluose paper by dot blot procedure. The filter was hybridized using a nick-translated psbD gene-specific probe.

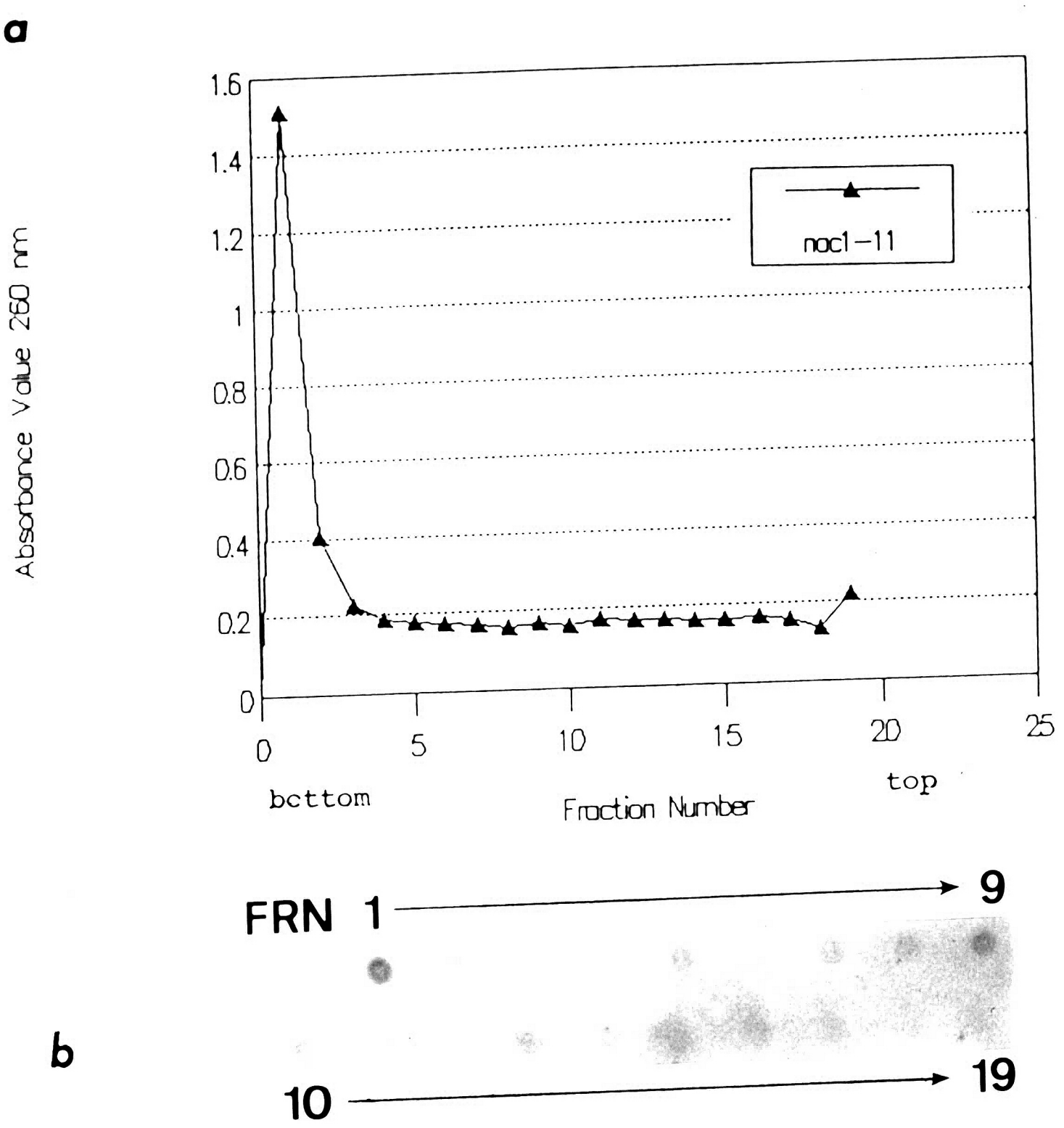
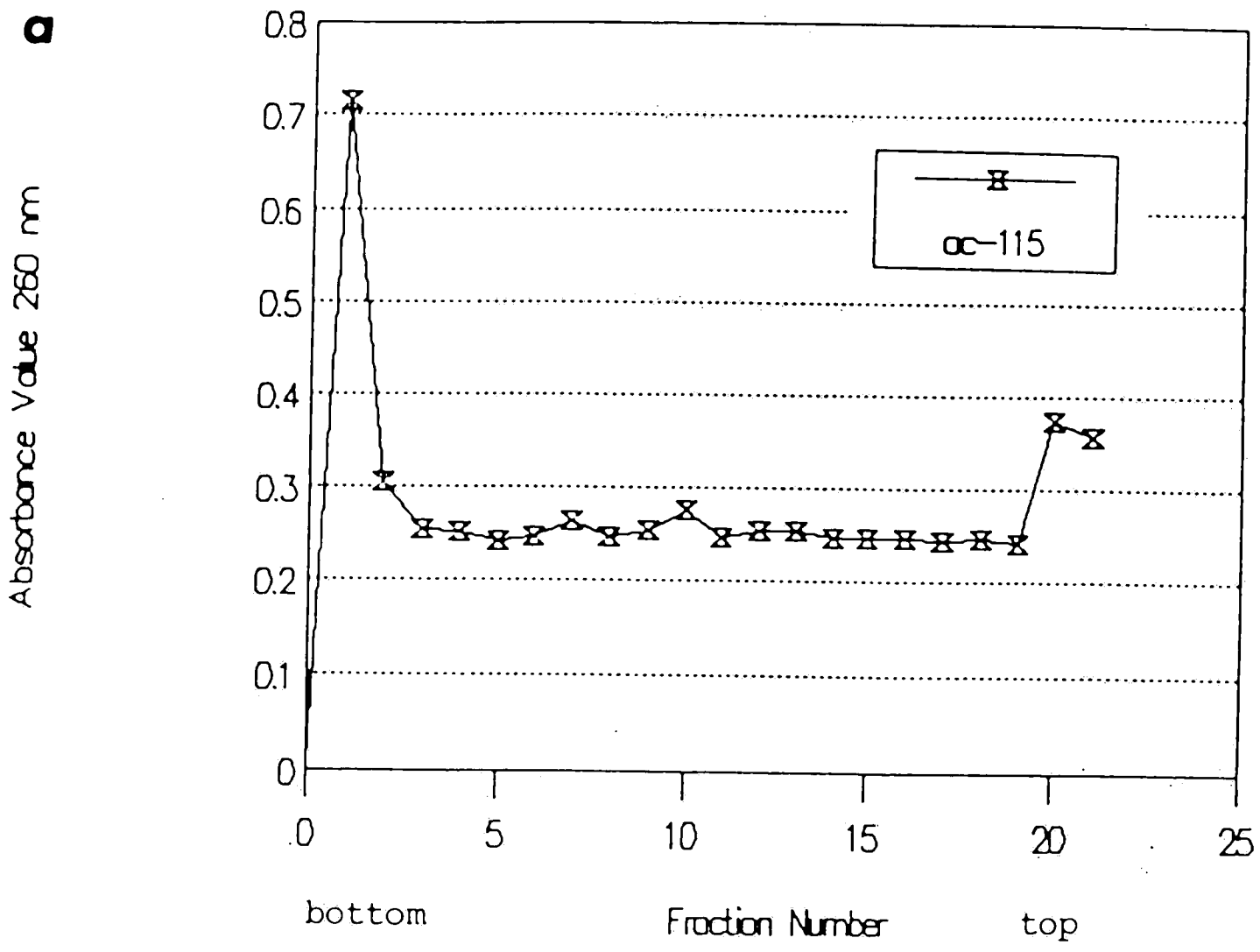


Figure 8. Polysome profile and dot blot analysis of nac1-11 ribosomes fractionated on a sucrose density gradient. Ribosomes isolated from nac1-11 cells were separated into polysomes and monosomes by centrifugation through a 10 - 30% sucrose gradient.

8a) A polysome profile was generated by measuring the A_{260} of each 0.5mL fraction collected from the gradient. Sedimentation is from right to left. Polysomes migrated to the bottom of the gradient due to their large size. Monosomes, being small in size, remained near the top of the gradient.

8b) Dot blot analysis of RNA extracted from ribosomal gradient fractions from nac1-11. Equal amounts of extracted RNA from ribosomal fractions were applied onto nitrocelluose paper by dot blot procedure. The filter was hybridized using a nick-translated psbD gene-specific probe.



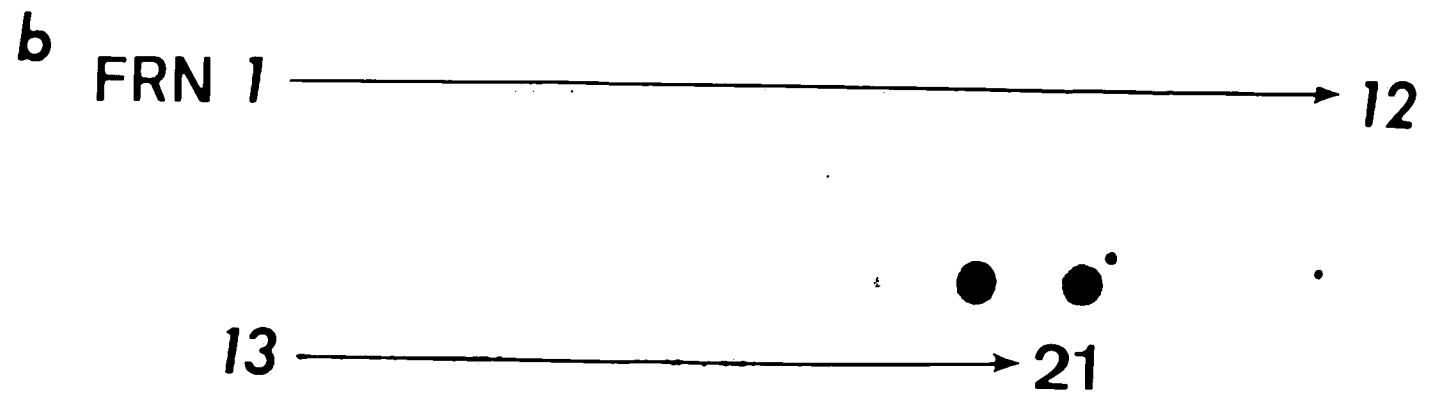


Figure 9. Polysome profile and dot blot analysis of ac-115 ribosomes fractionated on a sucrose density gradient. Ribosomes isolated from ac-115 cells were separated into polysomes and monosomes by centrifugation through a 10 - 30% sucrose gradient.

9a) A polysome profile was generated by measuring the A_{260} of each 0.5mL fraction collected from the gradient. Sedimentation is from right to left. Polysomes migrated to the bottom of the gradient due to their large size. Monosomes, being small in size, remained near the top of the gradient.

9b) Dot blot analysis of RNA extracted from ribosomal gradient fractions from ac-115. Equal amounts of extracted RNA from ribosomal fractions were applied onto nitrocelluose paper by dot blot procedure. The filter was hybridized using a nick-translated psbD gene-specific probe.

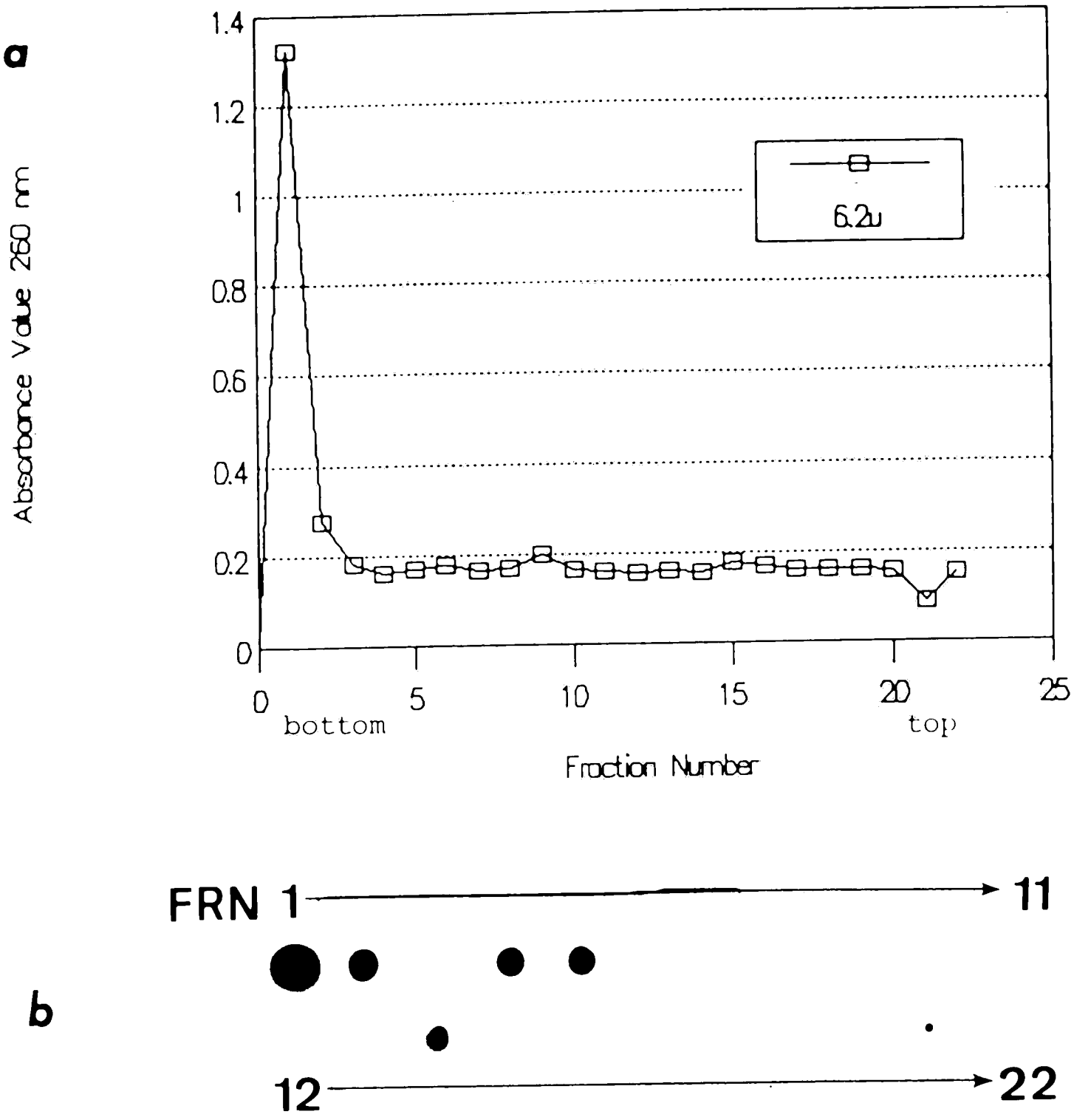


Figure 10. Polysome profile and dot blot analysis of 6.2u ribosomes fractionated on a sucrose density gradient. Ribosomes isolated from 6.2u cells were separated into polysomes and monosomes by centrifugation through a 10 - 30% sucrose gradient.

10a) A polysome profile was generated by measuring the A_{260} of each 0.5mL fraction collected from the gradient. Sedimentation is from right to left. Polysomes migrated to the bottom of the gradient due to their large size. Monosomes, being small in size, remained near the top of the gradient.

10b) Dot blot analysis of RNA extracted from ribosomal gradient fractions from 6.2u. Equal amounts of extracted RNA from ribosomal fractions were applied onto nitrocelluose paper by dot blot procedure. The filter was hybridized using a nick-translated psbD gene-specific probe.

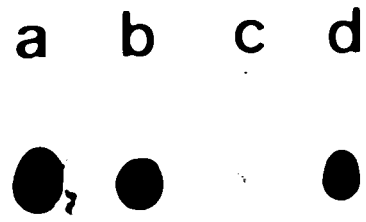


Figure 11. Dot blot analysis of RNA derived from thylakoid membrane preparations. Thylakoid membranes were isolated by discontinuous sucrose gradient centrifugation. RNA was extracted from thylakoid membrane preparations following DNase treatment. Equal amounts of RNA were dot blotted onto nitrocellulose paper and hybridized with nick-translated psbD gene-specific probe. (sample a - wild-type, sample b - nac1-11, sample c - ac-115, sample d - 6.2u)

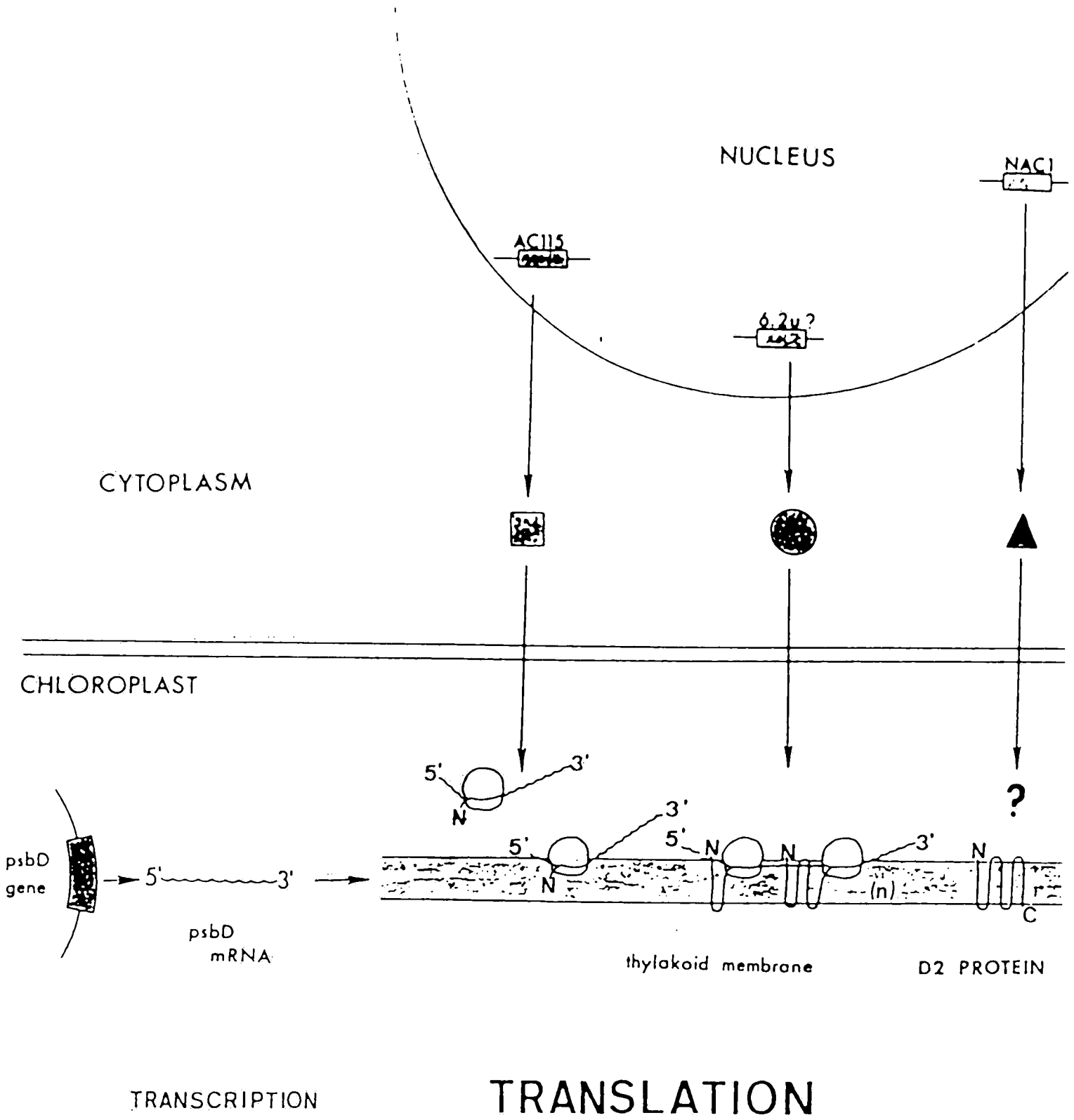


Figure 12. Proposed functions of nuclear encoded gene products involved in the expression of the D2 protein at the translational and/or post-translational level in C. reinhardtii. This model is based on data generated from dot blot analyses of crude ribosomes, fractionated ribosomes, and thylakoid membranes from wild-type, nac1-11, ac-115, and 6.2u. The AC-115 gene product may act at an early step of D2 translation, such as translation initiation or attachment of the nascent D2 polypeptide to the thylakoid membrane. The putative 6.2u gene product may participate in a late event of D2 translation including elongation or termination. The NAC1 gene product may be involved at the post-translational level of D2 synthesis.

REFERENCE CITATION:

Berry, J.O., Breiding, D.E., and Klessig, D.F. (1990). Light-mediated control of translational initiation of ribulose-1,5-bisphosphate carboxylase in amaranth cotyledons. Plant Cell 2, 795-803.

Berry, J.O., Carr, J.P., and Klessig, D.F. (1988). mRNAs encoding ribulose-1,5- bisphosphate carboxylase remain bound to polysomes but are not translated in amaranth seedlings transferred to darkness. Proc. Natl. Acad. Sci. USA 85, 4190-4194.

Bonham-Smith, P.C. and Bourque, D.P. (1988). The chloroplast genome and regulation of its expression. In Chromosomes: Eukaryotic, Prokaryotic and Viral. K. Adolph, ed. (Boca Raton, Florida: CRC Press).

Chua, N.H., (1980). Electrophoretic analysis of chloroplast proteins. Methods Enzymol. 69, 434-446.

Chua, N.H. and Bennoun, P. (1975). Thylakoid membrane polypeptides of Chlamydomonas reinhardtii: Wild-type and mutant strains deficient in photosystem II reaction center function and assembly. Proc. Natl. Acad. Sci. USA 72, 2175-2179.

Costanzo, M.C., and Fox, T.D. (1986). Product of Saccharomyces cerevisiae nuclear gene PET494 activates translation of a specific mitochondrial mRNA. Mol. Cell. Biol. 6, 3694-3703.

Costanzo, M.C, Seaver, E.C., and Fox, T.D. (1989). The PET54 gene of Saccharomyces cerevisiae: Characterization of a nuclear gene encoding a mitochondrial translational activator and subcellular localization of it product. Genetics 122, 297-305.

de Vitry, C., Olive, J., Drapier, D. Recouvreur, M. and Wollman, F.-A. (1989). Post-translational events leading to the assembly of photosystem II protein complex: A study using photosynthesis mutants from Chlamydomonas reinhardtii. J. Cell Biol. 109, 991-1006.

Dieckmann, C.L. and Tzagoloff, A. (1985). Assembly of the mitochondrial membrane system: CBP6, a yeast nuclear gene necessary for the synthesis of cytochrome b. J. Biol. Chem. 260, 1513-1520.

Eichacker, L.A., Soll, J., Lauterbach, P., Rudiger, W., Klein, R.R., and Mullet, J.E. (1990). In vitro synthesis of chlorophyll a in the dark triggers accumulation of chlorophyll a apoproteins in barley etioplasts. J. Biol.

Chem. 265, 13566-13571.

Erickson, J.M., Delepelaire, P., and Rochaix, J.-D. (1985). Analysis of genes encoding two photosystem II proteins of the 30 - 34 kD size class. In Molecular Biology of the Photosynthetic Apparatus, K.E. Steinback et al., eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory), pp. 53-66.

Erickson, J.M., Rahire, M., and Rochaix, J.-D. (1984). Chlamydomonas reinhardtii gene for the 32000 mol. wt. protein of photosystem II contains four large introns and is located entirely within the chloroplast inverted repeat. EMBO J. 3, 2753-2762.

Fox, T.D. (1986). Nuclear gene products required for translation of specific mitochondrially coded mRNAs in yeast. Trends in Genetics 11, 97-100.

Fu, L., Ye, R., Browder, L.W., and Johnston, R.N. (1991). Translational potentiation of messenger RNA with secondary structure in Xenopus. Science 251, 807-810.

Gamble, P.E. and Mullet, J.E. (1989). Translation and stability of proteins encoded by the plastid psbA and psbB genes are regulated by a nuclear gene during light-induced chloroplast development in barley. J. Biol. Chem. 264, 7236-7243.

Haffter, P., McMullin, T.W., and Fox, T.D. (1990). Functional interactions among two yeast mitochondrial ribosomal proteins and an mRNA-specific translational activator. Genetics 127, 319-326.

Harris, E. (1989). The Chlamydomonas Sourcebook: A Comprehensive Guide to biology and laboratory use. (San Diego, CA: Academic Press).

Harris, E.H., Boynton, N.W., and Gillham, N.W. (1987) Chlamydomonas reinhardtii. In Genetic Maps : A Compilation of Linkage and Restriction Maps of Genetically Studied Organisms. vol. 4, S.J. O'Brien ed. (Cold Spring Harbor, New York: Springer-Verlag, Berlin). pp. 257-277.

Herrin, D. and Michaels, A. (1985). The chloroplast 32 KDa protein is synthesized on thylakoid-bound ribosomes in Chlamydomonas reinhardtii. FEBS. Lett. 184, 90-95.

Herrin, D., Michaels, A., and Hickey, E. (1981). Synthesis of a chloroplast nenbrane polypeptide on thylakoid-bound ribosomes during the cell cycle of Chlamydomonas reinhardtii 137. Biochim. Biophys. Acta 655, 136-145.

Inamine, G., Nash, B., Weissbach, H., and Brot, N. (1985). Light regulation of the synthesis of the large subunit of ribulose-1,5-bisphosphate carboxylase in peas: Evidence for translational control. Proc. Natl. Acad. Sci. USA 82, 5690-5694.

Jensen, K.H., Herrin, D.L., Plumley, F.G., and Schmidt, G.W. (1986). Biogenesis of photosystem II complexes: Transcriptional, translational, and posttranslational regulation. J. Cell Biol. 103, 1315 - 1325.

Kindle, K.L. (1990) High-frequency nuclear transformation of Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. USA 87, 1228-1232.

Kirk, M.M. and Kirk, D.L. (1985). Translational regulation of protein synthesis, in response to light, at a critical stage of volvox development. Cell 41, 419-428.

Klein, R.R., Mason, H.S., and Mullet, J.E. (1988). Light-regulated translation of chloroplast proteins. I. Transcripts of psaA-psaB, psbA, and rbcL are associated with polysomes in dark-grown and illuminated barley seedlings. J. Cell Biol. 106. 289-301.

Klein, R.R. and Mullet, J.E. (1986). Regulation of chloroplast- encoded chlorophyll binding protein translation during higher plant chloroplast biogenesis. J. Biol. Chem. 261, 11138-11145.

Klein, R.R. and Mullet, J.E. (1987). Control of gene expression during higher plant chloroplast biogenesis. J. Biol. Chem. 262, 4341-4348.

Kuchka, M.R., Goldschmidt-Clermont, M., van Dillewijn, J., and Rochaix, J.-D. (1989). Mutation at the Chlamydomonas nuclear NAC2 locus specifically affects stability of the chloroplast psbD transcript encoding polypeptide D2 of PSII. Cell 58, 869-876.

Kuchka, M.R., Mayfield, S.P., and Rochaix, J.-D. (1988). Nuclear mutations specifically affect the synthesis and/or degradation of the chloroplast-encoded D2 polypeptide of photosystem II in Chlamydomonas reinhardtii. EMBO J. 7, 319-324.

Larkins, B.A. and Davies, E. (1975). Polyribosomes from peas. V. An attempt to characterize the total free and membrane-bound polysomal population. Plant Physiol. 55, 749-756.

Leaver, C.J. and Dyer, J.A. (1974). Caution in the interpretation of plant ribosome studies. Biochem. J. 144,

165-167.

Lehrach, H., Diamond, D., Wozney, J.M., and Boedtker, H. (1977). RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry 16, 4743-4751.

Levine, R.P. and Ebersold, W.T. (1960). The genetics of cytology of Chlamydomonas. Annu. Rev. Genet. 4, 397-408.

Levine, R.P. and Goodenough, U.W. (1970). The genetics of photosynthesis and of the chloroplast in Chlamydomonas reinhardtii. Ann. Rev. Genet. 4, 397-408.

Lindahl, L. and Zengel, J.M. (1986). Ribosomal genes in Escherichia coli. Annu. Rev. Genet. 20, 297-326.

Link, G. (1984). Hybridization study of developmental plastid gene expression in mustard (Sinapis alba L.) with cloned probes for most plastid DNA regions. Plant Mol. Biol. 3, 243-248.

Malnoe, P., Mayfield, S.P., Rochaix, J.-D. (1988). Comparative analysis of the biogenesis of photosystem II in the wild-type and y-1 mutant of Chlamydomonas reinhardtii. J. Cell Biol. 106, 609-616.

Mathews, M.B. (1986). Current Communications in Molecular Biology: Translational Control. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Mattoo, A.K., Marder, J.B., and Edelman, M. (1989). Dynamics of photosystem II reaction center. Cell 56, 241-246.

Mayfield, S.P., Rahire, M., Frank, G., Zuber, H., and Rochaix, J.-D. (1987). Expression of the nuclear gene encoding oxygen-evolving enhancer protein 2 is required for high levels of photosynthetic oxygen evolution in Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. USA 84, 749-753.

McCarthy, J.E.G. and Gualerzi, C. (1990). Translational control of prokaryotic gene expression. Trends in Genetics 6, 78-85.

Mishkind, M.L. and Schmidt, G.W. (1983). Posttranscriptional regulation of ribulose 1,5-bisphosphate carboxylase small subunit accumulation in Chlamydomonas reinhardtii. Plant Physiol. 72, 847-854.

Mullet, J.E., Gamble-Klein, P., and Klein, R.R. (1990). Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein

stability. Proc. Natl. Acad. Sci. USA 87, 4038-4042.

Mullis, K.B., Falloona, F.A., Scharf, S. Saiki, R., Horn, G., and Erlich, H. (1986). Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol. 51, 263-273.

Ranum, L.P.W., Thompson, M.D., Schloss, J.A., Lefebvre, P.A., and Silflow, C.D. (1988). Mapping flagellar genes in Chlamydomonas using restriction fragment length polymorphisms. Genetics 120, 109-122.

Rochaix, J.-D. (1987). Molecular genetics of chloroplast and mitochondria in the unicellular green alga Chlamydomonas reinhardtii. FEMS Microbiol. Rev. 46, 13-34.

Rochaix, J.-D. and Erickson, J. (1988). Function and assembly of photosystem II: Genetic and molecular analysis. Trends in Biol. Sci. 13, 56-59.

Rochaix, J.-D., Kuchka, M., Mayfield, S., Schirmer-Rahire, M., Girard-Bascou, J., and Bennoun, P. (1989). Nuclear and chloroplast mutations affect the synthesis or stability of the chloroplast psbC gene product in Chlamydomonas reinhardtii. EMBO J. 8, 1013-1021.

Rodel, G., Korte, A., and Kaudewitz, F. (1985). Mitochondrial suppression of a yeast nuclear mutation which affects the translation of the mitochondrial apocytochrome b transcript. Curr. Genet. 9, 641-648.

Rodermel, S.R. and Bogorad, L. (1985). Maize plastid photogenes: mapping and photoregulation of transcript levels during light-induced development.J. Cell. Biol. 100, 463-476.

Rouault, T.A., Hentze, M.W., Caughman, S.W., Harford, J.B., and Klousner, R.D. (1988). Binding of a cytosolic protein to the iron-response element of human ferritin messenger RNA. Science 241, 1207-1210.

Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). Molecular Cloning: A laboratory manual. 2nd edition. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

Sasaki, Y., Tomada, Y., and Kamilkubo, T. (1984). Light regulates the gene expression of ribulose bisphosphate carboxylase at the level of transcription and gene dosage in greening pea leaves. FEBS Lett. 173, 31-35.

Schaefer, E. M., Hartz, D., Gold, L., and Simoni, R.D. (1989).

Ribosome-binding sites and RNA-processing sites in the transcript of the Escherichia coli unc operon. J. Bacteriol. 171, 3901-3908.

Sieburth, L.E., Berry-Lowe, S., and Schmidt, G.W. (1991). Chloroplast RNA stability in Chlamydomonas: rapid degradation of psbB and psbC transcripts in two nuclear mutants. Plant Cell 3, 175-189.

Slovin, J.P. and Tobin, E.M. (1982). Synthesis and turnover of light-harvesting chlorophyll a/b binding protein in Lemma gibba grown with intermittent red light: possible translational control. Planta 154, 465-472.

Wulczyn, F.G. and Kahmann, R. (1991). Translational stimulation: RNA sequence and structure requirements for binding of Com protein. Cell 65, 259-269.

APPENDIX

Sedimentation Coefficient

$$s = \frac{210 \log (x_2/x_1)}{(rpm)^2 (t_2 - t_1)}$$

x_1 = the distance (in centimeters) from the axis of rotation to the particle at the start of centrifugation

x_2 = the distance (in centimeters) from the axis of rotation to the particle at the end of centrifugation

$(t_2 - t_1)$ = the elapsed time (in seconds) of centrifugation

S = Svedberg unit = 1×10^{-13} second

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ABSTRACTS: Kuchka, M., Wu, H., and Donahue, M. (1991). Nuclear gene products are required at distinct stages in the expression of the chloroplast-encoded D2 protein of Photosystem II in Chlamydomonas reinhardtii. In Molecular Interactions in Plant Development. Penn State 10th Summer Symposium in Molecular Biology.

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