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REGULATION OF THE PIGMENT-PROTEIN COMPLEXES BY LIGHT. MOLECULAR COORDINATION BETWEEN NUCLEAR AND PLASTID GENOMES

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I. Pigment-protein complexes.

Basically, there are three pigment-protein complexes: photosystem I, photosystem II and the LHCP (= Light Harvesting Chlorophyll Protein Complex). Both photosytsem I and II complex consists of a reaction center (a chlorophyll *a* dimer, P680 in PSII and P700 in PSI)), antenna pigments (chl *a* and *b*) and surrounded by accessory pigments (chl *a* and *b*, carotenes and xanthophylls). The antenna en accessory pigments are bound in a specific manner to polypeptides. The complexes are inbedded in the thylakoid membranes. Their main function is the aborption of light energy and the conversion into chemical energy (ATP). PSII and PSI are interconnected by redox systems such as plastoquinone, cytochroom b_6f-complex and plastocynine, making a vectorial arranged electron transport system.

Keywords: gene expression, genome, LHCP, light regulation, photosynthesis, photosystem.

The LHCP is a complex, like PSI and PSII, consisting of several polypeptide subunits to which pigments (chl*a*, *b*, and carotenoids) are bound. The main function of this complex is the "harvesting" of light and the fine tuning of the regulation of the photosynthetic apparatus towards rapidly changing light conditions.
The polypeptide subunits of all three complexes are synthesised by a complex interaction between the nuclear and the chloroplast genome.

II. Light regulation of nuclear-encoded thylakoid proteins.

II.1: *Introduction*

The formation of the thylakoid membrane system is the result of the coördination of two separate genomes, the chloroplast genome and the nuclear genome. The chloroplast genome is of the order of 120-170 kbp and carry about 100 genes. These genes encode for intrinsic membrane proteins, which form the core of the photosynthetic electron transport complexes, or chloroplast ribosomal and tRNAs and proteins required for chloroplast gene expression. Most chloroplast genes are expressed constitutively and regulated post-transcriptionally by factors other than light. Nevertheless, light can influence the accumulation of chloroplast transcripts. Different aspects of chloroplast and cytoplasmic metabolism, including post-transcriptional and post-translational processes, are controlled either directly or indirectly by light regulation of nuclear genes.
About 50% of the components of the electron transport chain proteins as well as almost all of the stroma-located enzymes involved in the biosynthetic pathways such as carbon fixation, lipid synthesis and amino acid synthesis are nuclear-encoded. These proteins are synthesized on cytoplasmic ribosomes and targeted to the chloroplast by the N-terminal transit peptide sequence, which is cleaved off during the transfer through the envelope membrane system.

II.2.: *Development of the thylakoid membrane system*

Undifferentiated meristems, seeds and non-green tissues contain proplastids (1μm in diameter), which have rudimentary internal membrane structure. Gradual increasing light exposure triggers the chlorophyll synthesis, the division and enlargement of plastids and the development of a highly organized thylakoid membrane system. Prothylakoid vesicles, budding from the internal envelop membrane, enlarge, flatten and become partially appressed to form grana stacks. The formation of grana is mediated by LHCII (light controlled) and results in a spatial separation of the PSII (located in the grana) and the PSI (located in the stroma lamellae) complexes.

In the absence of light, Angiosperms cannot synthesize chlorophyll. The proplastids are still able to enlarge, divide and they develop an extensive internal tubular para-crystalline network, the prolammellar body. This is called *skotomorphogenesis*, the normal development is called *photomorphogenesis*. The etioplasts accumulate large amounts of protochlorophyllide and of protochlorophyllide reductase. Upon illumination, Pchllide is rapidly converted into chlorophyll (Chl), the prolammellar body is fragmented and the PLB elements are rearranged to form the sacs of the thylakoid membrane system. Chl *a* is synthesized quickly, concomitant with the assembly of PSI and PSII cores. Photochemical activity is detected after 2 hours, the Chl *b* and Chl *a/b* antenna polypeptides appear after 4-6 hours.

II.3.: *Photoreceptors*

In chloroplast development and light-regulated transcription, three major classes of light receptors are involved: (a) phytochrome, (b) blue light/UV-A/B receptors, (c) protochlorophyllide oxidoreductase, which is a red-light receptor and an enzyme catalyst. Protochlorophyllide and chlorophyll function as photoreceptors, because light is required to convert Pchllide to Chl(lide) and the concentrations of Chl(lide) and Chl affect nuclear gene expression.

II.3.1. Phytochrome

Phytochromes are a family of 120 kDa proteins with a unique biliverdin chromophore. It exists in two photochemically interconvertible forms. It is synthesized as Pr, the inactive form for most responses; its absorption maximum is in the red (R), 665-667nm. On absorption with red light, 80% is converted to the active form, Pfr, whose absorption maximum is in the far-red (FR), 730nm. This conversion is reversed either upon absorption of far-red light or enzymatically in the dark. The phytochrome apoproteins are encoded by a gene family (*phyA-E* in *Arabidopsis*). Type I phytochrome (phyA) is abundant in dark-grown tissues and plays a major role in light-induced gene expression during the development of the etioplast or proplastid. Its conversion to the Pfr-form triggers for instance the up-regulation of the *cab*-genes and the down-regulation of the genes for the PChl(lide)-reductase and of PhyA itself. Type II phytochromes (PhyB and PhyC) are the prominent forms in green tissue; PhyB can mediate the increase of the *cab*-gene transcripts induced by red light. Phytochrome responses can functionally be divided into LF (low fluence, responses saturated at about 1 $mmol.m^{-2}$, far-red reversible) and VLF (very low fluence, threshold about 10^{-4} $\mu moles.m^{-2}$, not reversible by far-red but inducible by red pulse).

II.3.2. Cryptochroom

This class of photoreceptors mediates responses to B/UV-A. Blue-light action spectra (including UV-A light) coincides with the spectrum of receptors having flavin, pterin or carotenoid chromophores. The gene encoding the B-photoreceptor has been characterized as the *Arabidopsis thaliana HY4*-gene (on basis of genetical, photobiological and sequence studies). There is a striking sequence homology between HY4 and microbial photolyase (DNA-lyases), a unique class of flavo-enzymes that are dependent for their activity on B/UV-A. Studies with *blu*1 hypocotyl elongation mutant of *Arabidopsis* suggest that there may be separate blue and UV-A receptors. Since the DNA-lyases bind two chromophores, a flavin in the N-terminal domain and a pterin or deazaflavin in the C-terminal domain, with absorption maxima of 380 and 435nm respectively, both B- and UV-A receptors are attached to the same protein. Alternatively, the proteins could be members of the same gene family.

Recently, there is evidence for a correlation between the carotenoid zeaxanthin and blue-light-induced phototropism in corn coleoptiles. Zeaxanthin is produced from violaxanthin in the light

and is involved in responses to high light stress. Most of the zeaxanthin is located in the thylakoid membrane in association with several of the minor Chl *a*/*b*-light-harvesting antenna complexes.

II.3.3.: Protochlorophillide reductase

NADPH:protochlorophyllide oxidoreductase (POR) catalyses the reduction of the Pchllide to Chllide. Pchllide (A_{max} = 650nm) is the absorbing chromophore and also the substrate. The electron donor is NADPH, but the reaction is light dependent regardless of the NADPH levels. In etioplasts, the protein is strongly membrane-associated. Upon illumination, reductase activity and POR-protein decrease to 2-10% of the etioplast levels.

There exist two forms of the POR in angiosperms: one is a major component of the prolamellar body and is rapidly degraded during illumination. The other is found in low levels in green tissue and can be responsible for the maintenance of Chl-synthesis in the light. In *Arabidopsis thaliana*, two POR genes have been reported, one of which (*por*A) is expressed early in photomorphogenesis, while the other (*por*B) is present throughout development and in fully green leaves. In some green algae and gymnosperms, a light-independent POR with three polypeptide subunits, two of which are chloroplast-encoded, is present.

II.4. *Regulation of thylakoid membrane proteins.*

II.4.1.: General principles

Two double membrane barriers and the cytosol separate the nuclear and chloroplast genomes. This means that gene expression is regulated separately in the two compartments, and that signal interactions may be reactive rather than proactive. The nuclear genes are regulated in concert with the activities of the cell; light effects interact with hormonal effects and with developmental stage- and tissue-specificity. Many light-regulated genes are under the control of a circadian oscillator in mature leaf tissue. Frequently, there is no direct correlation between steady-state levels of mature transcripts and steady-state levels of mature protein in the appropriate place in the membrane environment. There is no regulatory effect of light on the import of proteins into chloroplasts (cleavage of the transit peptide). The energy for the transfer is delivered by ATP and proton gradients. The stability of the Chl-binding proteins is regulated by light because Chl-

synthesis is light dependent. Absence of Chl results in a fast turnover of the apoproteins of the complexes. The stability of other thylakoid polypeptides is dependent on their incorporation into complete macrocomplexes. Light can have a positive or negative effect on transcript levels and more than one photoreceptor can be involved in the expression of a single gene. That light is not a simple on-off switch can be illustrated by the complex regulation of the POR-system: POR mRNA levels are negatively regulated by another photoreceptor, phytochrome, which itself is negatively regulated by light at the transcriptional level.

II.4.2.: Positive regulation: antenna proteins

Cfr. LHCII-complex

The *Lhcb1*-genes are strongly light regulated at both transcriptional and post-transcriptional level via the phytochrome system. Some of these nuclear encoded genes are transcriptionally up regulated by blue light. In contrast to the response to blue/UV-A light, the level of *cab* transcripts in greened pea leaves decreased after treatment with UV-B light. This can be due to degradation of the *cab* message or to a drastic decrease in transcription.

The different *Lhcb*1-genes do not respond to light in the same way. One of the genes is induced to a much higher level by red light in etiolated *Arabidopsis* seedlings than the other ones. In green leaves, the steady-state expression level is much more the same for the different genes. In pea leaves the regulation is different; a single pulse of red light induces only two *Lhcb*1-genes, while the other ones are induced by continuous red or white light. The same group of genes shows different in response on the type of tissue and developmental stage.

The expression of the *Lhca*1-gene is regulated by phytochrome at the transcriptional level, and it has the same type of fluence response to red, far-red, blue and UV-light, as does the *Lhcb*1-gene. Since Chl *b* appears only after several hours of illumination of etiolated seedlings, it would make sense that *cab*-transcripts should only be made once seedlings have been exposed to light. However, low levels of all types of LHCII transcripts have been showed in the dark. This could be developmental regulating system acting independently of light enabling seedlings pushing up through the soil under normal conditions to assemble the photosynthetic apparatus more efficiently, thus avoiding photodamage.

A complicated factor in studying light-regulation of Chl-binding proteins is that several steps in Chl-biosynthesis are light regulated. Two enzymes involved in the biosynthesis of 5-aminolevulate, an intermediate in the synthesis of haem, phytochrome and chlorophyll are positively regulated by light.

II.4.3.: Positive regulation: early light-induced proteins (ELIPs).

This family of proteins is part of the extended family of proteins to which the CABs belong. After illumination of etiolated seedlings, ELIPs mRNAs rapidly turned on, reach a maximum after 2 – 4 hours and disappeared a few hours later. This proteins can also be induced in green leaves under high light conditions, especially by blue and UV-B light. The ELIP-proteins are degraded once plants are returned to low light.

II.4.4.: Negative regulation: protochlorophyllide reductase (POR).

POR is firmly associated with the prolamellar body of etioplasts. The major form of POR is being negatively regulated by red light both at the protein and RNA level.

II.4.5.: Proteins of the core complexes.

The nuclear encoded proteins of the core complexes play a supporting role in the electron transport chain. Three extrinsic proteins of PSII (*psb*O, *psb*P and *psb*Q) stabilize the water-splitting complex on the luminal side of the thylakoid membrane. Associated with PSI, there are several membrane-extrinsic proteins: plastocyanin (*petE*) on the luminal side; PSA-D (*psaD*) which stabilizes the interaction between the PSI core complex and ferredoxin on the stromal side; ferredoxin itself and ferredoxin-NADP-reductase (FNR). The PSA-E subunit is involved in the electron transport to alternative electron acceptors.

The only nuclear encode protein of the cytochrome b_6/f complex (*petC*) is the Rieske Fe-S protein. In the ATP-ase, the genes coding for the and the subunits of CF_1 and the CF_0 subunit are nuclear-encoded.

In spinach and tobacco, light exposure increases the mRNA level of the *psb*O tenfold, but the protein level only two to threefold and, although the fraction of polysome-associated RNA did increase twofold, light has no effect on the rate of synthesis of the protein. It appears that very

low levels of mRNA are sufficient for the synthesis of substantial amounts protein during development in the dark. This is supported by the observation that antisense constructs for *psb*O, *psb*P and *pet*C has a dramatic effect on the levels of the corresponding mRNAs but not on the steady-state levels of the proteins. For the *psa*D, high levels of mRNA can be induced by a short pulse of red light, accumulation of proteins depend on the association of mRNA with polysomes, which require high light levels.

Carotenoids play an important role in the photoprotection of the thylakoid membranes. In their absence even low light can cause photo-bleaching of Chl and dedifferentiation of the chloroplast. In the absence of Chl, the LHCII polypeptide are unstable; this is accompanied by a decrease of the steady state level of the nuclear encoded *Lhcb*1 mRNA. Also other nuclear-encoded genes involved in photosynthesis show a decreased transcription. This indicates that an intact chloroplast is required for correct expression of a number of nuclear genes encoding chloroplast proteins and that some signal originating in the chloroplast is sent to the nucleus to affect their transcription. There are some indications that haem or other porphyrins could be the signaling molecules.

II.5. *Signal transduction and gene expression*.

Eukaryote signal transduction chains involve some sort of receptor protein, often membrane bound when the signal originates outside the cell. The receptor is linked via a series of intermediates, which can include G-proteins, GMP, camp, Ca^{2+} and Ca^{2+}-binding proteins. The processes linking them, the effectors and receptors, often involve activation/inactivation by phosphorylation/dephosphorylation. Both the phytochromes and the putative *hy*4 blue-light receptor are soluble rather than membrane bound proteins.

II.6. *Genetic regulatory elements and transcription factors*

The nuclear genes that encode thylakoid structural proteins encode precursor polypeptides that are translated on cytoplasmic ribosomes and post-translationally imported into the chloroplast. The majority of the genes are single copy, some thylakoid proteins are encode by multigene families. The genes encoding the *Lhca*1-*Lhca*4 genes of the PSI antenna are present in one or two copies, the *Lhcb*1 genes have copy numbers ranging from 3 to 16. The other LHCII-genes, *Lhcb*2-6, are present in up to four copies. Also, since different members of the *cab* and the *rbc*Sb families are regulated differently, the existence of multiple copies does not necessarily affect the transcript or protein levels, but may provide more flexibility in response to complex environmental cues.

The upstream controlling regions of light-responsive genes have been extensively studied. A number of regulatory elements have been identified, and a number of DNA-binding proteins characterized. As a general observation, the upstream region adjacent to the gene itself is sufficient to confer light- and/or tissue-responsiveness to a transgenic reporter construct. The region can be defined as a series of elements which confer different effects, both negative and positive.

The signal transduction chain that activates/deactivates the DNA-binding proteins does not consists solely of a series of positive switches. The induction of light responses involves also derepression. The presence of negative as well as positive regulatory elements within promoters has been demonstrated by the use of transgenic deletion-reporter constructs.

III. Chloroplast mRNA: light activation.

III.1.: *Introduction*

The rate of gene transcription of nuclear-encoded photosynthetic proteins is affected by changes in illumination. Chloroplast mRNAs are transcribed at relatively constant rates and accumulate to similar levels under light and dark growth conditions, although fluctuations in plastid mRNA have been observed in response to developmental and environmental signals. A rapid induction of translation of chloroplast mRNA is noticed following an increase in illumination; this is indicative for a coordinate expression of both nuclear and chloroplast-encoded photosynthetic proteins.

III.2.: *Transcription as regulator*

The chloroplast translation machinery features characteristics of the prokaryotes, including 70S ribosomes and polycistronic messages that lack the 5' caps and polyadenylated tails. The promoters of plastid genes contain consensus bacterial-like –35 (TTGACA) and –10 (TATAAT) sequences. Several studies indicate that this consensus sequences are not always present as such in plastid mRNAs.

Plastid promoters contain only one or two consensus elements 5' of the transcription start. The transcription rates can differ a great deal from one promoter to the other. For a single gene, the transcription rate can vary between plant species: *psbA* transcription rate is 30% that of the 16S RNA in spinach, only 15% in *Chlamydomonas* and 150% in barley. As a general rule, genes that are transcribed at reduced rates in one species are also transcribed at reduced rates in other species. The plastid promoters contain only a –35 and a –10 element. This means that variations in transcription from different promoters must be affected by either downstream sequences or by the position of the gene within the chloroplast genome. Studies with chimeric genes in which foreign mRNA is transcribed from plastid promoters show that these transcription is independent

of the position of the gene within the plastid chromosome. In most of these chimeric constructs, sequences downstream of the transcription start were included. *In vivo* analysis of chimeric constructs containing the *atpB* promotor shows that downstream sequences up to +55 after transcription start are required for high levels of transcription. This indicates that sequences downstream of the –10 and –35 elements determine the rate of transcription from individual promoters. These elements can be regarded as transcription enhancers.

The rate of expression of plastid genes changes during development of barley seedlings; only a few genes are differentially transcribed. The relative transcription rate of several photosynthetic mRNAs during chloroplast development and in plastids of non-photosynthetic tissues is essentially the same in all plastid types. This suggests that transcription as such is of minor importance in determining the level of plastid mRNA accumulating in these cells. Transcription of plastid genes fluctuates in relation to plastid development, plastid type and cell cycle; the relative transcription of the individual genes varies only slightly compared to the overall change in total transcriptional activity. This suggests that transcription is a general regulator of plastid gene expression rather than the rate-limiting step for expression of individual genes.

The transcription of plastid genes changes markedly during light and dark grown phases. In tomato fruit, transcription of both plastid and nuclear-encoded photosynthetic genes increases during the night and early morning. In *C. reinhardtii* cells, a 10-fold increase in transcription can be observed during the dark. The diurnal fluctuations of transcription of specific genes are the result of light entrained circadian cycle. Illuminating 5 days old etiolated barley seedlings does not result in a marked shift in the transcription. When the seedlings are 8 days old, there is a transient but strongly marked increase in plastid gene expression, in which some mRNAs are more affected than others. Light-activated transcription can be clearly demonstrated in the *psbD-psbC* operon of barley. The *psbD* is encoded in a polycistronic mRNA that contains the *psbK*, *psbI*, *psbC*, *orf62* and *trnG* mRNAs. Transcription of this polycistronic message is normally light independent. A subset of this gene cluster containing the *psbD* and *psbC* mRNAs is transcribed in a light-dependent manner. This light-activated transcription raises the level of the *psbD-psbC* mRNA by 50% above dark conditions.

III.3.: *RNA-processing*

This process is not well defined in the regulation of chloroplast gene expression. Methyl jasmonate induces a reduction in translation of the *rbcL* transcript, due to a longer 5'-untranslated region (UTR). Many plastid genes, both monocistronic and polycistronic, give rise to multiple overlapping RNAs through a variety of processing steps. This depends on the developmental stages and environmental conditions. It does not appear to be a general rule for plastid gene expression because processing is not required for translation of individual coding regions within polycistronic mRNAs.
Similar to nuclear pre-mRNA, introns contribute to gene regulation also in the chloroplast genome. However, intron splicing has been shown to be a regulator of chloroplast gene expression. In bacteria, in rRNAs of both chloroplast and nucleus, in protein-encoding genes such as *psbA* in *C. reinhardtii* and sometimes in chloroplasts of higher plants, group I introns are found. Group II introns are found exclusively in organelles and most of those in chloroplasts are degenerate Group II introns. More complex introns, such as introns-in-introns termed twintrons, have been found. Several chloroplast introns contain open reading frames (ORFs) that encode nucleases or RNA-splicing maturases.

Trans-splicing of plastid mRNA is necessary to recombine gene segments that are discontinuous such as in *rps12* and *psaA* mRNAs. Three scattered exons encode each of these genes. The Group II introns are composed these exons and surrounding sequences. A fourth chloroplast RNA, *tscA*, is necessary for efficient splicing of *psaA*-gene presumably it completes the catalytic core of the introns. There are more than fourteen nuclear loci identified that are involved in *trans*-splicing of the chloroplast mRNA of the three exons of *psaA*.

RNA editing plays an important role in producing a fully functional RNA molecule. It involves the change of specific bases of an RNA from those that are encoded in the DNA. The ACG start codon in *rpl2* mRNA of maize and in *psbL* mRNA of tobacco and spinach is changed to a functional AUG start codon. Editing of the spinach *psbF-psbL* transcript is differentially down-regulated in seeds and roots; the unedited *psbL* transcript lacks an initiator codon and is untranslatable. Inside introns, editing sites have also been discovered, perhaps playing a role in

maintaining introns structure and/or function. Editing precedes both splicing and cleavage to monocistronic mRNA and does not require chloroplast translation, indicating that protein components of the editing machinery are encoded by the nucleus and imported into the chloroplast.

III.4.: *mRNA stability*

mRNA untranslated regions (UTRs) and stem-loop structures play a role in the regulation of the accumulation of mRNA and both the 3'- and the 5'-UTRs contribute to mRNA stability. Most chloroplast-encoded monocistronic and polycistronic mRNAs contain inverted repeats (IRs) within the 3'-stemloop and in the 5'-UTR. IRs have the potential to form stem-loop structures and play a role in RNA processing and /or as protective structures against nucleolytic degradation.

Several experiments give evidence that a 3'-IR element alone is not sufficient to confer RNA stability in vitro and that sequences within the 3'UTRs can have some impact on RNA accumulation. Also, deletions studies indicate that a complete IR is not essential for mRNA accumulation in vivo, but still that some portions of the 3'-UTR are required. In vivo analysis of the *psaB* and *rbcL*3'-IRs support this analysis. *C. reinhardtii* cells transformed with a chimeric construct fused to either of these 3'-IR sequences accumulate chimeric transcripts of a single size. Transformants containing constructs without 3'-IRs accumulate chimeric mRNA near wild-type levels but of heterogeneous length. Coupling of multiple 3'-IRs to a reporter gene results in an accumulation of transcripts that are terminated at the first IR in the forward orientation. This indicates that the stem-loop structure formed by the 3'-IR, or adjacent sequences, act as recognition sites in vivo for endonucleolytic cleavage distal to each stem-loop. Exonuclease digestion in the 3' to 5' direction cannot be involved in the 3'-end processing of these mRNAs in vivo because this mechanism would have yield transcripts of increased length when multiple IR sequences were added to the 3'-end of the chimeric gene. The rate of mRNA decay from these constructs in vivo is the same with and without the presence of a 3'-IR at the end of the mRNA. Transformants containing a chimeric *rbcL5'*-UTR fused to a reporter gene and two different 3'-ends produce mRNAs with identical half-lives, whereas chimerics containing different 5'-ends

and the same *psaB* 3'-UTR produce mRNAs with very different half-lives. These data indicate that 3'-UTRs do not play a critical role in determining mRNA half-live in vivo, but 5'-UTRs may have a role in determining the half-live of a message.

Proteins that bind to the mRNA 3'-IRs are involved in RNA processing. In spinach chloroplasts, both endo- and exonuclease activities that work on the 3'-IR of *petD* pre-mRNA have been identified.

III.5.: *Trans-acting protein factors*

Let's have a look now at the 5'-UTR. First we refer to the control of synthesis by epistasy. In this system, excess cytochrome *f*, that fails to be incorporated into the cytochrome b_6/f complex is able to attenuate translation of its own *petA* mRNA through interactions with the *petA* 5'UTR. It has been shown that multiple nuclear factors are required for the translation of specific chloroplast mRNAs. Many of these factors interact with the 5'UTRs of the chloroplast mRNAs. A photosystem I –deficient *C. reinhardtii* strain, F15, containing a mutation at the nuclear *TAB1* locus, fails to translate the *psaB* mRNA. A chloroplast suppressor of this mutation was mapped to a putative stem-loop element, adjacent to a potential Shine-Dalgarno (SD) sequence. The *TAB1* locus encodes a factor that normally alleviates local structures constraints in the mRNA around the SD-sequence, thereby enhancing *psaB* translation by providing initiation factors with access to a ribosome-binding site.

In nuclear mutants nac1-18 and ac-115 of *C. reinhardtii*, synthesis of the D2 protein (chloroplast encoded) from the *psbD* mRNA is reduced. The nac1 blocks translational elongation of the *psbD* mRNA, this mutation has also an effect on the translation initiation of *psbA*. Both mutations can be suppressed by a mutation to a third nuclear locus, *sup4b*. This indicates that several nuclear mutants are necessary for the translation of chloroplast messages. The translation of the *psbC* mRNA is affected by at least three nuclear loci: *TBC1*, *TBC2* and *TBC3*. Mutations of the *TBC3* locus results in the partial suppression of both a chloroplast and a nuclear mutation; its act via a stem-loop element within the *psbC* 5'UTR. Multiple nuclear mutations, spanning at least four nucleolar loci, specifically affect initiation of translation of the *psbA* mRNA (cfr. accumulation and activity of the binding proteins).

Trans-*acting factors differentially bind chloroplast 5' untranslated regions*

In this approach, proteins that bind the 5' UTRs are isolated. Proteins that crosslink to the 5' UTRs of *psbA*, *rbcL*, *atpB*, *rps7* and *rps12* mRNAs, showed, with respect to illumination, carbon source and level of protein synthesis activity within the chloroplast, a minimum set of six RNA-binding proteins with different binding patterns in response to changes in growth conditions.

A number of proteins that associate with the 5' UTR transcripts of *psbA*, *psbC* and *psbD* has been identified. The accumulation of a membrane associated 47 kDa protein that crosslinks to the *psbD* 5' UTR, is reduced by a nuclear mutation. Some of the proteins that bind the *psbD* 5' UTR are immunochemically related to *psbA*-associated complex, which has been shown to mediate light-dependent translational activation.

RNA-binding proteins of 95, 65 and 40 kDa were found after UV-crosslinking of chloroplast enriched-proteins to the 5' UTR. An A/U-binding protein of 46 kDa can block the psbC mRNA translation. Crosslinking proteins of 30, 32, 40, 46 and 80 kDa from *C. reinhardtii* chloroplasts bind the *psbC* 5' UTR. The *psbC*-associated 46 kDa protein is light dependent and can be inhibited by ADP and is indicative for a light-dependent translational regulation. Many of this proteins are associated with membranes. These factors localize chloroplast messages to the thylakoid membranes, thereby promoting the proper localization and insertion of the encoded integral membrane proteins. Nevertheless, these proteins are not associated with polyribosomes and have not been directly linked to translational regulation.

In contrast, a light-dependent translational regulation of the *psbA* 5' UTR mRNA is performed by the binding of a well characterized set of proteins: RB60, RB55, RB47 and RB38. The binding activity of this proteins is higher in illuminated cells compared with cells grown in the dark; this proteins are possible candidates for light-dependent translational regulators of *psbA* mRNA. The 47 kDa protein is homologous to the poly(A) binding proteins (PABP). This nuclear-encoded protein contains a chloroplast import signal. Several studies give evidence that cPABP accumulation and activity is required for normal *psbA* mRNA-ribosome association. The RB60 is a chloroplast-localized homolog of protein disulfide isomerases (cPDI). The oxidation potential of cPDI could allow for the oxidation of cPAPB when photosynthetic activity, and consequently the reducing potential of the chloroplasts, is low. This could mean that a chloroplast redox potential regulates the binding activity of translational activators through a simple redox switch.

III.6.: *Cis-acting RNA elements*

Prokaryotic ribosome binding sites are composed of six elements involve in translation: (i) an initiation codon; (ii) an SD sequence; (iii) well-defined spacing between the SD sequence and the initiation codon; (iv) translational enhancer sequences; (v) bias at the second codon and (vi) secondary structural elements that affect sequence accessibility.
The initiation of translation in Eukaryotes is located at a proper start codon via a ribosome scanning mechanism following the binding of an initiation complex at the 5' cap or through the recognition of a highly structured RNA element within the 5' UTR. The 5' UTRs of chloroplast mRNAs tend to be UA-rich and contain stem-loops elements as well as unstructured regions.

III.6.1.: Translational enhancers

Several enhancers that enhance translation are: (i) poly(U) binding site for the ribosomal S1 protein; (ii) regions that are complementary to the 16S rRNA; (iii) the downstream box; (iv) the -sequence; (v) the -sequence. A binding site for the S1 protein and several chloroplast 5' UTRs containing the UGAUC sequence, complementary to the 3' end of the 16S rRNA, has been identified. Moreover several regions upstream of chloroplast initiation codons has been shown to enhance translation, although the mechanisms by which these sequence enhancers affect translation has not been identified yet.

Site-directed mutations that disrupt a stem-loop element immediately upstream of a potential SD sequence greatly reduce D1 protein synthesis without affecting *psbA* mRNA accumulation. This same element has been proposed as binding site for the cPABP-containing complex of translational activators. In vivo the availability of the stem-loop element is complicated by a processing event that removes the stem-loop structure from the *psbA* messages, correlated with ribosome binding and related to putative SD sequences. This occurs before dynamical translational activation in response to illumination. Elements upstream of the SD sequence might affect *psbA* gene expression before or early in translation.

III.6.2.: Shine-Dalgamo sequences

Prokaryotic SD sequences comprise 3-9 nucleotides, which are complementary to the 3' terminus of 16S rRNA. During the early stages of initiation, the 30S ribosomal subunits binds mRNA at the SD sequence. The SD sequence is found 5 – 15 nucleotides upstream the initiation codon, the small ribosomal subunit will be positioned such that the fMet-$tRNA_f$ will bind to the correct initiation codon upon entering the ribosomal P-site. Most of the 5'-UTRs of chloroplast mRNAs contain potential SD sequences. However, less than half of these sequences are properly located, with respect to the prokaryotic consensus, upstream of the initiation codon. Deletion of potential SD sequences, or introduction of consensus SD sequences in the 5'-UTRs of chloroplast mRNAs might or might not result in substantial changes in translation.

Modified SD-dependent translation initiation process might use message specific nuclear-encoded translational activators to assist the small ribosomal subunit, held in a stand-by complex at a distant SD sequence to recognize the correct initiation codon. Moreover the location of translation initiation appears also to be determined by some spatial aspect of the mRNA and only by the codon at the translation initiation site.

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