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Transcriptional and post-transcriptional regulation of chloroplast gene expression in *Petunia hybrida*

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Summary. To study the control of differential gene expression during plastid biogenesis in *Petunia hybrida*, we have investigated the in vivo translation and transcription of the *rbc L* gene, coding for the large subunit of ribulose bisphosphate carboxylase (LSU), and the *psa A* gene, coding for P700 chlorophyll-*a* apoprotein (AP700). Differential expression of these plastid-encoded genes was studied in two developmentally different plastid systems, proplastid-like organelles from the green cell suspension AK2401 and mature chloroplasts from green leaves. In vivo translation of *rbc L* and *psa A* transcripts was analysed using specific antibodies. Specific transcript levels were analysed using internal fragments of the *rbc L* and *psa A* genes. A standardization procedure was used so that a direct correlation could be made between the amount of products and gene copy number. In *Petunia hybrida* the amount of LSU polypeptides present in both plastid types does not correspond to the amount of specific mRNA for the gene. Although the *rbc L* transcripts are present in both plastid types, the LSU protein is only present in green leaf plastids and not in cell culture plastids. In vitro translation of isolated *rbc L* transcripts give similar results, thereby suggesting that differences in the primary structure of the transcripts are responsible for the observed discrepancy. In contrast to this, the amount of AP700 polypeptides does correspond to the amount of the *psa A* transcripts. Therefore, our results indicate that the expression of chloroplast genes during plastid biogenesis takes place on at least two different levels: expression of the *rbc L* gene is regulated post-transcriptionally while expression of the *psa A* gene is regulated at the transcriptional level.

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Key words: *Petunia hybrida* – *rbc L* – *psa A* – Chloroplast biogenesis

Introduction

Chloroplast development in higher plants is a complex process that involves the cooperative expression of nuclear as well as plastid genes (for a recent review see Rochaix 1985). During the development of a proplastid into a mature chloroplast the accumulation of chlorophyll and thylakoid membranes, the accumulation of plastid mRNAs and the enhancement of de novo chloroplast protein synthesis can be observed. Not only is the expression of chloroplast DNA encoded genes affected, nuclear encoded genes are also influenced.

The effect of light on plant gene expression and chloroplast development has been extensively studied. For a number of nuclear-encoded plastid proteins, such as the light-harvesting chlorophyll a/b protein (Stiekema et al. 1983; Gallagher and Ellis 1982; Gollmer and Apel 1983), the small subunit of ribulose bisphosphate carboxylase (Stiekema et al. 1983; Smith and Ellis 1981) and the NADPH: protochlorophyllide oxidoreductase (Apel 1981; Santel and Apel 1981), a direct correlation has been demonstrated between the light-induced change in the levels of specific transcripts and altered amounts of the corresponding polypeptides during the light induced process of greening. The light-dependent changes in the relative abundance of the transcripts from these three genes appear to be under the control of the phytochrome receptor system.

So far, studies on developmentally regulated expression of plastid-encoded genes have been focussed mainly on the genes for the 32-kd protein of photosystem II and the large subunit of ribulose bisphosphate carboxylase. During plastid development, transcript levels for the *psb A* gene, coding for the 32 kd protein of photosystem II, increase markedly in maize (Bedbrook et al. 1978) and Spirodella (Reisfeld et al. 1978). In mustard the increase in the steady state level of the *psb A*

transcript also appears to be regulated by phytochrome (Link 1982). Fromm et al. (1985) conclude that in Spirodella the expression of this 32-kd protein is also regulated by light, but that this regulation occurs mainly at a translational level, instead of at the transcriptional level as suggested by the other authors mentioned.

The mechanisms of regulation of the *rbc L* gene, coding for the large subunit of ribulosebisphosphate carboxylase, seems to vary between different plant species. In mustard (Link 1982) and maize (Crossland et al. 1984) only minute differences can be observed between transcript levels in light-grown and etiolated plants. In pea, however, the amount of *rbc L* transcript is more abundant in light-grown seedlings than in etiolated seedlings (Sasaki et al. 1983; Smith and Ellis 1981). A special type of *rbc L* gene expression occurs in C4-plants like maize, where only bundle sheet chloroplasts contain *rbc L* transcripts and no transcripts are detectable in the mesophyll chloroplasts (Link et al. 1978). However, a direct linkage between increased *rbc L* transcript levels and LSU polypeptide amounts has been demonstrated only for pea (Smith and Ellis 1981). For the other species studied, a similar linkage is only suggested or assumed, but not proven.

Based on results studying the regulation of *rbc L* and *psb A* gene expression, it has become generally accepted that light affects chloroplast gene expression at the transcriptional level (Smith and Ellis 1981; Link 1982; Thompson et al. 1983).

Although the transcription of plastid ribosomal RNA is assumed to be constitutive (Poulsen 1983; Link 1984), the total amounts of plastid rRNA per cell change during the development of the plastids. This increase in rRNA amounts during greening is related to the concomitant increase in plastid genome copy number per cell. It has been found that chloroplasts contain different numbers of nucleoids, depending on their development stage (Lamppa et al. 1980), and that nucleoids in turn can contain different numbers of genome copies (Hansmann et al. 1985). Therefore, standardization of RNA samples according to their content of ribosomal RNA makes it possible to correlate the amount of transcript level indirectly to the transcript per gene level, thereby eliminating differences due to different plastid genome copy-number in specific preparations.

To characterize the control of the differential gene expression during chloroplast biogenesis, we have investigated the *in vivo* translation and transcription of certain plastid genes of *Petunia hybrida*. Previous studies, using in organello protein synthesis, showed a marked difference in polypeptides synthesized in plastids from green leaves and the previously described green cell suspension culture AK2401 (Colijn et al. 1982). This is most probably a reflection of the difference in developmental stages of the plastids. The plastids of the green cell suspension culture AK2401 are not able to perform photophosphorylation. They contain thylakoid membranes with some grana structures, and are proplastid-like. The plastids from green leaves are fully developed, normal chloroplasts.

Using these two plastid systems, further research was focussed on the expression of two chloroplast genes, *rbc L* and *psa A*, coding for the P700 chlorophyll-*a* apoprotein of photosystem I. Results obtained by Rodermel and Bogorad (1985) indicated that transcription of the *psa A* gene occurs predominantly in young developing plastids.

Our results indicate that the control of expression of plastid genes in *Petunia hybrida* in the studied plastid systems can take place on at least two different levels: transcriptional control occurs for the expression of the *psa A* gene, while the expression of the *rbc L* gene is regulated post-transcriptionally.

Material and methods

General techniques

Isolation of *Petunia hybrida* chloroplast DNA and physical mapping using restriction endonucleases, was as described in Overbeeke et al. (1984).

Cloning of fragments in vector pUC19, restriction enzyme analyses of DNA, isolation of plasmid DNA, nick-translation, Southern blotting, gel electrophoresis was performed according to standard procedures (Maniatis et al. 1982). *E. coli* strain JM83 [F$^-$ Δ (lac-pro) ara rps L $\varnothing$ 80 dlac ZM15] was used in all cloning procedures.

Protein blotting

Total proteins were isolated from leaves and cell suspension cultures. Samples were taken from the same filtrate as used for RNA isolation (see below) just prior to the phenol extraction, and mixed with an equal volume of twice concentrated Laemmli's sample buffer (Laemmli 1970). Proteins were run on 12.5% SDS-polyacylamide gels (Laemmli 1970) and electrophoretically transferred to nitrocellulose membranes. Specific proteins were detected by [^{125}I]-protein A antibody complexes, according to the procedures of Towbin et al. (1979).

RNA preparation

Total RNA was extracted from young leaves of green house grown *Petunia hybrida* R27 plants, or from a green cell suspension culture AK2401 (Colijn et al. 1982), using the following protocol. Ten gram of material was homogenized in ice-cold extraction buffer (0.1 M Tris, 0.4 M NaCl, 10 mM EDTA pH 9.0) in a Braun blendor. The homogenate was passed through two layers each of 200 µm and 30 µm mesh nylon gauze. The filtrate was immediately extracted with one volume phenol saturated with extraction buffer containing 5% v/v butanol-2, 1% w/v SDS and 2% w/v para-aminosalicyclic-acid. After mixing vigorously for 15 min, one volume chloroform, satured with extraction buffer, was added, and mixing was continued for another 15 min. After recovery of the aqueous layer by centrifugation (8,000 × g, 15 min) the phenol/chloroform extraction was repeated 4 times. The resulting aqueous phases, containing total nucleic acids, were combined and precipitated with ice-cold ethanol. The pellet was washed with 70% ethanol, and resuspended in 10 mM Tris, 1 mM EDTA pH 7.6.

Northern blot analysis

RNA samples were denatured in the presence of 1.2 M glyoxal, 2 mM Na_2HPO_4/NaH_2PO_4, 0.2 mM EDTA pH 6.0 at 60 °C for 40 min. Samples were run on a 1.5% agarose gel in 10 mM Na_2HPO_4/NaH_2PO_4, 1 mM EDTA pH 6.0, at 4 V/cm. The electrode buffers were circulated to maintain the pH below pH 8 in order to prevent dissociation of the glyoxal from the RNA. After electrophoresis the glyoxylated RNA was transferred to GeneScreen membranes (New England Nuclear), using the procedures as provided by the supplier.

Gene probes

In order to construct a plasmid containing an internal fragment of the *psa A* gene of *Petunia hybrida*, we performed heterologous hybridization studies using the cloned spinach *psa A* gene as a probe (kindly provided by Dr. J.C. Gray, Cambridge, UK). The gene for the P700 chlorophyll-*a* apo-

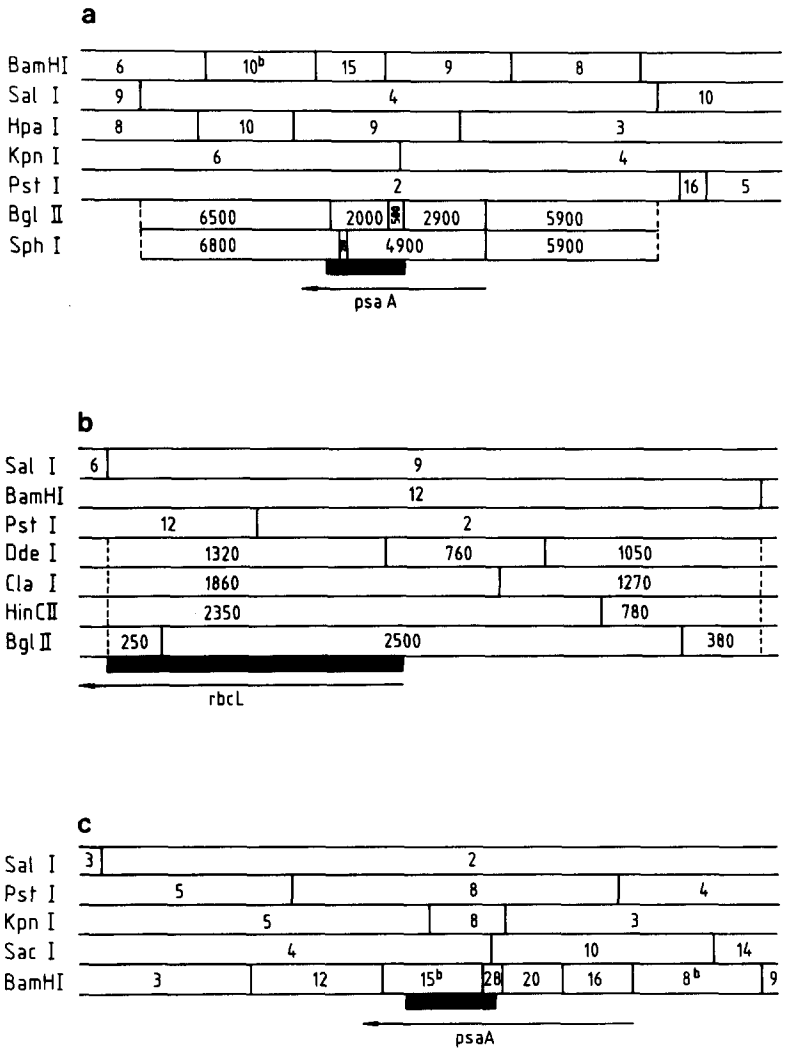

Fig. 1. Diagrams showing the regions of the *Petunia hybrida* chloroplast DNA encoding the P700 chlorophyll-*a* apoprotein (**a**) and the large subunit of ribulose-bisphosphate carboxylase (**b**). The location of the structural genes, estimated transcript size and direction of transcription are indicated. The BamHI, SalI, HpaI, KpnI and PstI fragments are numbered in decreasing molecular weight, according to the nomenclature of Bovenberg et al. (1981). Fragments generated by other restriction enzymes are indicated by their molecular weight in basepairs. **c** shows a diagram of the 8.3 kb fragment of spinach chloroplast DNA encoding the P700 chlorophyll-*a* apoprotein. The restriction fragments are numbered in decreasing molecular weight (Westhoff et al. 1983). The location of the structural gene for the P700 chlorophyll-*a* apoprotein, approximate transcript size and direction of transcription are indicated (adapted from Westhoff et al. 1983)

protein of photosystem I has been localized in the center of the large single copy region of the *Petunia hybrida* chloroplast genome, on fragment HpaI 9 (Fig. 1 a). This location is similar to that reported for spinach (Westhoff et al. 1983). When fragment HpaI 9 was used to program an *E. coli* transcription/translation system, it could be shown by immunoprecipitation with specific antibodies against AP700 from spinach (gift of Dr. J.C. Gray, Cambridge, UK) that it indeed encoded the AP700 protein (results not shown). To determine which subfragment of the HpaI-9 fragment could be used as an internal *psa A* gene probe, we performed heterologous hybridization experiments with various fragments of the spinach *psa A* gene (Fig. 1 c; Westhoff et al. 1983). A spinach 1.1 kb BamHI-KpnI fragment containing the carboxy terminus of the P700 chlorophyll-*a* apoprotein hybridized with the 6.8 kb SalI-KpnI fragment from petunia, but not with the adjacent 0.2 kb SphI-SphI fragment. When the petunia 1.5 kb SphI-BglII fragment was used to probe the cloned spinach *psa A* region, it only hybridized to the 1.3 kb spinach BamHI-KpnI fragment, a fragment that only contains internal *psa A* coding sequences (Fig. 1 c; Westhoff et al. 1983). Since the petunia 0.5 kb BglII-BglII fragment also hybridized to the spinach 1.3 kb BamHI-KpnI fragment, the amino terminus of the P700 chlorophyll-*a* apoprotein must be localized in the adjacent 2.9 kb BglII-BglII fragment. Therefore, the petunia 1.5 kb BglII-SphI fragment contains only internal *psa A* coding sequences. Cloning of this fragment in pUC19 resulted in the plasmid pPhcBS9, further referred to as the internal P700 chlorophyll-*a* apoprotein probe of *Petunia hybrida*. We also constructed, using a similar strategy, a probe containing only coding sequences for the *rbc L* gene. This plasmid pPhcPS1, contains a 700 bp PstI-SalI fragment (Fig. 1 b). As a probe for the ribosomal operon we used the petunia Pst I-4 fragment, cloned in plasmid pPhcP4.

Hybrid released translation

Specific *rbc L* mRNA's were purified from total RNA extracts using a petunia internal *rbc L* gene fragment, immobilized on nitrocellulose membranes (Maniatis et al. 1982). The specific mRNA's were translated in a mRNA dependent rabbit reticulocyte cell-free system (NEN) in the presence of [^{35}S]-methionine (NEN), as recommended by the supplier. Immunoprecipitations were performed as described before (Bovenberg et al. 1984). Protein samples were run on 12.5% SDS-polyacrylamide gels (Laemmli 1970) and proteins were visualized by fluorography.

Results

In vivo synthesis of LSU and AP700

Steady state levels of LSU and AP700 proteins were studied using western blot analysis. Since protein samples were taken from the same homogenate that was also used to determine the transcript levels of these genes, the protein samples could be adjusted, by standardization with the rRNA probe, in such a way that they contained identical amounts of plastid mRNA. Since the synthesis of plastid ribosomal RNA is supposed to be constitutive (Poulsen 1983; Link 1984) this standardization will correct for differences in plastid genome copy number per cell. The adjusted protein samples, therefore, reflect the amount of protein produced per gene copy.

Protein samples were run on 12.5% SDS-polyacrylamide gels and electrophoretically transferred to nitrocellulose. The western blots were incubated with specific antibodies raised against LSU (Fig. 2a) or AP700 proteins (Fig. 2b). Bound antibodies were detected using [^{125}I]-protein A. The autoradiogram of the blots shows that LSU protein could only be detected in mature chloroplasts from the green leaves (Fig. 2a, lane 2). The proplastid-like plastids, isolated from the green cell suspension culture AK2401, contained no detectable amounts of LSU protein, as is shown in lane 1 of Fig. 2a. This situation is comparable with proplastids in dark grown seedlings of many plant species where the LSU polypeptide is also absent.

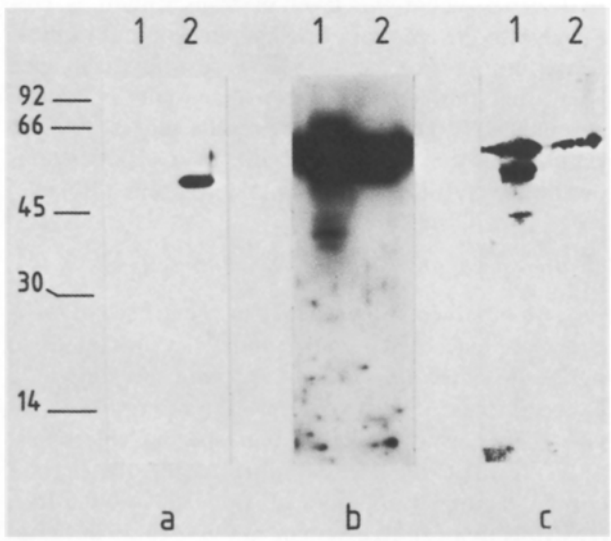

Fig. 2. Western blot analysis. Total protein samples, from cell suspension culture AK2401 (*1*) or green leaves (*2*), standardized according to their contents of plastid rRNA were separated on a 12.5% SDS-polyacrylamide gel and electrophoretically transferred to nitrocellulose membranes. The membranes were incubated with antibodies raised against the large subunit of ribulose-bisphosphate carboxylase (**a**), P700 chlorophyll-*a* apoprotein (**b**) or with preimmune serum (**c**). Immobilized proteins were detected after incubation with [^{125}I]-protein A. Autoradiography was for 2 h for *panel a*, and 16 h for *panels b* and *c*. Molecular weight scale is indicated *on the left*, in kd

Figure 2b shows the amount of P700 chlorophyll-*a* apoprotein detectable in plastids from green leaves and green cell suspension culture AK2401. Since the absolute amount of AP700 protein is low with respect to the LSU protein level, a longer exposure time was needed to detect the specific AP700 proteins immobilized on the western blots. The autoradiogram shows that the P700 chlorophyll-*a* apoprotein is only detectable in protein samples from the cell suspension culture. No P700 chlorophyll-*a* apoprotein can be detected in protein samples from green leaves. During this prolonged exposure a specific artifact band of the [^{125}I] protein A detection method becomes visible (Tasheva et al. 1983). The same artifact band is observed when pre-immune serum is used (Fig. 2c). The observation that AP700 protein is only observed in green cell culture plastids is in complete contrast to the results found for the large subunit protein where the polypeptides could only be detected in plastids from green leaves.

In vivo transcription of rbc L and psa A genes during chloroplast biogenesis

To establish whether the observed differential synthesis of the large subunit of ribulose-biphosphate carboxylase and P700 chlorophyll-*a* apoprotein is the result of the differential transcription of these genes, we have analyzed the steady state level of mRNA for these specific genes in both plastid systems. The approach we followed to assess the extent of developmental gene expression of the *rbc L* and *psa A* genes is to isolate total RNA from green leaves and green cell suspension culture AK2401. Samples of these RNA preparations were run on denaturing glyoxal gels and transferred to GeneScreen membranes. The different RNA samples were adjusted in such a way that they contained equal amounts of plastid ribosomal RNA.

Since it is commonly accepted that the transcription of plastid ribosomal RNA is constitutive (Poulsen 1983; Link 1984) standardization on rRNA levels makes it possible to correlate the amount of a specific transcript indirectly to the transcript level per gene, thereby eliminating differences due to different plastid genome copy numbers in specific preparations. To our knowledge this is the first report in which this accurate method for quantification of specific transcripts is used.

Figure 3a shows the hybridization pattern that is obtained when total RNA from green leaves and green cell suspension is probed with nick-translated pPhcP4, a plasmid containing the rrn A operon from *Petunia hybrida* on fragment PstI 4 (Bovenberg et al. 1984). Hybridization occurs with the plastid specific ribo-

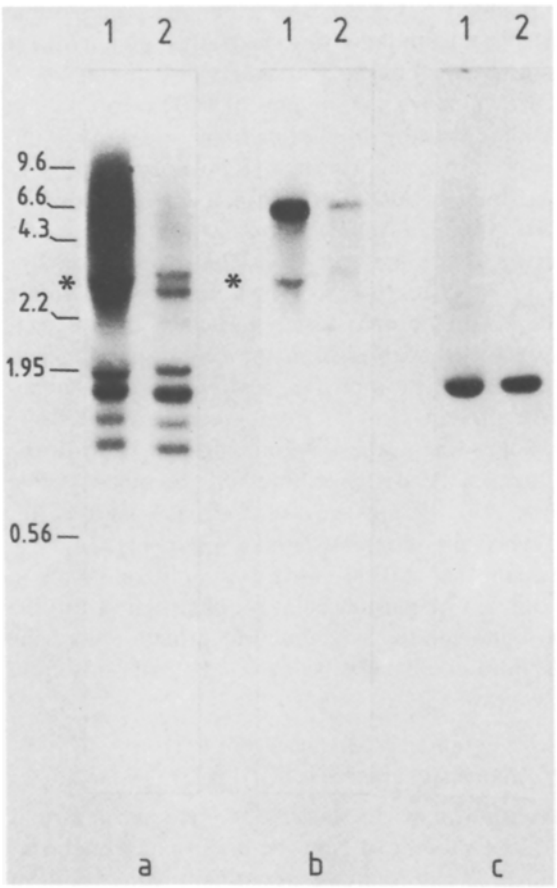

Fig. 3. Northern blot analysis of total RNA. Total RNA was isolated from the green cell suspension culture AK2401 (*1*) and from green leaves (*2*), glyoxylated, electrophoretically transferred to GeneScreen membranes and hybridized with different probes. **Panel a** pPhcP4, containing the *rrn A* operon was used as a probe. **Panel b** probe pPhcBg9 was used to detect the *psa A* transcripts. *Panel c: rbc L* transcripts were detected by hybridization with pPhcPS1, the internal probe for the *rbc L* gene. Glyoxylated HindIII fragments of phage lambda DNA were used as molecular weight standards. * denotes the artifact band, described in the text

somal 23S and 16S RNA. Due to the specific breakdown of the large 23S rRNA (Leaver 1982), two additional bands are also visible on the autoradiogram. The background hybridization, present in the high molecular weight region of the gels, is most probably due to the presence of nuclear DNA. This is particularly striking in the lanes representing green cell culture derived RNA where large amounts of total RNA (and thus large amounts of contaminating nuclear DNA) are loaded on the gels in order to obtain equal amounts of plastid RNA per lane.

In Figure 3 b, results are shown, after hybridization with the nick-translated internal probe for the *psa A* gene, pPhcBS9. The estimated size of the unique *psa A* transcript is 5.3 kb, which is similar as observed in spinach (Westhoff et al. 1983) and maize (Fish et al. 1984). The size of the *psa A* transcript is large enough to accomodate two adjacent coding frames in tandem as was suggested by Fish et al. (1984).

The amount of *psa A* transcripts present in the lane representing the RNA from green cell suspension culture AK2401 and standardized on plastid rRNA transcripts, is at least one order of magnitude higher in these plastids than in mature chloroplasts. The probe of the *psa A* gene also hybridizes with a second transcript (marked with an asterix) in lane 2. We have also seen this hybridization several times in RNA samples from leaves and also with other probes. We do not have an explanation for this artifact band.

Figure 3 c shows the obtained hybridization pattern when the internal fragment of the *rbc L* gene, pPhcPS1, is used to probe the glyoxylated total RNA preparations. The probe hybridizes with a 1.7 kb transcript, which implies that the size of the *Petunia hybrida rbc L* transcript is of a similar size to those found in tobacco (Shinozaki et al. 1982), maize (McIntosh et al. 1980; Erion 1985) and spinach (Zurawski et al. 1981). The hybridization signals for the *rbc L* transcripts are about the same in both plastid preparations (Fig. 3 c). This result implicates that the *rbc L* gene is also transcribed in plastids from the green cell suspension culture although no LSU polypeptides can be detected in those plastids. This is in contrast to the expression of the *psa A* gene, where the level of transcription is correlated with the amount of polypeptides found in these plastid forms. Since the samples were adjusted in such a way that they contained equal amounts of plastid ribosomal RNA (Fig. 2 a), these results suggest that the synthesis of the *Petunia hybrida rbc L* transcript is constitutive, as is the synthesis of the ribosomal RNA.

In vitro translation of hybrid selected transcripts

Since we observed a discrepancy in occurrence of *rbc L* transcripts and LSU polypeptides, we wanted to analyze whether this difference could be due to a change in translatability of the *rbc L* transcripts isolated from both plastid types, or that specific chloroplast factors regulate the translatibility of the *rbc L* transcripts. In vitro translation of isolated, protein-free, transcripts in a cell-free system can give some information about the translatability of transcripts. Specific *rbc L* transcripts from mature chloroplasts (Fig. 4 a) and from plastids from the green cell suspension culture AK2401 (Fig. 4 b) were isolated from total RNA preparations, by hybrid selection. Immobilized internal *rbc L* gene fragment, pPhcPS1, was used as the selective agent. Aliquots of these hybrid selected RNA samples were tested for translation in the rabbit reticulocyte cell-free system. The authenticity of the synthesized LSU proteins was shown by immunoprecipitation, fol-

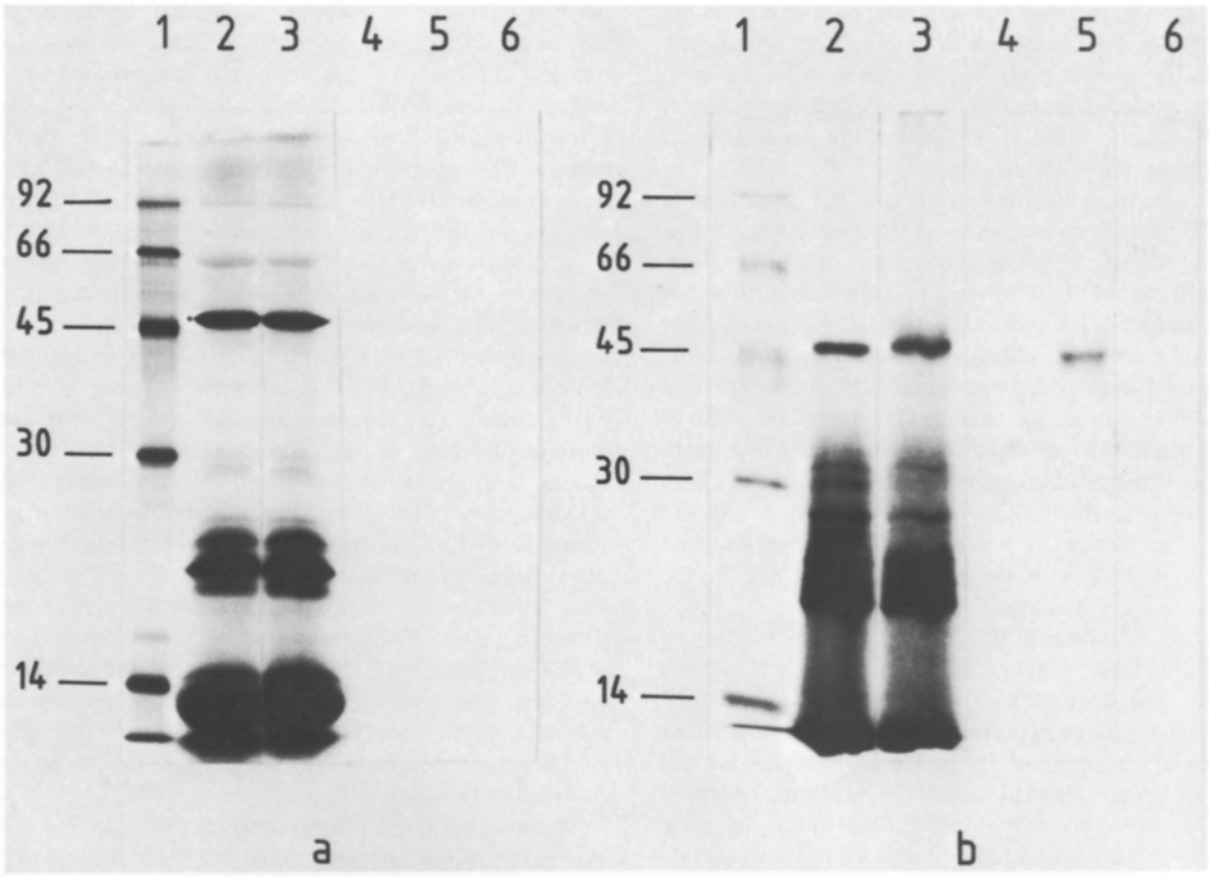

Fig. 4. Analysis of in vitro synthesized proteins. Hybrid selected *rbc L* transcripts from green cell suspension culture AK2401 (**panel a**) or from green leaves (**panel b**), were used to program the mRNA-dependent rabbit reticulocyte cell-free system. Synthesized [^{35}S]-methionine labelled proteins were separated on a 12.5% SDS-polyacrylamide gels and visualized by fluorography. Molecular weight markers indicated in kd (*lane 1*), total proteins synthesized without added RNA (*lane 2*) or with specific *rbc L* mRNA (*lane 3*). Immunoprecipitations of synthesized proteins with LSU antibodies in systems programmed without added RNA (*lane 4*) or with specific *rbc L* mRNA (*lane 5*). Immunoprecipitation when pre-immune serum is used in a system programmed with specific *rbc L* mRNA (*lane 6*)

lowed by SDS-polyacrylamide electrophoresis. Although equal amounts of *rbc L* transcript were used to programme the mRNA dependent reticulocyte cell-free system, only LSU proteins were synthesized when *rbc L* transcripts, isolated from mature chloroplasts were used. This result suggests that differences in the primary structure are responsible for the observed variation in translation of the *rbc L* transcripts from both plastid types. Also, this result is in agreement with the observation that in vivo LSU polypeptides are only present in leaf mesophyll chloroplasts and not in green cell culture plastids (Fig. 2).

Discussion

We have investigated the *in vivo* translation and transcription of two plastid-encoded genes, *rbc L* and *psa A*. Our purpose was to characterize, at least in part, the control levels of the differential regulation of these genes during chloroplast biogenesis. The reason for studying these *rbc L* and *psa A* genes was that preliminary results suggested that these genes are subject to different mechanisms of regulation during chloroplast biogenesis. Differential regulation of plastid-encoded genes was studied in two developmentally different plastid systems, the proplastid-like organelles from the green cell suspension culture AK2401 and the mature chloroplasts of green leaves.

To obtain insight in the in vivo protein level, we isolated total proteins from both plastid systems, and screened them for the presence of the large subunit of ribulose bisphosphate carboxylase and P700 chlorophyll-*a* apoprotein. The presence of a vast amount of LSU polypeptides was clearly determined. However, in our experiments LSU proteins could not be detected in plastids obtained from green cell culture AK2401, which implies that CO_2 fixation is turned off in these proplastid-like organelles from cell culture. This conclusion is supported by measurements of ribulose-biphosphate carboxylase activity in vivo, since we could

only detect a very low level of enzyme activity in the cell culture AK2401 (results not shown). Since the amount of specific LSU proteins does differ between the two plastid systems, this suggests that regulation of the *rbc L* gene must take place at the level of transcription or post-transcriptionally.

Analysis of in vivo transcription of the *rbc L* gene in our two plastid system, showed that regulation of this gene does not take place at the level of transcription. The amount of *rbc L* transcripts does not differ between mature chloroplast and proplastid-like organelles from cell suspension culture AK2401. This seems to be in contrast with some previous results of Smith and Ellis (1981), who suggested a light inducible increase in *rbc L* transcripts. On the other hand, Sasaki et al. (1984) correlated the observed increase in amounts of *rbc L* mRNA's during greening of pea leaves with the increase in plastid copy number. This seems to be in good agreement with the results we obtained in the *Petunia hybrida* system.

Recently, Erion (1985), Mullet et al. (1985) and Crossland et al. (1984) observed the occurrence of multiple transcripts for *rbc L* mRNA in maize chloroplasts. These transcripts could only be detected after S1 analysis and they could not be separated under normal RNA gel electrophoresis conditions. The two observed mRNAs were colinear and differed only in their 5′ ends. Crossland et al. (1984) and Mullet et al. (1985) suggested that only the larger *rbc L* transcript (1.8 kb) is the product of in vivo initiation of transcription, the smaller transcript (1.6 kb) seems to result from processing of the original larger transcript by site-specific cleavage. However, the biological function of the existence of multiple *rbc L* transcripts is not clear. Our results suggests that this heterogeneity in 5′ ends of the *rbc L* transcripts may be related to differences in translatability of the various transcripts of the *rbc L* gene. This is supported by the observation that the *rbc L* transcripts from the green cell cultures can not be translated in an in vitro rabbit reticulocyte cell-free system.

The regulation of the *psa A* gene, however, seems to take place at the transcriptional level. The level of steady state mRNA is high in plastids from the green cell suspension culture AK2401, when compared to the level as found in mature chloroplasts. Vierling and Alberte (1983) observed that de novo synthesis of the P700 chlorophyll-*a* apoprotein is required for the onset of photosystem I activity in greening tissue. They hypothesized, therefore, that the synthesis of this protein should be regulated at the transcriptional level. The observed high amounts of *psa A* transcripts in green cell culture plastids could then represent the onset of synthesis of the protein to be assembled in the membrane bound photosystem I because the cell culture plastids represent the stage of chloroplast development where thylakoid membranes are being formed. In mature chloroplasts, all thylakoid membranes are already present, so that there is no longer need for high levels of the de novo synthesized membrane proteins like the P700 apoprotein. This decreased need of the de novo synthesis is reflected in the observed decreased amount of mRNA for the *psa A* gene. A striking observation was that we could barely detect AP700 protein in the samples representing leaf chloroplasts. However, one should realize that these protein samples were adjusted in such away that they contain equal amounts chloroplast DNA template. During plastid development the amount of chloroplast template amount increases steadily while the AP700 protein is mainly synthesized in proplastid-like organelles and not afterwards. Therefore, this will result in the observed low AP700 protein/template ratio in leaf chloroplast. Although the plastids from the green cell suspension culture are not actively photosynthesizing, the presence of the P700 apoprotein is of biological significance. The P700 apoprotein and the nuclear encoded plastocyanin, which is already present in etiolated leaves, form a complex that ensures the light electron transport to start immediately when leaves are exposed to light (Nechustai and Nelson 1985).

Summarizing, the results obtained indicate that the expression of plastid genes takes place on at least two different levels: transcriptional control of the *psa A* gene and posttranscriptional, most likely translational regulation of the *rbc L* gene. At present, experiments are in progress to analyze the 5′ end of the *Petunia hybrida rbc L* mRNA to obtain an explanation for the observed difference in translatability of the *rbc L* transcript. This could answer the question whether multiple transcripts, as observed for the *rbc L* gene in maize, play a role in the differential regulation of plastid genes during chloroplast development.

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