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Studies of psbD mRNA Stability in *Chlamydomonas reinhardtii*

by

Maureen E. Donahue

A Thesis

Presented to the Graduate Committee

of Lehigh University

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in

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Abbreviations

DNA	deoxyribonucleic acid
RNA	ribonucleic acid
mRNA	messenger RNA
tRNA	transfer RNA
rRNA	ribosomal RNA
UTR	untranslated region
IR	inverted repeat
ATP	adenosine triphosphate
NADP	nicotinamide adenosine diphosphate
UTP	uridine triphosphate
KDa	kilodalton
PSII	photosytem II
bp	base pair
DTT	dithiothreitol
NTP	nucleoside triphosphate
EDTA	ethylene diamine tetraacetic acid

Abstract

Post-transcriptional control of mRNA stability is an important regulatory mechanism of plastid gene expression. However, the molecular mechanisms involved in mRNA stability are not well defined. In order to elucidate the possible mechanisms involved in plastid mRNA stability the nuclear nac2-26 mutant of Chlamydomonas reinhardtii was studied. This mutant fails to accumulate any levels of the chloroplast encoded psbD mRNA which encodes the D2 protein of photosystem II. Analysis of this mutant has indicated that the psbD message is initially transcribed but rapidly degraded. The focus of this thesis was to elucidate the mechanisms involved in psbD mRNA stability in Chlamydomonas reinhardtii. Transcription run on experiments demonstrate that the lack of the psbD mRNA in nac2-26 cells is not the result of abortive transcription initiation. Results also show that the message is still present in wild type cells six hours after treatment with cycloheximide, a cytoplasmic protein synthesis inhibitor. Previous data suggest that sequences other than the 3′ end of the message are involved in conferring message stability. Experiments were performed in order to isolate and clone the 5′ sequences of the psbD gene. The 5′ UTR and some coding sequences of the gene were amplified by the polymerase chain reaction. The 5′ sequences of the psbD gene were subsequently cloned into a transcription vector. This construct was then used as a template for an in vitro

transcription reaction to synthesize 5′ psbD transcripts. Chloroplast soluble and membrane proteins were isolated from the cell wall-less strain (cw15) of Chlamydomonas reinhardtii. These proteins together with the 5′ psbD transcript will be used in future experiments to identify trans acting proteins which associate specifically with the 5′ UTR of the psbD transcript. These experiments will help to identify the cis and trans acting elements involved in psbD message stability.

Introduction

Post-transcriptional regulation of differential mRNA stability is an important control step in plastid gene expression. Controlling the rate of mRNA turnover contributes significantly to the steady state level of an mRNA in the organelle. The molecular mechanisms involved in determining mRNA stability in plastids are largely unknown. Certain cis acting elements present on the mRNA itself may be important determinants of plastid transcript stability (reviewed in Gruissem,1989). The association of trans acting factors with these sequences has also been implicated in conferring message stability (Gruissem,1989). The ultimate goal of this research is to understand the molecular mechanisms of plastid mRNA stabilization. This thesis is aimed at elucidating specific aspects of these mechanisms.

The Chloroplast

The chloroplast is the organelle in plant cells that is responsible for the photochemical events of photosynthesis. Photosynthesis is the process by which the light energy from the sun is converted into biochemical energy. The reactions of photosynthesis can be divided into two distinct categories, the light and the dark reactions. The light reactions involve the transfer of electrons through various electron carriers in order to generate a proton gradient which drives the synthesis of ATP. Also during the light reactions, water is oxidized to reduce NADP+ to NADPH and liberate O_2. During the dark reactions the energy from ATP and the reducing power of NADPH is then used to convert CO_2 from the air into organic compounds such as carbohydrates.

The photosynthetic apparatus consists of multi-subunit complexes which are embedded in the thylakoid membrane of the chloroplast (Rochaix and Erickson,1988). These multi-subunit complexes are composed of both nuclear and chloroplast encoded proteins. The nuclear encoded proteins are synthesized on cytoplasmic ribosomes and are transported into the chloroplast where they then function.

Chloroplasts contain their own genome which encodes products involved in transcription and translation, such as tRNAs, rRNAs, RNA polymerase, as well as genes encoding proteins necessary for photosynthesis. Although chloroplasts contain their own genome, they are only semi-autonomous

organelles which require an interaction with the nuclear genome for chloroplast biogenesis. For example, certain chloroplast genes require the expression of nuclear gene products in order to be expressed (reviewed in Rochaix and Erickson, 1988). Several nuclear mutants of the unicellular green alga Chlamydomonas reinhardtii have been isolated that are defective in the expression of certain chloroplast genes (Rochaix et al.,1989;Kuchka et al.,1988;Kuchka et al.,1989;Jensen et al.,1986;Sieburth et al.,1991). These studies demonstrate in part the involvement of the nuclear genome in chloroplast function.

mRNA Stability During Chloroplast Development

Chloroplasts develop from undifferentiated organelles called proplastids which lack pigmentation and internal membrane structure (Hoober,1984). Like chloroplasts, proplastids contain their own genome but they have not yet begun synthesizing the proteins required for photosynthesis. In the presence of light proplastids differentiate into etioplasts which have some internal membrane structure and have begun synthesizing chlorophyll and other photosynthetic pigments and proteins (reviewed in Tobin and Silverthorn,1985). Mature chloroplasts contain all of the pigments and proteins necessary for the reactions of photosynthesis.

During light induced development, many chloroplast mRNAs

and proteins are found to accumulate to high levels as compared to their levels in the dark (Mullet and Klein, 1987; Deng and Gruissem, 1987). However, not all plastid mRNAs accumulate to the same levels during the developmental process. For example, in spinach chloroplasts, the mRNA encoding the D1 protein of photosystem II (psbA) accumulates to high levels relative to the levels of the mRNA encoding the large subunit of the rubisco 1,5-bisphosphate carboxylase enzyme (rbcL), whose levels remain constant during development (Deng and Gruissem,1987). Earlier studies have shown that the accumulation of several plastid mRNAs is uncoupled from the transcriptional activity of their genes (Deng and Gruissem,1988; Deng et al.,1987; Gruissem et al.,1988). Alternatively, other studies indicate that the psbA and rbcL genes can also be controlled at the translational level (Berry et al.,1986; Klein and Mullet,1986). This suggests that post transcriptional and/or translational control is important in regulating plastid gene expression during development.

mRNA stability appears to be involved in regulating the expression of plastid genes during different stages of development. Recently, changes in the stabilities of the psbA and rbcL mRNAs during development have been shown in vivo (Klaff and Gruissem,1991). Results from this study show that the half life of the psbA mRNA increases in mature leaves relative to the half life of rbcL. These studies suggest that post transcriptional control of mRNA stability plays an

important role in regulating plant gene expression during development.

Cis Acting Elements Involved in mRNA Stability

Plastid protein coding genes are often flanked by inverted repeat sequences (IR) at their 5′ and 3′ ends which when transcribed may form stem loop structures in vivo (Gruissem,1989). Plastid mRNAs are prokaryotic in nature in that they do not contain a 5′ methyl cap or a poly A tail. It is possible that the secondary structure present at the 5′ and 3′ untranslated regions (UTR) may be important in protecting plastid transcripts from degradation.

Secondary structure present at the 3′ ends of nuclear encoded mRNAs has been shown to be involved in transcript stability in many systems. For example, in eukaryotes histone mRNAs lack a poly A tail and instead contain a special stem loop structure in the 3′ region that confers stability to the message (Ross and Kobs,1986). AU rich sequences present at the 3′ ends of certain eukaryotic transcripts may be responsible for determining the steady state levels of the mRNA. c-fos mRNA contains such an AU sequence at its 3′ end that is implicated in destabilizing the message by enhancing the removal of the poly A tail (Shyu et al., 1991).

Plastid mRNAs also contain IR sequences in their 3′ UTR which do not serve as transcription terminators as they do in prokaryotic cells, but rather serve as RNA processing and stabilizing elements (Stern and Gruissem, 1987). Transcripts

which contain these 3′ IR sequences are more stable in chloroplast extracts than are transcripts without these sequences. In fact, the 3′ IR has been identified as having a potential role in mRNA stability in vivo. In a recent study, deletions of all or part of the 3′ IR sequence of the atpB transcript (which encodes the ß subunit of the ATP synthase complex) results in a decrease in atpB transcript accumulation even though the transcriptional activity of the gene remains the same in Chlamydomonas reinhardtii transformants (Stern et al.,1991). These results suggest that the 3′ IR sequences may be involved in mRNA stabilization. Perhaps plastid 3′ IR sequences fold into stem loop structures in vivo which act to stabilize the mRNA by protecting against 3′ to 5′ nucleolytic degradation.

RNA processing events may also be involved in the stability of mRNAs in plastids. It has been shown that the 3′ IRs serve as accurate processing signals in vitro and that processed transcripts are more stable in chloroplast extracts than those transcripts that have not been processed (Stern and Gruissem, 1987). The 3′ IRs may function as RNA processing sites or as protective structures against nucleolytic degradation. Perhaps processing and stability are overlapping events in plastids and without mature 3′ ends plastid transcripts are rapidly degraded.

Secondary structure present at the 5′ ends of plastid mRNAs may also be important in transcript stability by

protecting against 5′ to 3′ nucleolytic degradation. Although no 5′ to 3′ exonuclease has yet been isolated from chloroplasts, Sieburth et al have reported a 5′ to 3′ exonuclease activity in chloroplast extracts of Chlamydomonas reinhardtii (Sieburth et al, 1991). If such a nuclease exists it would suggest that certain chloroplast transcripts are degraded in a 5′ to 3′ direction. Mutants of Chlamydomonas reinhardtii have been isolated that are affected in the stability of chloroplast transcripts (Kuchka et al., 1989;Sieburth et al., 1991). It has been suggested that these transcripts are degraded in a 5′ to 3′ direction. Although the exact mechanism by which these plastid mRNAs is degraded is unclear, the characterization of such mutants is important because it will help to elucidate the mechanisms of mRNA stability in plastids.

In prokaryotic cells many examples are known of mRNAs being degraded in a 5′ to 3′ direction. For example, in Escherichia coli, ompA and bla mRNA degradation is initiated by site specific cleavages at their 5′ ends which is catalyzed by a novel endonuclease, RNase K (Lundberg et al., 1990). Also in E. coli, the mRNA for the tryptophan operon is degraded in a 5′ to 3′ direction (Morikawa and Imamoto, 1969). In bacteriophage T4, the gene32 mRNA is stabilized by sequences at its 5′ UTR which interact with a trans-acting factor which confers stability to the message (Gorski et al.,1985).

Trans-Acting Factors Involved in Plastid mRNA Stability

In addition to sequences present on the mRNA itself, other factors are likely to be involved in the stability of plastid mRNAs. Such trans-acting factors include RNA-binding proteins that recognize either specific sequences or secondary structure present on the mRNA. Perhaps these proteins act to protect the mRNA from nucleolytic degradation, or they may serve a more indirect role such as guiding the message to ribosomes and this association stabilizes the message.

Trans acting factors that bind to sequences in the 3′ UTR of mRNAs and play a role in transcript stability have been identified in many systems. For example, human transferrin mRNA stability is regulated by a specific cytoplasmic factor that binds to the 3′ UTR (Mullner et al.,1989) A cytosolic factor has been identified that binds to the c-myc AU rich element and destabilizes the message (Shaw and Kamen,1986)

Proteins have been identified that bind to the 3′ IR sequences of plastid transcripts in vitro (Stern and Gruissem,1989). The binding of some of these proteins to RNA is believed to be involved in chloroplast transcript stability possibly by covering nuclease sensitive sites present on the message. Some of the proteins identified appear to be transcript specific and it has been suggested that these may function in determining the differential stability of the mRNA Other proteins have been identified which bind to the 3′ end of all plastid transcripts. It has been implied that these

proteins may be involved in 3′ end processing of plastid transcripts. Recently, a nuclear encoded 28 KDa protein was isolated from spinach chloroplasts that binds to both precursor and processed 3′ ends of psbA, rbcL, and petD mRNA (Schuster and Gruissem,1991). This protein copurifies with a 3′ end processing activity in chloroplast extracts. Depletion of this protein from chloroplasts results in the lack of 3′ end processing and therefore decreases the stability of chloroplast transcripts. This suggests that this protein is involved in the processing and/or stability of plastid 3′ RNAs. Perhaps the 28 KDa protein has an RNA processing activity that recognizes plastid 3′ end precursors and converts them into 3′ end products that are more stable.

It is also possible that trans-acting factors play a role in transcript stability by binding to the 5′ UTR. This has been found to be the case in some systems. For example, as previously described the bacteriophage T4 gene32 mRNA is stabilized by the binding of a specific trans acting factor to the 5′ UTR (Gorski et al.,1985). Additionally, there are known examples of trans acting factors which bind to the 5′ UTRs of mitochondrial transcripts in order to confer transcript stability (Dieckmann and Mittelmeier,1987;Papadopoulou et al.,1990).

The association of the mRNAs with ribosomes may be important in determining the rate of turnover of certain transcripts. In eukaryotes, the stability of the ß-tubulin

mRNA depends on the recognition of free αß dimers with the first four amino acids of the nascent polypeptide chain as they emerge from the ribosome (Yen et al.,1988). As the concentration of free αß tubulin dimers increases, free dimers bind (or activate other factors which bind) directly to the nascent polypeptide chain. This binding appears to activate a nuclease which then degrades the ß tubulin mRNA while it is still associated with polysomes. Alternatively, the binding of factors to the nascent polypeptide chain could induce the ribosome to stall on the mRNA which would then leave the RNA in an altered configuration so that nuclease sensitive sites are exposed.

Perhaps the association of plastid mRNAs with ribosomes also plays a role in transcript stability. Ribosomes could protect the mRNA from degradation by covering possible nuclease cleavage sites. However, in spinach chloroplasts, ribosome association of psbA and rbcL mRNAs is not required to increase the stability of the transcripts (Klaff and Gruissem,1991) In fact, ribosome association may actually be involved in the degradation of both mRNAs. Perhaps the polysome complex is associated with a nuclease which degrades the mRNA, or the association of the message with ribosomes alters the secondary structure of the mRNA so that it is more susceptible to nucleases.

The nac2-26 mutant of Chlamydomonas reinhardtii is affected in psbD mRNA stability

The nac2-26 mutant of Chlamydomonas reinhardtii is affected in the stability of the chloroplast encoded psbD mRNA which encodes the D2 protein of photosystem II (see figure 1)(Kuchka et al.,1989). Northern analysis has indicated that this mutant does not accumulate any steady state levels of the psbD transcript. However, in vivo pulse labeling of total RNA from nac2-26 cells has shown that the psbD mRNA is initially transcribed. In addition, this mutant fails to accumulate any PS II core or OEE (oxygen evolving enhancer) proteins. This phenotype is unique to the nac2-26 mutant because all other mutants that are deficient in the D2 protein accumulate trace amounts of the PSII core proteins and they accumulate OEE proteins at wild type levels. This suggests that the nuclear gene product may have a dual role in wild type cells. First it may serve to stabilize the psbD mRNA and second, it may serve to anchor the other PS II core proteins to the thylakoid membrane. Alternatively, it may be that the nac2-26 mutant is the tightest D2 mutant in that it allows for no D2 expression. Other nuclear mutants which affect D2 expression may allow for low levels of D2 expression. Perhaps the D2 protein itself is required for PS II assembly and in the absence of the D2 protein no other PS II proteins can assemble on the thylakoid membrane. It is also possible that the NAC2 gene product may affect the expression of other unidentified proteins which are involved in PS II assembly.

Understanding the involvement of the NAC2 gene product in psbD message stability will further elucidate one role of the nuclear genome in chloroplast biogenesis. It is possible that the NAC2 gene product interacts with secondary structure present on the psbD mRNA. The 5′ and 3′ ends of the psbD message are like most plastid transcripts in that they contain sequences that can be folded into stem loop structures to which the NAC2 gene product may interact in order to prevent nucleolytic degradation. As mentioned earlier, in some cases the 3′ ends of plastid mRNAs have been found to contain inverted repeat sequences and these IRs serve as RNA processing and stabilizing elements (Stern and Gruissem,1987). However several lines of evidence suggest that it is not the 3′ end that is important in psbD transcript stability. A study performed by Rochaix et al. (in press) seems to indicate that the 3′ end of the psbD mRNA is not likely to be involved in transcript stability. In this experiment a chimeric gene was constructed in which the 3′ untranslated region of the psbD gene replaced the 3′ end of a reporter gene, atpB, which encodes the ß subunit of the ATP synthase complex. This was done in order to determine if the transcript from this chimeric construct would be stable in the nac2-26 background. The results from this experiment have shown that the chimeric transcript is present in wild type levels in the nac2-26 mutant background. This indicates that the 3′ UTR of the psbD transcript alone is not sufficient to destabilize the message

in nac2-26 cells.

In Chlamydomonas reinhardtii the psbD gene is co-transcribed with the psaA exon 2. The psaA message is transcribed from three widely spaced exons that are trans-spliced in order to form the mature message, which then encodes a protein of photosystem I (Kuck et al.,1987). Splicing mutants have been isolated which fail to slice psaA correctly and result in splicing intermediates which contain the psbD transcript upstream of psaA sequences (figure 2). These splicing intermediates are not present however in nac2-26/psaA splicing double mutants. This suggests that when no free 3′ end of the psbD mRNA is present, the message is still destabilized in nac2-26 cells. Therefore, it is possible that other regions of the psbD mRNA are important in conferring message stability.

In other organellar systems, it has been shown that nuclear encoded proteins interact with the 5′ UTR of transcripts. For example, the 5′ UTR of the mitochondrial cytochrome b transcript interacts with the nuclear encoded CBP1 protein to stabilize the cytochrome b transcript (Dieckmann and Mittelmeier,1987). Also in yeast mitochondria, a 40 KDa nuclear encoded protein has been found to interact with the 5′ UTR of the cox II transcript and this protein may also serve to confer message stability (Papadopoulou et al.,1990). It is not unlikely that the nuclear encoded NAC2 gene product is a protein that interacts with the 5′ UTR of

the psbD transcript. Perhaps the NAC2 gene product interacts with a putative stem loop structure present at the 5′ end of the message and confers stability to the message. The NAC2 gene product may stabilize the psbD mRNA by preventing 5′ to 3′ nucleolytic degradation or by altering the secondary structure of the message so that it is more stable. Alternatively, the NAC2 gene product may affect the stability of the psbD message in a more indirect manner. It is possible that the NAC2 gene product affects other unidentified trans-acting factors which bind in order to stabilize the message. Another indirect role of the NAC2 gene product in conferring message stability may be to guide the psbD message to chloroplast ribosomes. Perhaps in the absence of the NAC2 gene product the message no longer associates with ribosomes and is therefore degraded.

The focus of this thesis was to elucidate possible mechanisms involved in the destabilization of the psbD mRNA in the nac2-26 strain of Chlamydomonas reinhardtii. Transcription run on of the psbD gene was performed in order to determine the extent of transcription in nac2-26 cells as compared to wild type. Also, experiments were performed in which cytoplasmic protein synthesis was inhibited to test the effect of this inhibition on psbD mRNA accumulation. Additionally, the 5′ sequences of the psbD gene were amplified by the polymerase chain reaction and cloned into a transcription vector in order to synthesize a 5′ psbD

transcript. Chloroplast soluble and membrane proteins were isolated from Chlamydomonas reinhardtii cells so that they may be used in future experiments. These future experiments are designed to identify chloroplast proteins that interact specifically with 5′ sequences of the psbD mRNA. Understanding the mechanisms in which the psbD mRNA is stabilized in Chlamydomonas will strengthen our understanding of post transcriptional regulation of transcript stability in plastids.

Materials and Methods

1) Strains, Culture Conditions and Inhibitor Treatment

Chlamydomonas reinhardtii strains wild type (WTCC125), cw15 (a cell wall-less mutant) and the photosynthetic mutant nac2-26 were grown on Sager-Granick media supplemented with acetate (Harris,1988). Cells were grown in the presence of continuous dim light at 25° C to a concentration of 2×10^7 cells/ml.

For protein inhibitor studies WTCC125 cells were treated with 10μg/ml of cycloheximide to inhibit cytoplasmic protein synthesis (Harris,1988). To inhibit chloroplast transcription WTCC125 cells were treated with rifampicin at a concentration of 250μg/ml (Surzycki,1969). Times of incubation are indicated under the results section of this thesis.

2) RNA Isolation and Northern Analysis

Total RNA was isolated by the miniprep procedure described by Rochaix et al. (1988). Equal amounts of RNA (5μg) were separated on 1.2% denaturing formaldehyde agarose gels (Lehrach et al.,1977). RNA was transferred to nitrocellulose filters (Goldshcmidt-Clermont,1986) and hybridized with a psbD specific nick translated probe. The source for the psbD probe was the plasmid pH3 which contains the entire psbD gene, a kind gift from Dr. Jeanne Erickson (Erickson et al.,1986). Prehybridization and hybridization

conditions are those described by Kuchka et al. (1988).

3) In Vivo Pulse Labeling of Total RNA

Five-hundred milliliters of C. reinhardtii cells were grown to mid log phase, concentrated to 7 x 10^7 cells in one milliliter and permeabilized by toluene treatment (Guertin and Bellemare,1979). RNA was pulse labeled for five minutes with ^{32}P-UTP . Total RNA was extracted by the miniprep procedure (Rochaix et al.,1988) and hybridized to psbD HincII restriction fragments immobilized on nitrocellulose filter paper. Transfer conditions as well as prehybridization and hybridization conditions were carried out as previously described under section 2, materials and methods.

4) Plasmid Construction and in vitro transcription

The plasmid pGR$_1$220 was constructed in the following manner: A 220 b.p. fragment containing the 5′ UTR sequences and some coding sequences of the psbD gene was amplified by the polymerase chain reaction (PCR). The primers used for the PCR were synthesized in a DNA synthesizer (Biosearch) by the phosphoramidite chemical synthesis, and purified by elution through a Sephadex G10 column. These primers are complementary to sequences present on the pH3 plasmid and flank the sequences we wished to amplify (for sequences see table 1). Both primers contain EcoR$_1$ linkers at both ends so that when the PCR product is digested with EcoR$_1$ it will

contain $EcoR_I$ ends. Twenty-five cycles of the polymerase chain reaction were used to amplify this sequence. The cycle conditions for the PCR were as follows: Round 1- 94° C for 1 minutes; Round 2- 37° C,1 minutes; Round 3- 72° C,2 minutes. The 220 bp fragment was purified by extraction from an agarose gel using the Clean-A-Gene method (Andes Scientific Company).

The 220 b.p. PCR fragment carrying the 5′ sequences of the psbD gene was ligated into the transcription vector pGEM 3z (Promega) which was linearized with $EcoR_I$. E. coli JM109 cells were transformed with the ligation mixture and transformants were grown in LB media containing 25μg/ml ampicillin (Sambrook et al.,1989). Transformants which contained the 220 b.p. insert were identified and the orientation of the insert determined by restriction digestions. The pGR_I220 plasmid was isolated from the cells by the procedure described in Sambrook et al. (1989).

pGR_I220 was digested with appropriate restriction endonucleases in order to provide a template for the generation of sense and anti-sense RNAs in vitro. RNA was transcribed from these templates in vitro by the following reaction: 1μg of template DNA, 20 units of SP6 or T7 RNA polymerase, 2.5mM NTPs, 1mM DTT, and 50 μCi ^{32}P-UTP were incubated for one hour at 37° C in a 5x transcription buffer (Promega). The DNA template was removed by digestion with 1 unit of RNase free DNase at 37° C for 15 minutes. RNA was isolated by ethanol precipitation in the presence of one tenth

volume of sodium acetate and collected by centrifugation. The concentration of RNA was determined by spectrophotometry at a wavelength of 260 nm. RNA was visualized on 5% denaturing polyacrylamide gels (Sambrook et al.,1989). Gels were autoradiographed overnight at -70° C.

5) Isolation of Intact Chloroplasts and Soluble Chloroplast Proteins

Intact chloroplasts were isolated from cw15 cells by the Mendiola-Morgenthaler method (1985). Chloroplasts were separated from intact cells and broken plastids on 40-85% Percoll step cushions as described by Weissbach and Weissbach (1988).

Soluble chloroplast proteins were isolated by a procedure adapted by Gruissem et al. (1986) Briefly, chloroplasts were resuspended in Buffer A (10mM Tris-Hcl,pH 7.9, 1mM EDTA, 5mM DTT), broken in a Yeda press at 1000 psi, and released into a cold graduated cylinder. Lysed chloroplasts were spun at 80,000g for 30 minutes at 4° C to remove membrane material. The supernatant from this spin contains soluble proteins and the pellet contains membrane proteins. Soluble proteins were precipitated with 55% ammonium sulfate, incubated on ice with gentle stirring for 1 hour, and collected by centrifugation at 85,000g for 30 minutes at 4° C. The protein pellet was resuspended in Buffer D (20mM Hepes,pH7.9, 60mM KCl, 12.5 mM MgCl2, 0.1mM EDTA, 2mM DTT, 17% glycerol) and dialyzed at 4°

C against 2 changes of Buffer D. Proteins were separated on 7.5%-15% polyacrylamide gels according to the protocol of Chua (1980) and visualized by silver staining as described by Goldman et al. (1981). Protein concentrations were quantified using the Bio-Rad protein assay (Biorad).

Results

The nac2-26 mutant of Chlamydomonas reinhardtii does not accumulate psbD mRNA

Northern analysis performed by Kuchka et al. (1989) demonstrated that the nac2-26 mutant of Chlamydomonas reinhardtii does not accumulate any psbD mRNA which encodes the D2 protein of PS II. This experiment was repeated to confirm the phenotype of the nac2-26 mutant. Equal amounts of total RNA from wild type and nac2-26 cells were separated on denaturing agarose gels, transferred to nitrocellulose filters, and hybridized with a psbD specific nick translated probe. As shown in the Northern blot pictured in figure 3, the psbD mRNA is present in wild type cells but missing in the nac2-26 mutant, which is consistent with the finding of Kuchka et al. (1989). Other studies of Kuchka et al. (1989) indicate that messages encoding other chloroplast proteins are present in wild type levels in this mutant. It therefore appears that the mutation at the nuclear NAC2 locus affects only the accumulation of the chloroplast encoded psbD mRNA.

Transcription of the psbD gene in wild type and nac2-26 cells

Although there is no accumulation of the psbD mRNA in nac2-26 cells, previous studies have shown that the psbD mRNA is initially transcribed in this mutant (Kuchka et al.,1989). It is therefore possible that the lack of the psbD mRNA in nac2-26 cells is the result of abortive transcription

initiation or increased message turnover. In order to test the first possibility, wild type and nac2-26 cells were permeablilized by treatment with toluene and RNA was pulse labeled for five minutes with ^{32}P-UTP (Guertin and Bellemare,1979). Labeled transcripts were isolated from the cells and hybridized to psbD restriction fragments immobilized on nitrocellulose filters (see figure 4). If psbD transcription initiation is aborted prematurely in nac2-26 cells, we would expect to see hybridization of run on transcripts with only psbD restriction fragments which represent the 5' end of the gene. Alternatively, if psbD transcription procedes past the initiation step, we would expect to see hybridization with restriction fragments across the gene. The results shown in figure 4 demonstrate that pulse labeled RNA from wild type and nac2-26 cells hybridizes similarly to the psbD restriction fragments. Indeed, labeled RNA from nac2-26 cells hybridizes to restriction fragments which span the entire psbD coding sequences. This experiment suggests that the lack of the psbD mRNA in nac2-26 cells is not the result of abortive transcription initiation. It is therefore very likely that the psbD mRNA is turned over at an increased rate in the nac2-26 cells. Perhaps the NAC2 gene product serves to stabilize the psbD mRNA and in its absence the message is rapidly degraded.

Effect of inhibiting cytoplasmic protein synthesis on the accumulation of psbD mRNA

The mutation at the nuclear NAC2 locus affects the accumulation of the psbD mRNA. It is possible that the nuclear gene product is a protein that is required for psbD transcript accumulation. In order to test this possibility, cytoplasmic protein synthesis was inhibited with cycloheximide to see the effect of this inhibition on psbD mRNA accumulation. WTCC125 cells were treated with 10μg/ml of cycloheximide and total RNA was isolated after 0 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours, and 6 hours of treatment. RNA was separated on denaturing agarose gels and hybridized with a psbD specific probe. The Northern blot pictured in figure 5 demonstrates that the psbD mRNA is present six hours after treatment with cycloheximide. However, the levels of the message seem to decrease after four hours of exposure to the antibiotic. Additionally, the levels of the psbD message appear to increase slightly at six hours as compared to the four hour time point. This result suggests that the psbD mRNA is normally quite stable in wild type cells for at least two hours after the inhibition of cytoplasmic protein synthesis. The decreased levels of the message after four and six hours of treatment may be due to an increased turnover of the message as a result of the turnover of cytoplasmic proteins which are involved in psbD message accumulation. Slight variations in RNA loadings may account

for the increased levels of the psbD mRNA at six hours. Alternatively, the concentration of cycloheximide may have been limiting which could result in an increase in the accumulation of the message six hours after treatment.

Construction of the pGR_1220 plasmid and synthesis of in vitro transcripts

The 5' UTR of certain mRNAs is implicated in transcript stability in some systems (Dieckmann and Mittelmeier,1987; Papadopoulou et al.,1990; Gorski et al.,1985). To determine if the 5' sequences of the psbD mRNA bind to specific trans acting factors which may serve to stabilize the mRNA. To this end an in vitro transcript was synthesized that contains 5' end sequences of the psbD mRNA. To synthesize this transcript, the plasmid pGR_1220 was constructed (for cloning strategy see figure 6). This plasmid consists of a 220 base pair insert of psbD sequences cloned into the transcription vector pGEM-3z . The 220 base pair insert contains the 5' UTR and some coding sequences of the psbD gene. This insert DNA was generated by the polymerase chain reaction in the following manner: Synthetic oligonucleotides were used as primers for the PCR which are complementary to sequences of the pH3 plasmid (Erickson et al.,1986) and flank the 220 base pair region of interest (for primer sequences see table 1). Figure 7 shows a picture of an Ethidium Bromide stained gel indicating the size of the PCR product which is 220 base pairs. The 220 base pair fragment was cloned into the $EcoR_1$

restriction site of the pGEM-3z plasmid and the orientation was determined by restriction digestions. The map of this construct is illustrated in figure 8.

pGR_1220 was digested with the restriction endonuclease Taq_1 and used as a template for an in vitro transcription reaction. Using the T_7 promoter a 160 base transcript was synthesized that contains the 5' UTR and some coding sequences of the psbD mRNA (see figure 9, lane 3). A 5' psbD anti-sense transcript was also synthesized by digesting pGR_1220 with Nde_1 and using the SP_6 promoter. This transcript will be used as a competitor RNA to determine the specificity of proteins binding to sequences of the 5' psbD mRNA. The anti-sense transcript is 300 bases in size and is also pictured in figure 9. The concentrations of these transcripts were determined by spectrophotometry at a wavelength of 260 nm. The yield for both transcripts was approximately 1μg/μl. These transcripts will be used for gel shift assays and UV crosslinking experiments in order to determine if proteins interact with the 5' UTR of the psbD gene.

Isolation of soluble chloroplast proteins from Chlamydomonas

Gel shift assays and UV crosslinking experiments can be performed in order to determine if proteins bind specifically to the 5' UTR of the psbD mRNA. For these experiments chloroplast soluble proteins were isolated from cw15 cells by the method described in section 5 of Materials and Methods.

The concentration of the chloroplast soluble proteins was approximately 1μg/μl as determined by the BioRad protein assay(Biorad). The silver stained gel pictured in figure 10 shows total soluble proteins, total membrane proteins, chloroplast soluble proteins and chloroplast membrane proteins from cw15 cells. This picture demonstrates that soluble chloroplast proteins from cw15 cells were isolated by the identification of the large subunit of RUBISCO, a soluble chloroplast protein. The RUBISCO large subunit was identified based on its characteristic mobility through a gradient gel to its molecular weight of 55 KDa which is noted in figure 10 (M. Kuchka, personal communication). Also indicated in figure 10 are the LHC (light harvesting complex) proteins which are membrane proteins of the chloroplast (Harris,1988). Gel shift assays with soluble chloroplast proteins incubated with the 5' psbD sense and anti-sense transcript are currently underway. These experiments will identify proteins which specifically interact with the 5' region of the psbD mRNA. At present preliminary data are unclear as to whether or not proteins bind to this region. The identification of RNA binding proteins that bind specifically to the psbD mRNA may help to elucidate a possible mechanism involved in psbD transcript stability.

Discussion

The control of mRNA stability in plastids is an important post-transcriptional regulatory mechanism of plant gene expression. Several nuclear mutants have been isolated that are affected in the stability of chloroplast mRNAs (Kuchka et al.,1989; Sieburth et al., 1991). The characterization of these mutants will increase our understanding of how chloroplast mRNAs are specifically stabilized. The nac2-26 mutant of Chlamydomonas reinhardtii is affected in the stability of the psbD mRNA (Kuchka et al.,1989). The focus of this thesis is to define possible mechanisms by which the psbD mRNA is stabilized in Chlamydomonas reinhardtii.

The nac2-26 mutant of Chlamydomonas reinhardtii does not accumulate psbD mRNA. Previous in vivo pulse labeling of total RNA has shown that the psbD mRNA is initially transcribed in this mutant (Kuchka et al.,1989). It has been suggested that the lack of the psbD mRNA in nac2-26 cells is either due to abnormal transcription or increased message turnover (Kuchka et al.,1989). Transcription run off experiments suggest that the lack of the psbD mRNA in nac2-26 cells is not the result of abortive transcription initiation because RNA from the mutant hybridizes to psbD sequences that extend half way into the coding region of the gene. Therefore it is possible that the psbD mRNA is turned over at abnormally high rates in this mutant. However, another possibility exists that psbD transcription is terminated at sequences

closer to the 3' end of the gene, i.e. the psbD gene may not be transcribed in full in nac2-26 cells. Future experiments may be performed in order to test this possibility. For these experiments pulse labeled RNA from wild type and nac2-26 cells will be hybridized to restriction fragments that represent 3' end sequences of the psbD gene. These experiments will further elucidate the extent of transcription in nac2-26 cells.

The psbD mRNA is missing in nac2-26 cells due to a single nuclear mutation at the NAC2 locus (Kuchka et al.,1989). Perhaps cytoplasmic proteins are involved in the accumulation of the psbD message by either affecting the transcription or stability of the message. To test the effect of inhibiting cytoplasmic protein synthesis on the accumulation of the psbD mRNA, cells were treated with cycloheximide. Results show that the psbD transcript is still present in wild type cells six hours after treatment with cycloheximide. However, the levels of the psbD message appear to decrease after four hours of exposure to cycloheximide. This result suggests the psbD message is normally quite stable for at least two hours after the inhibition of cytoplasmic protein synthesis. This result does not exclude the possibility that nuclear encoded proteins are involved in transcription and/or accumulation of the psbD message during this time period. Perhaps proteins involved in transcript accumulation are stable during the two hours of treatment with the antibiotic. These proteins may then be

turned over after four hours which may account for the decrease in the accumulation of the message. It is unclear why the levels of the psbD message seem to increase slightly after six hours of treatment as compared to the four hour time point. The possibility exists that there were slight variations in the loadings of the RNA. Alternatively, it is possible that the concentration of cycloheximide used was limiting. Perhaps cytoplasmic protein synthesis was reinitiated after six hours resulting in the accumulation of proteins that are involved in psbD transcript accumulation. Given that these results are preliminary, the possibility cannot be ruled out that nuclear encoded proteins are not involved in psbD message accumulation. Additional experiments will have to be performed in order to approximate the steady state levels of the psbD transcript in wild type cells. By inhibiting chloroplast transcription with rifampicin (Surzyski,1964), any level of message present will be due to the steady state levels of the message in the chloroplast. Preliminary data suggest that the psbD transcript is still present after four hours of treatment with rifampicin (data not shown). This is consistent with the results of Malnoe et al. (1988) who demonstrated that the D2 protein accumulates to wild type levels when cells are treated with rifampicin for one hour. This study shows the steady state levels of the psbD transcript present after a one hour treatment with rifampicin is sufficient to produce wild type levels of the D2

protein. This study again suggests that the psbD mRNA is very stable in Chlamydomonas reinhardtii. Additional studies will need to be performed in order to further elucidate the affects of these inhibitors on the accumulation of the psbD transcript.

It is possible that secondary structure present at the 5' end of the psbD mRNA plays a role in message stability. The 5' end of this message contains sequences that may form stem loop structures in vivo. Also, a 5' to 3' exonuclease activity has been identified in chloroplast extracts of Chlamydomonas reinhardtii which suggests that chloroplast transcripts may be degraded in a 5' to 3' direction. The NAC2 gene product may be a protein that interacts with secondary structure present at the 5' end of the psbD message and protects the message from 5' to 3' nucleolytic degradation. This idea is further supported by known examples of nuclear encoded proteins that interact with the 5' UTR of certain transcripts to confer transcript stability (Dieckmann and Mittelmeier, 1987; Gorski et al.,1985). In order to determine if proteins associate with the 5' UTR of the psbD mRNA, gel shift assays and UV crosslinking studies will be performed with the 5' specific psbD transcript incubated with soluble chloroplast proteins. These experiments are designed to determine if there are soluble proteins present in the chloroplast of Chlamydomonas reinhardtii which interact physically with the 5' region of the psbD transcript. Through

UV crosslinking studies it will be possible to determine if there are differences between wild type and nac2-26 chloroplasts proteins which associate with the 5' UTR of the psbD mRNA. Identification of proteins from these two strains may show differences in the number of proteins which associate with the message. It is possible that we will see fewer proteins which interact with the psbD transcript in nac2-26 cells as compared to wild type cells . Because of the nuclear mutation in this strain, perhaps proteins that would normally associate with the message are no longer able to. If no protein differences are found between wild type and nac2-26 cells it may be that all of the proteins that associate with the transcript are still present in the mutant, however due to the nuclear mutation they may be altered in such a way that they are not detectable by these methods. These studies will also be performed with chloroplast membrane proteins to see if proteins associated with the thylakoid membrane associate with the psbD transcript.

Sequences other than those present on the 5' end of the message may important in transcript stability. The IR sequences present at the 3' ends of plastid transcripts have been found to be involved in transcript stability (Stern and Gruissem, 1987). It has also been demonstrated that proteins bind to these sequences in vitro and that the binding of these proteins may be involved in transcript stability (Stern and Gruissem,1989). However, it is not believed that the 3' end

of the psbD mRNA is involved in transcript stability for two reasons. First, a chimeric transcript that contains the 3' end of the psbD message fused to a reporter gene, atpB, is stable in the nac2-26 background (Rochaix, unpublished results). Second, transcripts that share the 5' sequences of the psbD mRNA, such as psbD-psaA-exon2 and psbD-psaA-exon2/exon3, are unstable in nac2-26 cells (Kuchka et al.,1989). These studies suggest that the 3' end of the psbD transcript alone is not involved in destabilizing the message in nac2-26 cells. Alternatively, sequences present in the coding region of the message may be important in transcript stability. This has been found to be the case for certain transcripts including c-fos and c-myc. Sequences in the coding region of these transcripts have been identified as playing an important role in controlling transcript stability (Shyu et al.,1991; Wisdom et al.,1991). Perhaps the binding of proteins to sequences other than those present at the 5' end are important in transcript stability. To test this, the entire psbD transcript will be synthesized and used in gel shift assays and UV crosslinking studies to determine if proteins bind specifically to sequences throughout the transcript.

Another indirect role of the NAC2 gene product may be to guide the psbD message to ribosomes. Perhaps the association of transcripts with ribosomes acts to protect the message from degradation. Several examples exist in which translation is

related to mRNA stability (Graves et al.,1987; Yen et al., 1988). However, it has been demonstrated that the association of plastid transcripts with ribosomes may actually be involved in the destabilization of the message (Klaff and Gruissem,1991).

Based on the evidence discussed, a model has been proposed as to how the NAC2 gene product may serve to stabilize the psbD mRNA (see figure 11). The NAC2 protein is transported into the chloroplast where it may function to stabilize the psbD mRNA by binding to secondary structure present at the 5' end of the message and protecting the message from 5' to 3' nucleolytic degradation. This model illustrates one possible mechanism by which chloroplast mRNAs are specifically stabilized. Obviously more evidence needs to be collected in order to substantiate this model. Future experiments are planned to test this model. These experiments include gel shift assays and UV crosslinking experiments with a 5' psbD transcript and a full length psbD transcript in order to determine if there are proteins which associate with the psbD mRNA.

Primer 1	5'	GGAATTCTAATTTTATTTTATATAAG 3'
Primer 2	5'	GGAATTCTGACCAACCTACGAATACG 3'

Table 1. Sequences of oligonucleotides used for PCR primers. Both primers contain ECOR1 linkers at their 5' ends which are underlined.

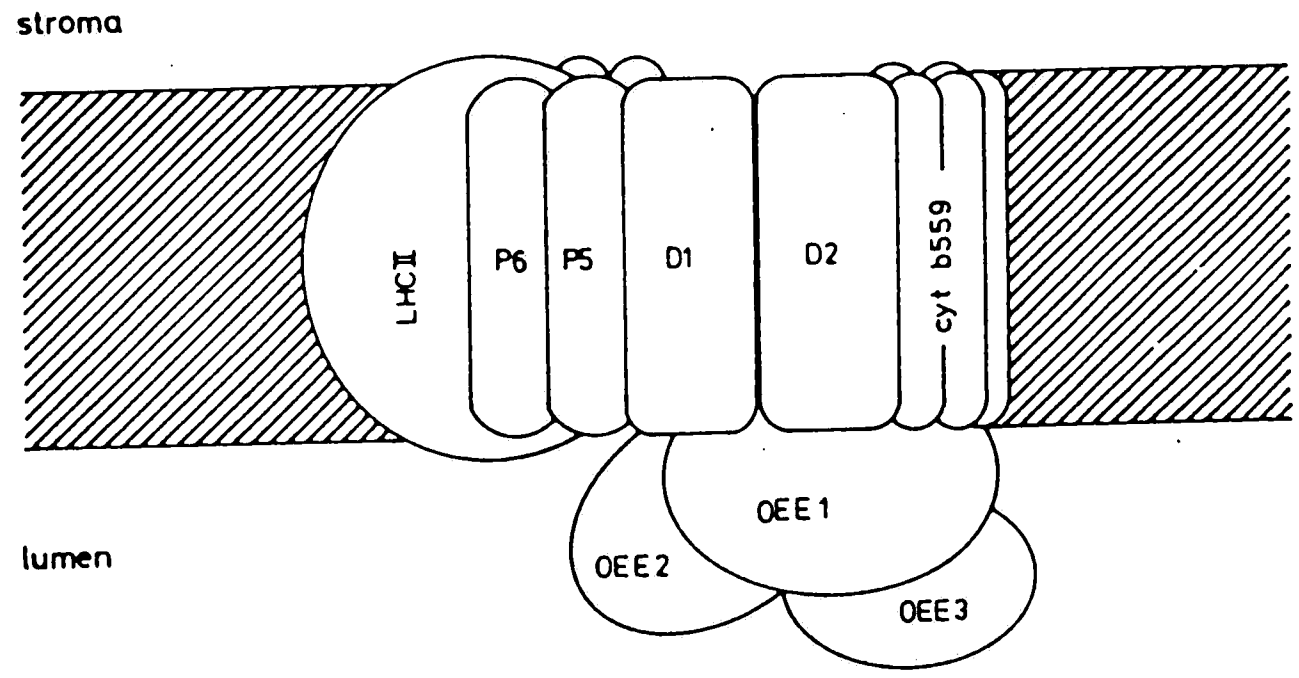


Figure 1. Schematic diagram of photosystem II in the thylakoid membrane of the chloroplast. Photosystem II is a multi-subunit complex composed of both nuclear and chloroplast encoded proteins. The proteins D1, D2, P5, P6 and the apoproteins of cytochrome b_{559} are encoded by chloroplast genes. The oxygen evolving enhancer proteins, OEE 1, -2, and -3 and the light harvesting complex, LHC II, are nuclear encoded. From Kuchka et al. (1989)

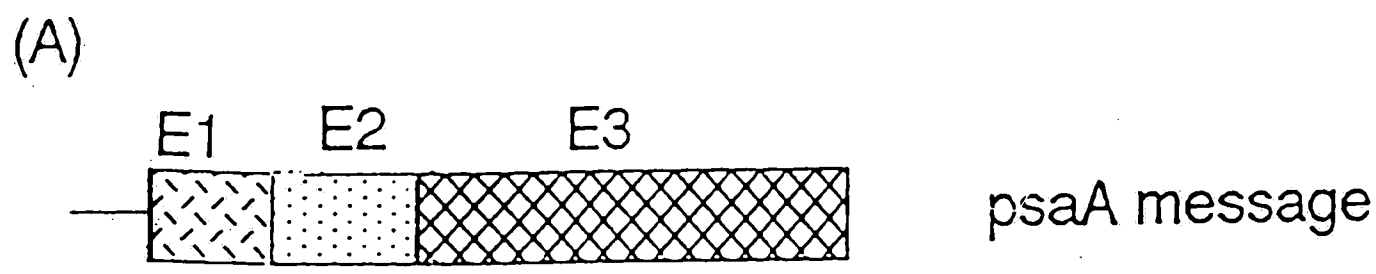


Figure 2. Splicing intermediates that contain the psbD transcript upstream of psaA sequences. (A) Diagram of the mature psaA message which is formed by the trans-splicing of three exons. (B) The psbD transcript is co-transcribed upstream of psaA exon 2. Splicing mutants exist that fail to splice the psaA exons correctly and result in splicing intermediates which contain the psbD transcript upstream of psaA sequences. (E1-psaA exon1, E2-psaA exon2, E3-psaA exon3)

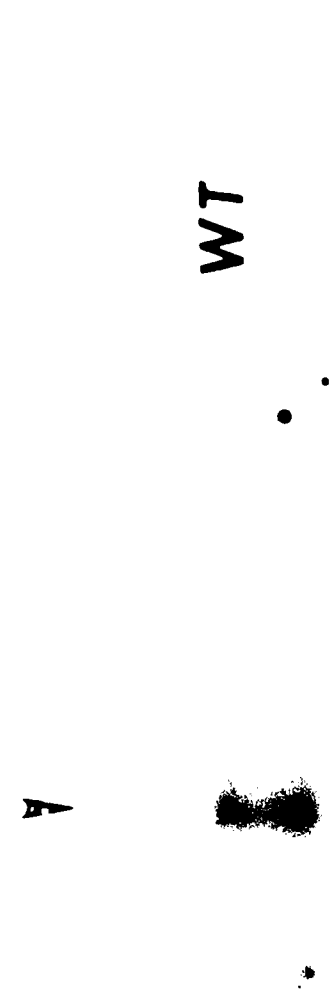


Figure 3. Northern Analysis of total RNA from wild type and nac2-26 cells. Equal amounts of RNA from wild type (WT) and nac2-26 cells were separated on denaturing formaldehyde gels, blotted to nitrocellulose filters, hybridized with a psbD specific probe, and autoradiographed.

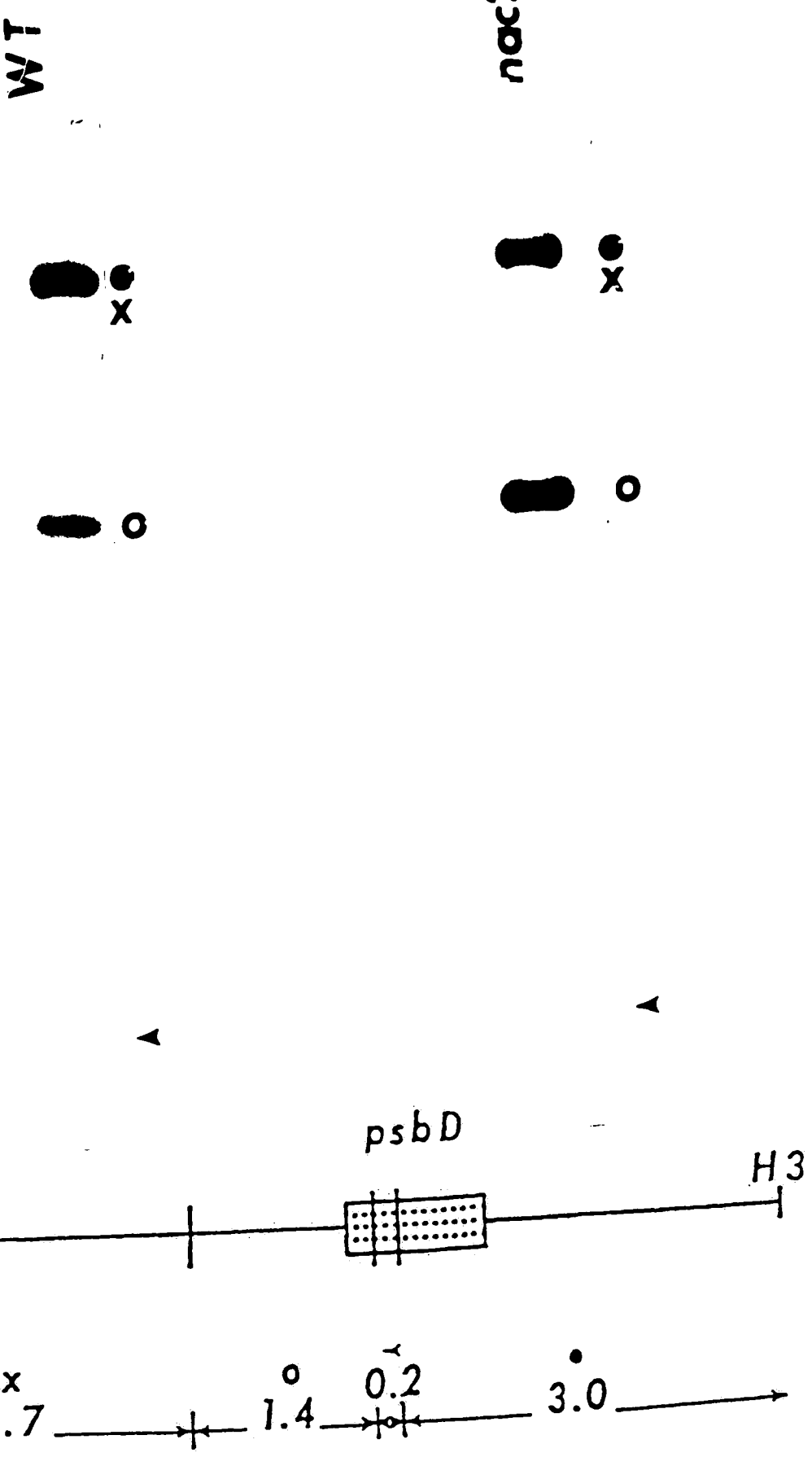


Figure 4. psbD transcription run on experiment. Pulse labeled RNA from toluene permeabilized wild type and nac2-26 cells was hybridized to restriction fragments of chloroplast DNA carrying psbD sequences.

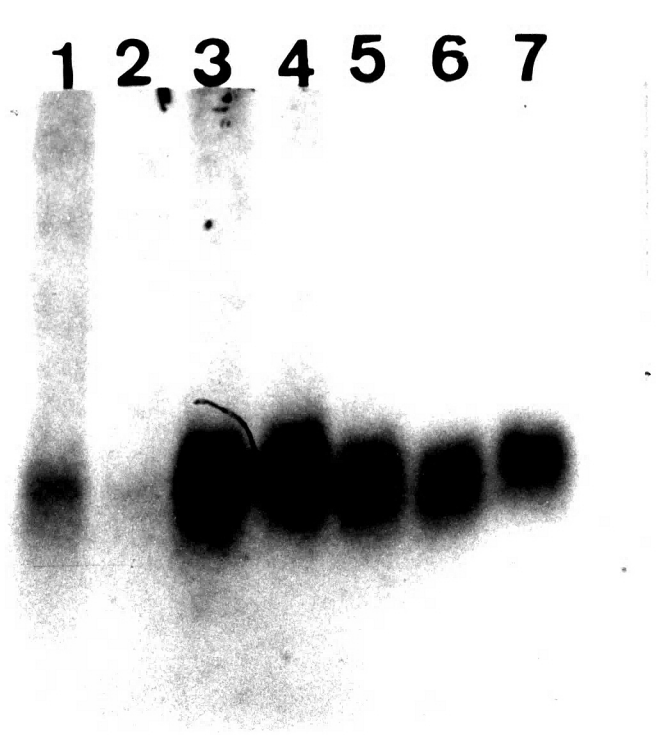
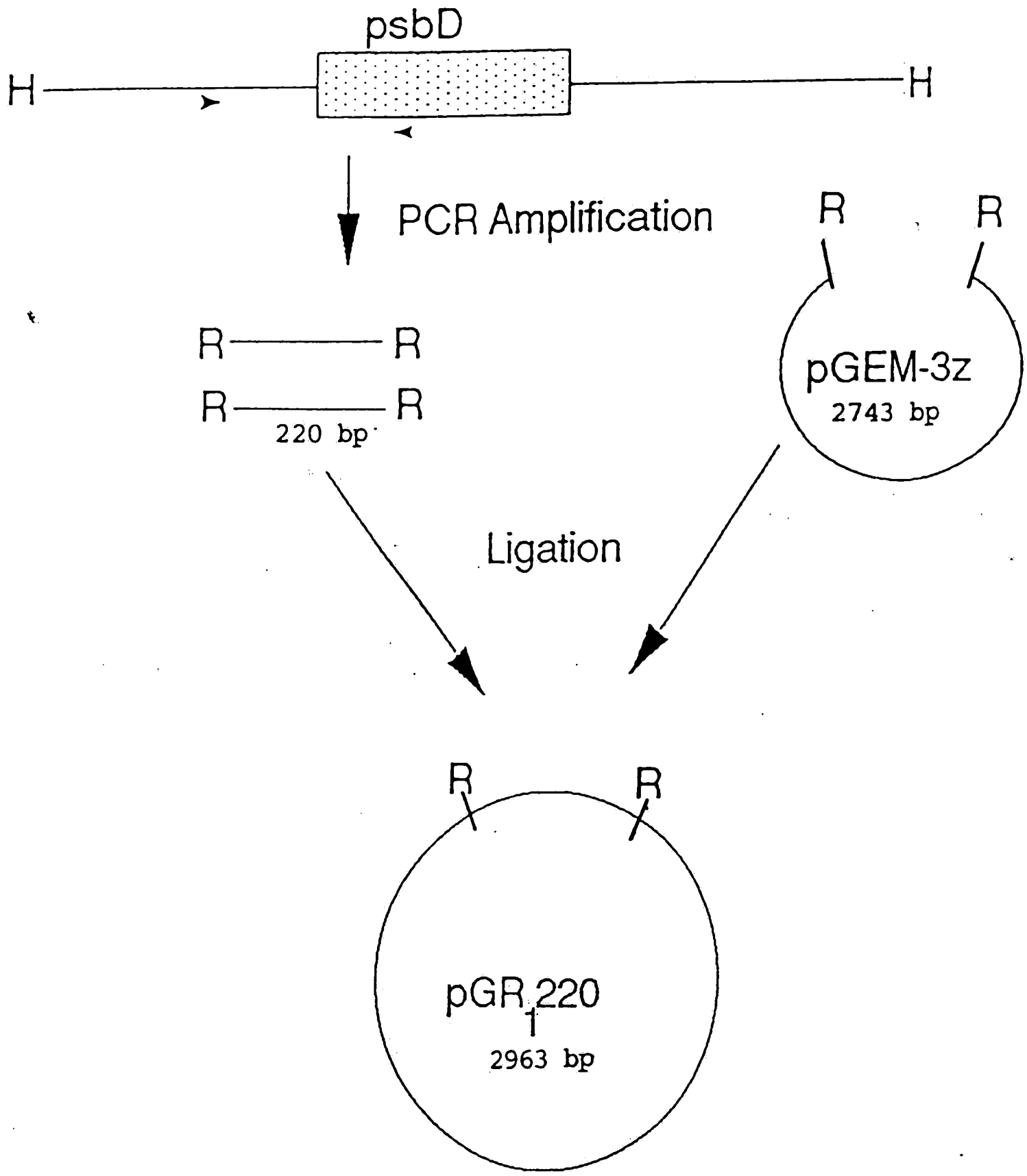


Figure 5. Effect of cycloheximide treatment on psbD mRNA accumulation. Wild type cells were treated with 10μg/ml of cycloheximide. Total RNA was isolated from these cells after 0 minutes, 30 minutes, 60 minutes, 2 hours, 4 hours, and 6 hours of treatment. RNA was separated on denaturing formaldehyde gels, transferred to nitrocellulose filters, hybridized with a psbD specific probe and autoradiographed. Lane 1-6 hours; Lane 2- 4 hours; Lane 3- 2 hours; Lane 4- 1 hour; Lane 5- 30 minutes; Lane 6- 0 minutes; Lane 7- wild type.



Not to scale

Figure 6. Strategy for the construction of the pGR_1220 plasmid. A 220 base pair fragment of 5' psbD sequences was amplified by the polymerase chain reaction and cloned into the EcoR1 site of the transcription vector pGEM-3z. (►) indicates PCR primers with ECOR1 linker ends. See table 1 for sequences. (HindIII-H, EcoR1-R)

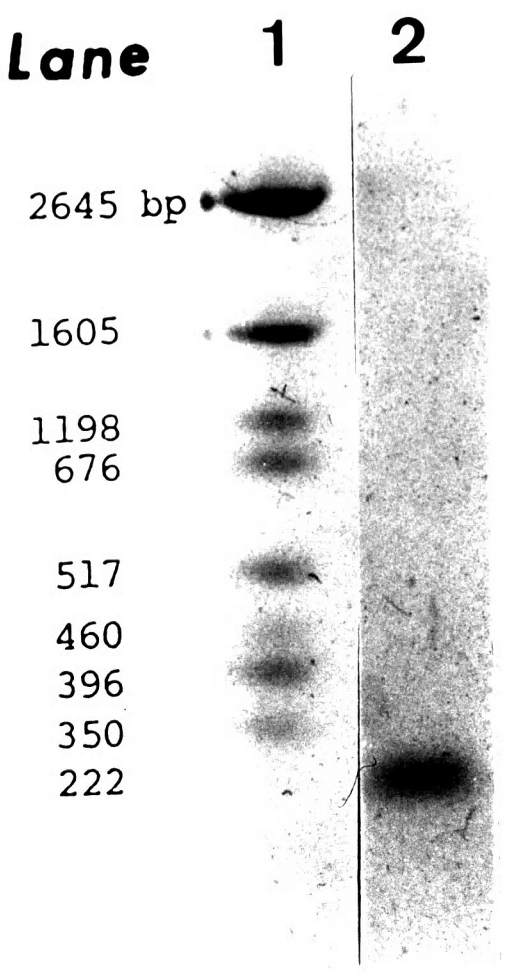
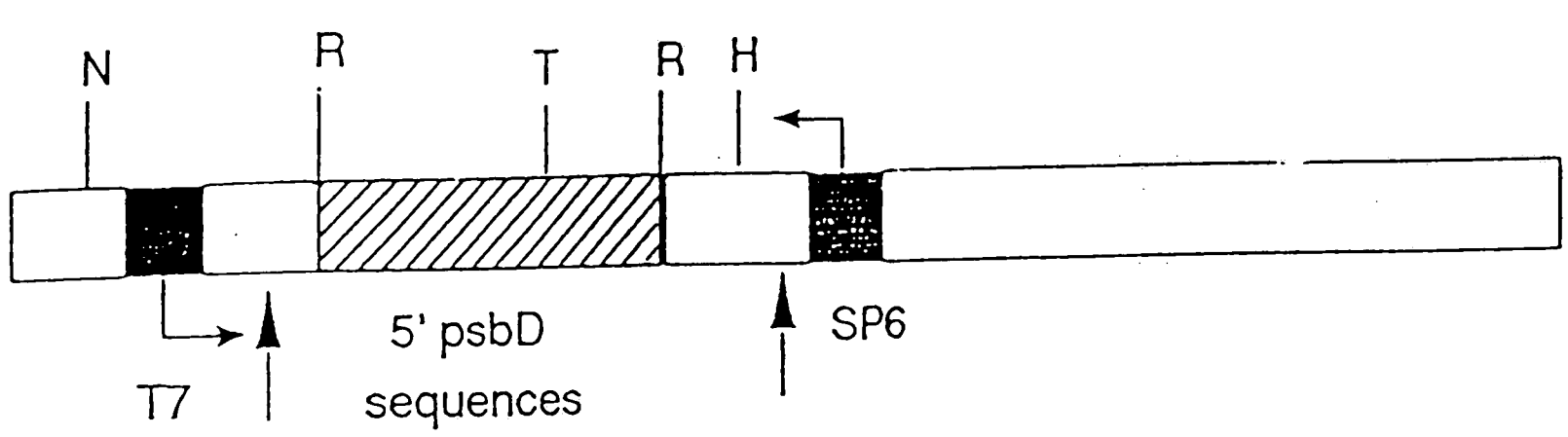


Figure 7. Ethidium bromide stained gel of the 220 base pair PCR product. The PCR product was separated onto a 2% agarose gel and stained with ethidium bromide. Lane 1- pGEM Markers; Lane 2-220 base pair PCR product.



Not To Scale

Figure 8. Map of the pGR_1220 plasmid. The pGR_1220 plasmid consists of a 220 base pair insert of 5' psbD sequences cloned into the vector pGEM-3z. psbD sequences are noted by the hatched box and vector sequences are indicated by the unshaded areas. The arrows indicate the transcription start sites, and the dark boxes represent the T7 and SP6 promoters. The restriction sites for Hind III (H), EcoR1 (R), Taq1 (T), Nde1 (N) are as indicated.

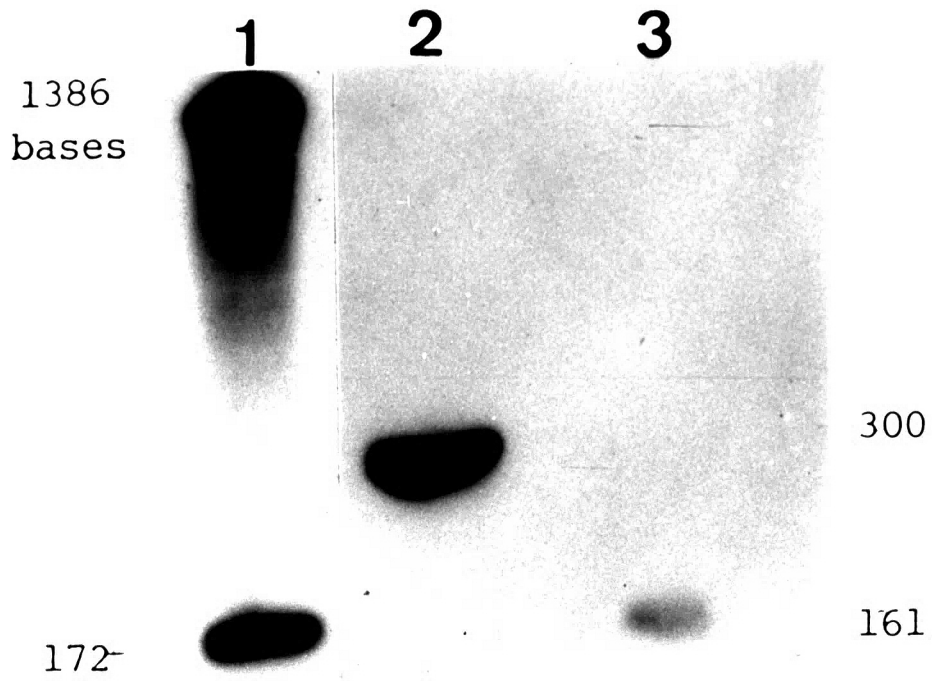


Figure 9. Autoradiograph of a 5% sequencing gel indicating the relative sizes of the psbD sense and anti-sense in vitro transcripts. RNA from in vitro transcription reactions were separated on a 5%-polyacrylamide/Urea gel. Lane 1- RNA markers; Lane 2- 5' psbD anti-sense transcript; Lane 3- 5' psbD sense transcript.

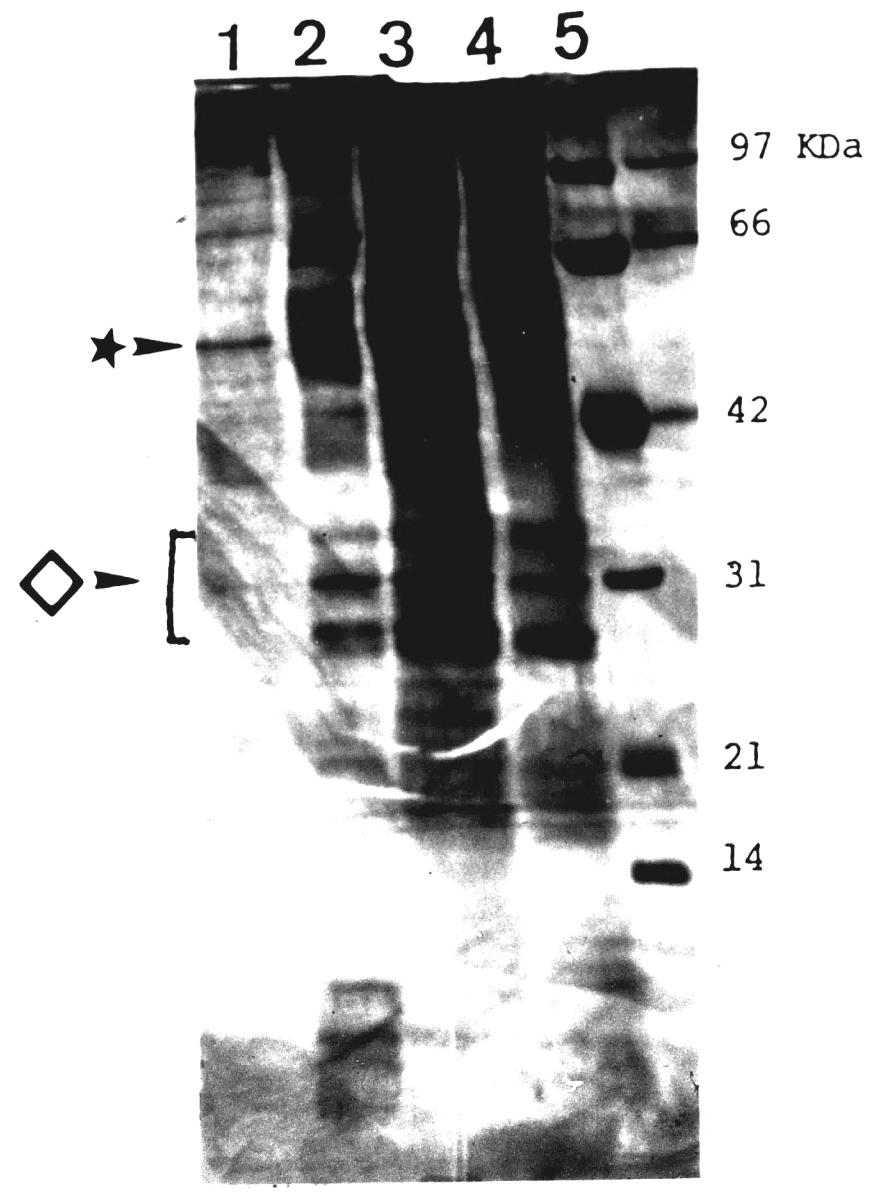


Figure 10. Silver stained gel of total proteins and chloroplast proteins from the cw 15 strain of <u>Chlamydomonas reinhardtii</u>. Proteins were isolated from cw 15 cells, separated on a 7.5% - 15% SDS-PAGE gel, and silver stained. Lane 1- chloroplast soluble proteins; Lane 2- chloroplast membrane proteins; Lane 3-total soluble proteins; Lane 4-total membrane proteins; Lane 5- molecular weight standards. (★) represents the large subunit of RUBISCO, and (◇) represents the LHC proteins.

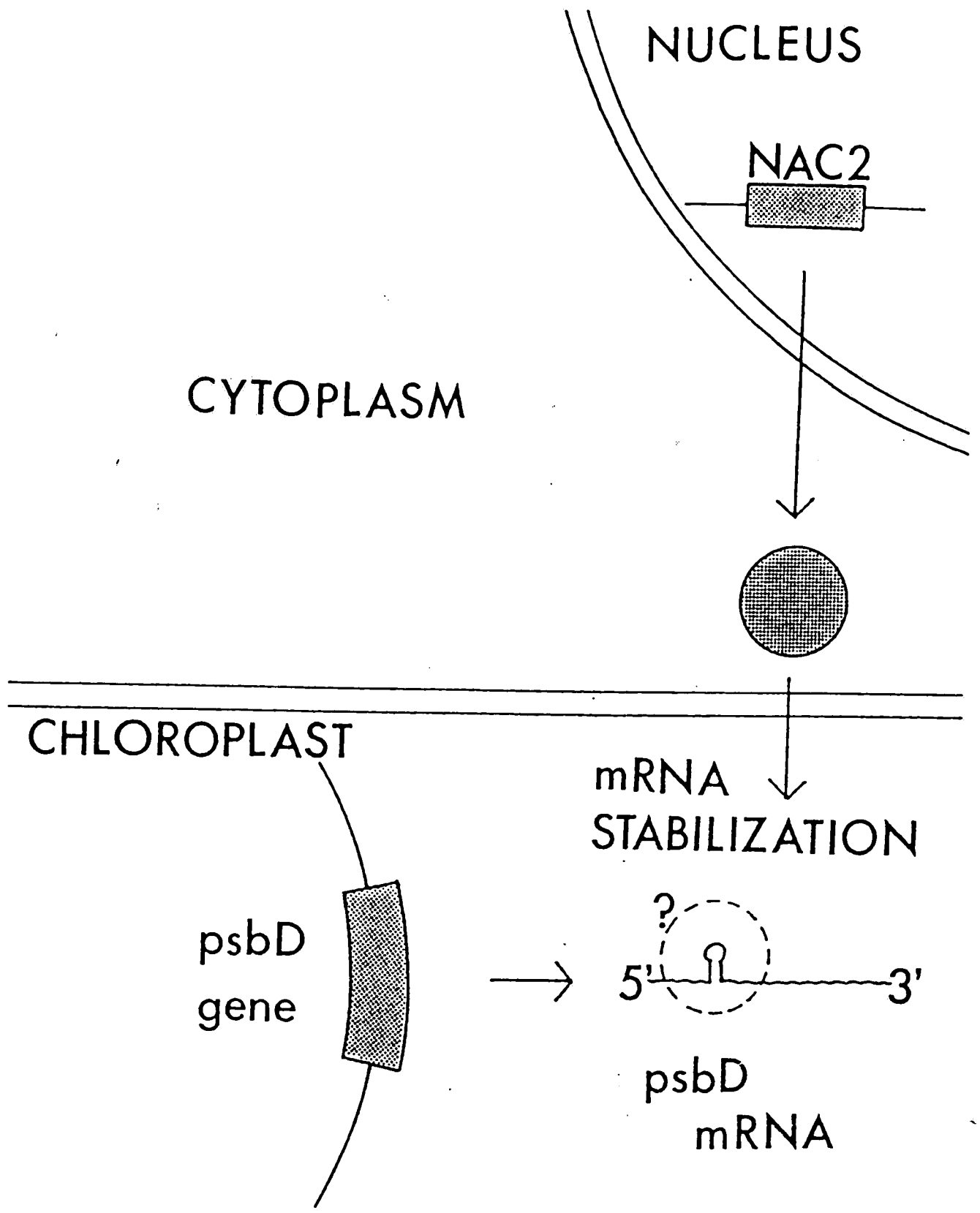


Figure 11. Proposed model of how the NAC2 gene product may act to stabilize the psbD mRNA. The NAC2 gene product may be a protein that is transported into the chloroplast where it binds specifically to 5' sequences of the psbD mRNA. Perhaps the binding of this protein protects the message from 5' to 3' nucleolytic degradation.

References

Berry, J.O., Nikolau, B.J., Carr, J.P., anf Klessig, D.F. (1986) Translational regulation of light induced ribulose 1,5- bisphosphate carboxylase gene expression in Amaranth. Mol. Cell. Biol. **6**:2347-3353.

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

Deng, X.-W., and Gruissem, W. (1987) Control of plastid gene expression during development: The limited role of transcriptional regulation. Cell **49**:379-387.

Deng, X.-W., Stern, D.B., Tonkyn, J.C. and Gruissem, W. (1987) Plastid run on transcription: Appication to determine the transcriptional regulation of spinach plastid genes. J. Biol. Chem. **262**:9641-9648.

Deng, X.-W., and Gruissem, W. (1988) Constitutive transcription and regulation of gene expression in non-photosynthetic plastids of higher plants. EMBO J. **7**:3301-3308.

Dieckmann, C.L., and Mittlemeier, T.N. (1987) Nuclearly encoded CBP1 interacts with the 5' end of mitochondrial cytochromeb pre-mRNA. Current Genetics **12**:391-397.

Erickson, J.M., Rahire, M., Malnoe, P., Girard-Bascou, J., Bennoun, P., and Rochaix, J.-D. (1986) Lack of the D2 protein in a Chlamydomonas reinhardtii psbD mutant affects photosystem II stability and D1 expression. EMBO J. **5**:1745-1754.

Goldman, D., Sedman, S.A., and Ebert, M.H. (1981) Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science **211**:1437-1439.

Goldschmidt-Clermont, M. (1986) The two genes for the small subunit of the RuBP carboxylase/oxygenase are closely linked in Chlamydomonas reinhartii. Plant Mol. Biol. **6**:13-21.

Gorski, K., Roch, J.-M., Prentki, P., and Krisch, H.M. (1985) The stability of bacteriophage T4 gene 32: A 5' leader sequence that can stabilize mRNA transcripts. Cell **43**:461-469.

Graves, R.A., Pandeyk N.B., Chodohoy, N., and Marzluff, W.F. (1987) Translation is required for regulation of histone mRNA degradation. Cell **48**:615-626.

Gruissem, W., Greenberg, B.M. Zurawski, G., and Hallick, R.B. (1986) Chloroplast gene expression and promoter identification in chloroplast extracts. Methods in Enzymology **118**:253-271.

Gruissem, W., Barkan, A., Deng, X.-W., and Stern, D. (1988)Transcriptional and post-transcriptional control of plastid mRNA levels in higher plants. Trends in Genetics **4**:258-263.

Gruissem, W. (1989) Chloroplast gene expression: How plants turn their plastids on. Cell **56**:161-170.

Gruissem, W., and Zurawski, G. (1985) Analysis of promoter regions for the spinach chloroplast rbcL, atpB, and psbA genes. EMBO J. **4**:3375-3383.

Guertin, M., and Bellemare, G. (1979) Synthesis of chloroplast ribonucleic acid in Chlamydomonas reinhardtii toluene treated cells. Eur. J. Biochem. **96**:125-129.

Harris, E.H. (1989) The Chlamydomonas Sourcebook: A Comprehensive Guide to Biology and Laboratory Use. (San Diego, CA: Academic Press)

Hoober, K.K. (1984) Chloroplasts. (New york, NY: Plenum Press)

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

Klaff, P., and Gruissem, W. (1991) Changes in chloroplast mRNA stability during leaf development. Plant Cell **3**:517-529.

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.

Kuchka, M.R., Mayfield, S.D. 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 reinhartii. EMBO J. **7**:319-324.

Kuchka, M.R., Goldschmidt-Clermont, M., van Dillewijin, 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 PS II. Cell **58**:869-876.

Kück, U., Choquet, Y., Schneider, M., Dron, M., and Bennoun, P. (1987) Structural and transcriptional analysis of two homologous genes for the P700 chlorophyll a-apoproteins in Chlamydomonas reinhartii: Evidence for trans-splicing. EMBO J. 2185-2195.

Lehrach, H., Diamond, D., Wozney, J.M., and Boldetter, H. (1977) RNA molecular weight determination by gel electrophoresis under denaturing conditions, a critical examination. Biochemistry **16**:4743-4751.

Lundberg, U., von Gabain, A., and Melefors, O. (1990) Cleavages in the 5' region of the ompA and bla mRNA control stability: Studies with an E. coli mutant altering mRNA stability and a novel endoribonuclease. EMBO J. **9**:2731-2741.

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

Mendiola-Morgenthaler, L., Leu, S., and Boschetti, A. (1985) Isolation of biochemically active chloroplasts from Chlamydomonas. plant Sci. **38**:33-39.

Mittelmeier, T.M., and Dieckmann, C.L. (1990) CBP1 function is required for stability of a hybrid cob-oli1 transcript in yeast mitochondria. Current Genetics **18**:421-428.

Morikawa, N., and Imamoto, F. (1969) On the degradation of the messenger RNA for the tryptophan operon in Escherichia coli. Nature **223**:37-40.

Mullet, J.E., and Klein, R.R., (1987) Transcription and RNA stability are important determinants of higher plant chloroplast RNA levels. EMBO J. **6**:1571-1579.

Mullner, E.N., and Kuhn, L.C. (1988) A stem loop in the 3' untranslated region mediates iron dependent regulation of transferrin receptor mRNA stability in the cytoplasm. Cell **53**:815-825.

Nelson, T., Harpster, M.H., Mayfield, S.D., and Taylor, W.C. (1984) Light regulated gene expression during maize leaf

development. J. Cell Biol. **98**:558-564.

Papadopaolou, B., Dekker, P., Blom, J., and Grivell, L.A. (1990) A 40 Kd protein binds specifically to the 5' untranslated region of yeast mitochondria. EMBO J. **9**:4135-4143.

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.

Rochaix, J.-D., Mayfield, S., Goldschmidt-Clermont, M. and Erickson, J. (1988) Molecular biology of Chlamydomonas. In Plant Molecular Biology- A practical approach. C.H. Shaw ed. (New York:IRL press), pp. 253-275.

Rochaix, J.-D., and Erickson, J. (1988) Function and assembly of photosystem II: Genetic and molecular analysis. TIBS **13**:56-59.

Ross, J. and Kobs, G. (1986) H4 histone messenger RNA decay in cell free extracts initiates at or near the 3' terminus. J. Mol. Biol. **188**:579-593.

Sambrook J., Fritsch, E.F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual.2nd ed. (NY:NY;Cold Spring Harbor.

Schuster, G., and Gruissem, W. (1991) Chloroplast mRNA 3' end processing requires a nuclear encoded RNA binding protein. EMBO J. **10**:1493-1502.

Sieburth, L.E., Berry-Lowe, S., 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.

Shaw, G., and Kamen, R. (1986) A conserved AU sequence from the 3' untranslated region of GM-CSF mRNA mediates selective mRNA degradation. Cell **46**:659-667.

Shyu, A.-B., Belasco, J.G., and Greenberg, M.E. (1991) Two distinct destabilizing elements in the c-fos message trigger deadenylation as a first step in rapid mRNA decay. Genes and Devel. **5**:221-231.

Stern, D.B., and Gruissem, W. (1987) Control of plastid gene expression: 3' inverted repeats act as mRNA processing and stabilizing elements, but do not terminate transcription. Cell **51**:1145-1157.

Stern, D.B., Jones, H., and Gruissem, W. (1989) Function of plastid 3' inverted repeats: RNA stabilization and gene specific protein binding. J. Biol. Chem. **264**:18742-18750.

Stern, D.B., Radwanski, E.R. and Kindle, K.L. (1991) A 3' stem/loop structure of Chlamydomonas chloroplast atpB gene regulates mRNA accumulation in vivo. The Plant Cell **3**:285-297.

Surzyski, S.J. (1969) Genetic functions of the chloroplast of Chlamydomonas reinhardtii:Effect of rifampicin on chloroplast DNA dependent RNA polymerase. Proc. Natl. Acad. Sci. USA **63**:1327-1334.

Tobin, E.M., and Silverthorn, J. (1984) Light regulation of gene expression in higher plants. Annu. Rev. Plant Physiol. **36**:569-593.

Wisdom, R., and Lee, W. (1991) The protein coding region of c-myc mRNA contains sequences that specifies rapid mRNA turnover and induction by protein synthesis inhibitors. Genes and Devel. **5**:232-243.

Weissback, A. and Weissback ,H. (1988) Methods for Plant Molecular Biology. (San Diego, CA:Academic Press) pp.128-129.

Yen, T.J., Machlin, P.S., and Cleveland, D.W. (1988) Autoregulated instability of ß tubulin mRNAs by recognition of the nascent amino terminus of ß tubulin. Nature **334**:580-585.

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TEACHING EXPERIENCE

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