Plant Molecular Biology 12: 201–211, 1989

The components of the plastid ribosome are not accumulated synchronously during the early development of spinach plants

Cordelia Bisanz-Seyer, You-Fang Li, Patrick Seyer and Régis Mache
Laboratoire de Biologie Moléculaire Végétale, CNRS URA 57, Université Joseph Fourier, BP 53X, F-38041 Grenoble cédex, France

Received 1 August 1988; accepted in revised form 1 November 1988

Key words: early plant development, germination, plastid ribosome, ribosomal proteins, *Spinacia oleracea*

Abstract

The expression of components of the 70S plastid ribosome has been determined during the first 13 days of spinach plant development. Total cellular RNA and proteins were used to determine the relative steady-state levels of mRNA for ribosomal proteins (r-proteins) by dot blot hybridization and the relative amounts of proteins by immunodetection with specific antibodies. The 16S rRNA as well as mRNAs for 9 out of 11 proteins studied, including those for the 32 kDa polypeptide of photosystem II and the large subunit (LSU) of ribulose-1,5-bisphosphate carboxylase (Rubisco) show a marked increase at the beginning of the germination (day 5). At this time the plastid DNA content increases from 4% to 6% of total DNA content and so the plastome copy number can only in part account for the important increase in mRNA steady-state levels. Interestingly the transcripts of the *rpl*23 and *rps*19 genes show a different accumulation pattern, indicating either a differential gene transcription and/or an increased stability of the transcripts. In the western blot analysis a group of r-proteins can be detected in dry seeds or after 24 hours of imbibition while a second group of proteins accumulates after 3 to 5 days of development. The differential accumulation pattern of r-proteins and mRNA for r-proteins indicates that post-transcriptional control plays an important role in plastid r-protein synthesis.

Introduction

Plastid ribosomes of higher plants are composed of 4 rRNA species and 52–55 r-proteins. The 4 rRNA species and about one third of the r-proteins are encoded by the plastid DNA (ptDNA), while the remaining r-proteins are encoded on nuclear DNA. Therefore ribosome biogenesis requires the expression of nuclear and plastid genes. With one exception, all components are present in the ribosome in equimolar amounts and we wonder how synthesis and assembly of such a protein-RNA complex may be regulated.

Until now, the effect of light on differential gene expression has been extensively studied essentially for the synthesis and assembly of Rubisco and photosystem reaction centers (for a recent review of the literature see [13]). Seedlings have usually been grown in the dark for several days and then transferred to light. But these etiolated seedlings have already undergone a considerable development and critical molecular events have already occurred. Therefore, in addition to this system, we also studied the synthesis of components of the 70S ribosome from the very earliest stages of spinach plant development, i.e. the imbibition and germination of seeds, as the most crucial phases in plant development. We determined for nuclear- and plastid-coded

r-protein genes the changes in the relative mRNA levels and the sequence of appearance of the proteins.

We studied one ribosomal protein gene for which the transcript and the gene product had been determined. For the other genes, the identification of the gene products is not yet done. We show that ribosomal protein genes are expressed at a very early stage of seedling development and that transcriptionally as well as post-transcriptionally operating regulatory mechanisms are implicated in the synthesis of components of the 70S ribosome.

Materials and methods

Plant material

Spinach seeds (*Spinacia oleracea* L. cv. Géant d'hiver) were imbibed, germinated and grown in soil at 25 °C in the dark.

Two grams of dry seeds (day 0) and of seeds swollen for 1–4 days until germination, i.e. the protrusion of the radicle, were collected for protein and nucleic acid extraction. Material (whole plantlets with seed residues) was further collected at each successive day during the germination period (day 5–day 8). Dark-grown seedlings with fully developed cotyledons (day 9) were transferred to continuous light and 2 g of plants (cotyledons and stems) were taken before exposure to light (day 9) and during the greening period (day 10–day 13) for chlorophyll, protein and nucleic acid extraction. All samples were frozen immediately in liquid nitrogen and stored at −80 °C until use.

Isolation of total cellular DNA, RNA and protein

Total cellular RNA was extracted from 2 g of material as described by Westhoff *et al.* [38] with the following modifications: after ethanol precipitation, the nucleic acids were dissolved in 80 mM Tris-borate, pH 8.3, 1 mM EDTA to a final concentration of 1 $\mu g/\mu l$. RNAs were precipitated twice with 2 M LiCl and, after dissolution in sterile water, stored at −80 °C until used for dot blots. Total cellular DNA was precipitated from the LiCl supernatants with 0.3 M sodium acetate and ethanol and cellular proteins were isolated according to Nechushtai and Nelson [21].

Determination of plastid DNA content

Determination of plastid DNA content was as described by Aguettaz *et al.* [1], using as radioactive DNA probe an intragenic fragment coding for the LSU of Rubisco (Table 1).

Detection of relative steady-state levels of r-proteins

To determine relative amounts of specific r-proteins in seeds and seedlings, aliquots of the protein extracts were electrophoresed on 12.5% polyacrylamide-SDS gels [14]. The proteins were transferred from the gels to nitrocellulose papers (BA 85, Schleicher and Schüll or Hybond C-Extra, Amersham) by electrotransfer. The blots were immunodetected with antibodies raised against individual or groups of 70S r-proteins, which had been separated and purified by HPLC ([18] and unpublished results). Reacting proteins were visualized either with ^{125}I-protein A or with the enzymatic peroxidase reaction (anti-mouse IgG sheep immunoglobulins conjugated with peroxidase; Bio-Sys).

Preparation and labelling of the DNA probes

Table 1 summarizes the different gene probes used in this study. Nuclear coded r-protein genes were obtained as follows: Poly(A)$^+$ RNA was selected by chromatography on oligo-dT cellulose. Integrity and enrichment of poly(A)$^+$ species were assessed by electrophoresis in highly denaturing conditions and by *in vitro* translation in the reticulocyte system.

Double-stranded cDNA was synthesized using the "cDNA synthesis kit" of Boehringer. cDNA libraries were constructed using the Amersham kit

Table 1. List of spinach and tobacco genes used as hybridization probes to determine relative amounts of mRNA and rRNA during the early development of spinach plants.

Genes	Gene products	Coded for by[1]	Origin of the probe	Specification of the probe	Reference
psbA	herbicide binding, "32 kDa photogene" of photosystem II	pt	spinach	*Sal* I-*Pst* I, 1200 bp	[42]
rbcL	large subunit of Rubisco	pt	spinach	*Pst* I-*Eco* RI, 1500 bp	[41]
rps1	ribosomal proteins of the small ribosomal subunit (30S)	n	tobacco	*Eco* RI-*Eco* RI, 1100 bp	P. Seyer (unpublished results)
rps3		pt	tobacco	*Sal* I-*Eco* RI, 216 bp	[34]
rps7 + rps12		pt	tobacco	*Pst* I-*Pst* I, 1300 bp	[30]
rps11		pt	spinach	*Sal* I-*Xba* I, 600 bp	[31]
rps19		pt	tobacco	*Sma* I-*Bgl* I, 400 bp	[30]
X[2]		n	spinach	*Eco* RI-*Eco* RI, 700 bp	P. Seyer (unpublished results)
rpl16	ribosomal proteins of the large	pt	tobacco	*Eco* RI-*Bam* HI, 300 bp	[34]
rpl23	ribosomal subunit (50S)	pt	spinach	*Eco* RI-*Xho* I, 510 bp	[43]
16S rDNA	16S ribosomal RNA	pt	spinach	*Eco* RI-*Pvu* II, 1100 bp	[5]

[1] n = nuclear-coded, pt = plastid-coded.

[2] Not determined; the cDNA reacts with the antibody against the spinach r-protein CS-S7, but sequence data did not yet allow us to determine a homology with an *E. coli* r-protein.

of λgt 10 and λgt 11 as vectors. The λgt 11 libraries were screened immunologically with anti-30S serum as described [9]. The cDNA clones obtained were recloned in pUC18 and further analysed by sequencing. Information of the identification of these cDNAs is given in Table 1.

Plasmid DNAs containing cloned spinach or tobacco ptDNA or cDNA fragments were purified according to Birnboim and Doly [3]. The plasmids were cut with appropriate restriction enzymes and gene-specific DNA fragments were purified by electroelution from agarose gels [20]. DNA fragments were radiolabelled with (^{32}P)dCTP using the nick-translation kits from Amersham or Boehringer. Specific activities ranging from 10^4 to 10^6 cpm/μg DNA were obtained.

Preparation of RNA dot blots and hybridization

Dilution series of 1−0.125 μg of total cellular RNA were bound to nitrocellulose filters (BA 85, Schleicher and Schüll) according to White and Bancroft [39]. Each radioactive probe was hybridized to 2−4 independent dot blots. Prehybridization and hybridization of the filters was according to Thomas [36] omitting dextran sulfate in the hybridization mixture. After hybridization for 48 h filters were washed, dried and exposed to Kodak XAR-5-Omat or Fuji RX films for 1−3 days using intensifying screens. After autoradiography, radioactive dots were cut out and counted in a Beckman scintillation counter.

Nomenclature of plastid r-proteins and r-protein genes

In this study we used two different kinds of nomenclature: 70S r-proteins from spinach and their corresponding antibodies are named according to Mache *et al.* [17]: CS-S1, CS-S2,..., CS-L1, CS-L2,..., whereas r-protein genes are designated according to their homology with r-proteins from *Es-*

Table 2. List of spinach r-proteins, their coding compartment and their corresponding counterparts in *E. coli* as identified until now (R. Mache, unpublished results).

Spinach r-protein	Coded for by[1]	*E. coli* r-protein	Gene
CS-S23	pt	S19	*rps*19
CS-L4	pt	L2	*rpl*2
CS-L20	n	L7/L12	*rpl*12
CS-L29	pt	L14	*rpl*14

[1] pt = plastid-coded, n = nuclear-coded.

cherichia coli [8, 30]: *rps*1, *rps*2, ..., *rpl*1, *rpl*2, ...

Table 2 summarizes the spinach r-proteins for which the corresponding *E. coli* r-protein has been identified.

Results

The steady-state levels of components of the plastid ribosome during the first 13 days of spinach plant

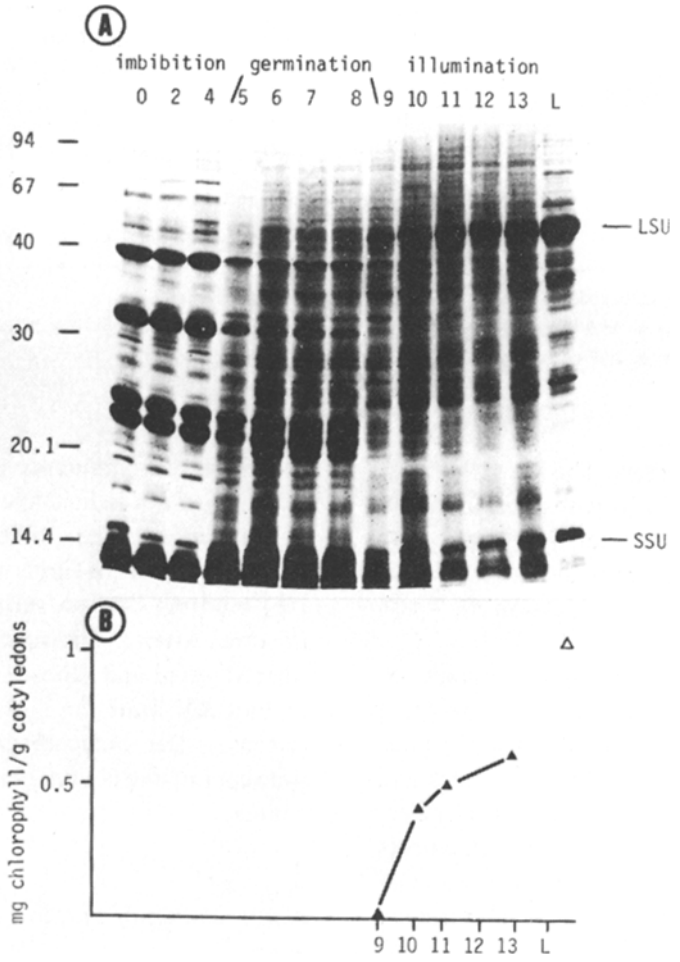

Fig. 1. (A) Changes in total cellular polypeptide content during imbibition, germination and illumination of dark grown spinach plants. Total cellular proteins were isolated as described in Materials and methods. Aliquots of 3 μl were electrophoresed in 12.5% polyacrylamide-SDS gels and proteins were visualized by Coomassie blue staining. Numbers on top of the slots indicate the developmental stage (in days) as described in Materials and methods. Lane L shows the protein pattern of green spinach leaves. Numbers on the left margin refer to molecular weight of protein standards in kDa. The protein bands corresponding to the two subunits of Rubisco (LSU and SSU) are indicated on the right. (B) Time course of changes in chlorophyll content after illumination of etiolated spinach seedlings. Chlorophyll determination was according to Vernon [37]. Numbers indicate the stages of development in days. The open triangle in lane L indicates the chlorophyll content in green leaves of spinach plants.

development have been determined. Relative concentrations of 16S rRNA, mRNAs for 8 r-proteins, the large subunit of Rubisco and the 32 kDa protein of photosystem II as well as changes in the relative concentrations of 14 r-proteins have been followed during imbibition and germination of seeds and during subsequent greening of seedlings.

Figure 1A shows the changes in the protein pattern of the different developmental stages studied. The four most intensive stained protein bands of approximately 49, 35, 23, and 21 kDa, respectively, representing probably the main spinach storage proteins, decrease drastically with the protrusion of the radicle at day 5 of development and disappear completely in the course of germination. During the following continuous illumination of etiolated seedlings, the amounts of large and small subunit of Rubisco (LSU and SSU) increase considerably.

Chlorophyll synthesis accompanying the illumination period is shown in Fig. 1B. Chlorophyll synthesis starts after a lag phase of about 2 hours after exposure to light. Until 24 h in the light (day 10) there is a rather large increase, then the chlorophyll synthesis slows down to reach 0.65 mg/g of cotyledons after 4 days of illumination.

Relative transcript levels of mRNAs for 70S r-proteins and from psb*A and* rbc*L genes*

Figure 2 shows a typical series of dot blot experiments in which 1 μg of total cellular RNA from dry

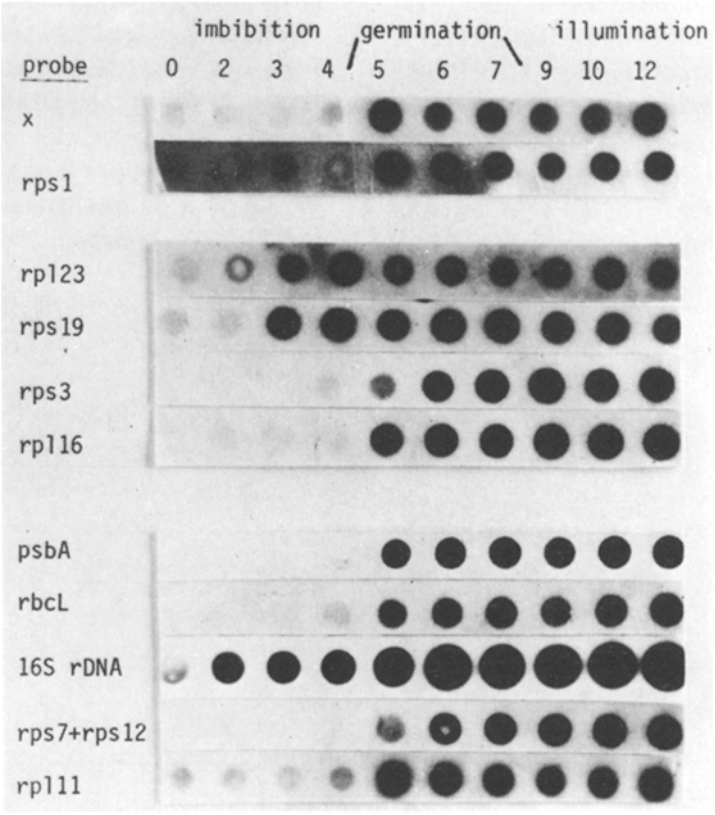

Fig. 2. Hybridization of developmental dot blots. RNA was extracted from seeds during several stages of imbibition and germination and from seedlings during de-etiolation. Dilution series of 1–0.125 μg of total cellular RNA were dot-blotted on nitrocellulose filters and hybridized with the radioactive DNA probe listed on the left margin. "x" = not determined; the cDNA clone reacts specifically with the spinach r-protein antibody CS-S7, but partial sequencing of the clone did not yet allow us to determine a homology with an *E. coli* r-protein. Typical results obtained with 1 μg RNA are shown. Numbers on top represent the developmental stages (in days) as described in Materials and methods.

(0), imbibed (2–4), germinating (5–8) seeds and from etiolated (9) and greening (10–12) seedlings were bound to nitrocellulose and hybridized with the radioactive DNA fragments corresponding to the probes noted on the left margin. The probe named "x" (not determined) represents the cDNA encoding the spinach r-protein CS-S7 for which a corresponding *E. coli* r-protein could not yet been identified.

Because autoradiographies only give a visual, rather imprecise, impression of the radioactivity bound to the filters, we cut out the dots and counted them in a liquid scintillation counter. The values obtained show that RNA species for all genes studied are present in dry seeds, though in minute amounts for some of them. A significant burst in accumulation of the different mRNAs is observed at two different stages of development. Most of the mRNAs studied show an important increase in accumulation at the beginning of germination (day 5, Fig. 2, lane 5). Among these mRNAs are not only those for plastid and nuclear coded ribosomal proteins (*rps*3, *rpl*16, *rps*11, *rps*7, *rps*12, *rps*1, "x") but also the mRNAs for the 32 kDa polypeptide of photosystem II (*psb*A) and for the LSU (*rbc*L). Yet there are two mRNAs showing an increased steady-state level in seeds at day 3 of development under our growth conditions (Fig. 2, lane 3). These are the transcripts of the *rpl*23 and *rps*19 genes which are clustered on the plastid DNA in an arrangement similar to the S-10 operon of *E. coli* [34, 35].

Besides the different mRNAs, we looked also at changes in the steady-state levels of the 16S rRNA (Fig. 2). Already present in dry seeds, the 16S rRNA is much more abundant than all the mRNAs studied, but there is also an increase in accumulation at days 5 and 6 of development. The results shown in Fig. 2 are obtained by hybridizing the probe to 1 μg of total cellular RNA and at this concentration the dots are saturated. Results obtained with 0.125 μg total RNA show, that the 16S rRNA increases between day 6 and day 12.

We next asked the question if the increase in transcript levels we observe during germination could be due to an increased number of ptDNA molecules. Figure 3A shows that in dry seeds the amount of ptDNA is 4% of total DNA. This amount remains constant during the four days of imbibition, but doubles between day 4 and day 6 of development. Plastid DNA content further increases to reach 16% in fully green cotyledons. The comparison of the in-

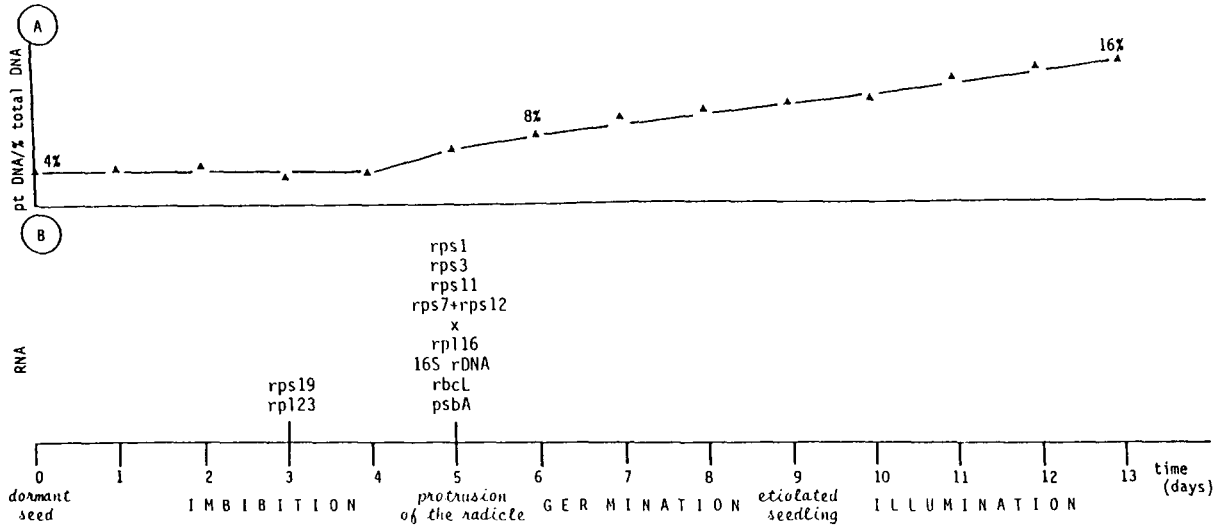

Fig. 3. (A) Changes in the percentage of ptDNA in total cellular DNA during the first 13 days of spinach plant development. Determination of the percentage was according to [1], using as radioactive probe an intragenic fragment of *rbc*L. (B) Summary of the developmental RNA dot blot hybridizations, indicating the time of accumulation of the mRNAs of the different genes studied. "x" = not determined; the cDNA clone reacts with the spinach r-protein antibody CS-S7, but sequence data did not yet allow us to determine a homology with an *E. coli* r-protein.

crease in ptDNA content (Fig. 3A) with the burst in accumulation of the different mRNAs (Figs. 2 and 3B) shows, that the increase observed for the ptDNA level is much less than the increase observed in the mRNA steady-state levels. This suggests that plastome copy number only plays a minor role in the augmentation of the mRNA levels.

Sequence of appearance of 70S r-proteins

We studied changes in the steady-state levels of some of the 70S r-proteins during imbibition, germination and subsequent greening of etiolated spinach plants with an immunological approach [21], using specific antibodies raised against individual or groups of r-proteins from spinach ([18] and unpublished results).

Figure 4A shows some typical results. It is obvious that the sequence of appearance of the 70S r-proteins is more complex than the increase in accumulation of the different mRNAs as shown in Fig. 2. The mRNAs accumulated at two different times of development (days 3 and 5), while the different r-proteins studied show rather each an individual accumulation pattern. Using different protein con-

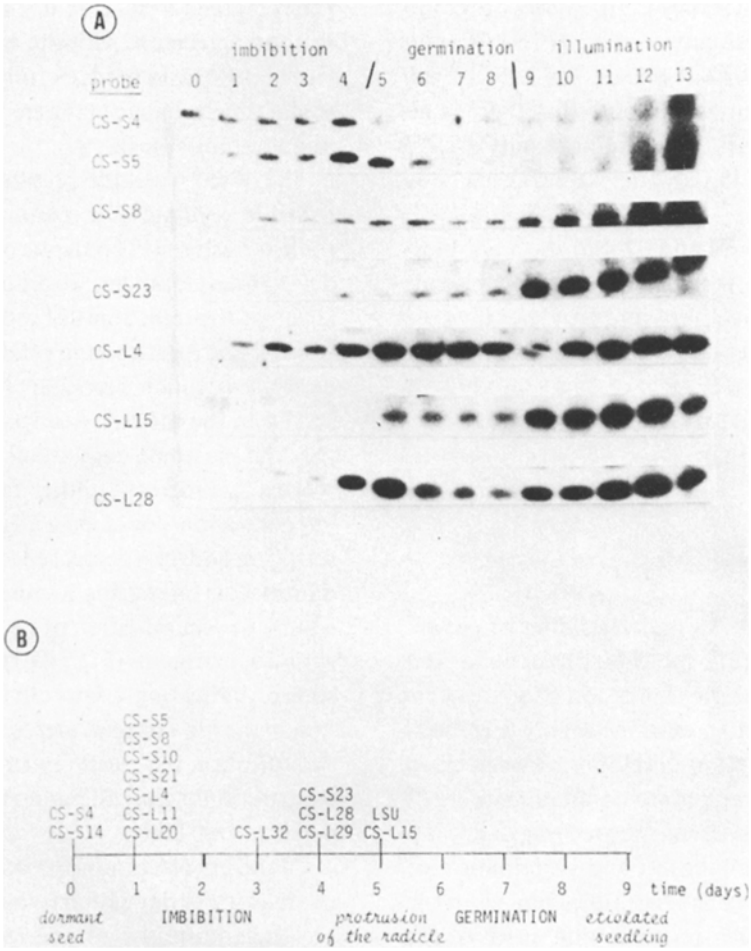

Fig. 4. (A) Sequence of appearance of some of the r-proteins studied. Total cellular proteins were separated on 12.5% polyacrylamide-SDS gels and electrotransferred to nitrocellulose. After immunodetection with the specific antibodies noted on the left margin, the protein-antibody complex was revealed with ^{125}I-protein A. Numbers on top of the lanes correspond to the development stages in days as described in Materials and methods. (B) Summary of the developmental western blot analysis for r-proteins and the LSU of Rubisco, indicating the first detection for the different proteins studied.

centrations and different gel systems (data not shown), we always observed the same pattern of sequential appearance. Thus we conclude that the differential pattern we observe is not due to a different sensitivity of the various antibodies used, but reflects differences in the steady-state levels of the proteins studied.

Fig. 4B summarizes all results obtained in our developmental western blot analysis (not all patterns are shown in Fig. 4A). A rather important number of r-proteins can be detected at the very earliest stages of development: either they are already present in dry seeds (CS-S4 and CS-S14) or they accumulate after one day of imbibition of the seeds (CS-S5, CS-S10, CS-S21, CS-L4, CS-L20). A second group of proteins accumulate between day 3 (CS-L32) and day 5 (CS-S23, CS-L28, CS-L29, CS-L15) of development. It is interesting to note that there is no preferential early accumulation of nuclear (CS-L20) versus plastid (CS-S23, CS-L4, CS-L29) encoded r-proteins.

Two r-proteins (CS-S4 and CS-S5) show an unexpected pattern: their steady-state levels decrease after day 4 or day 6, respectively, and only start increasing again after day 11, i.e. after 48 hours of illumination. The significance of this reproducible observation is difficult to explain and we tend to consider them as artifactual.

Discussion

As a first step towards an understanding of plastid ribosome synthesis at the molecular level we looked for information about the expression of some of its components. In order to examine highly metabolically active stages in plant development we decided to extend the classical physiological approach of light-induced changes during the greening of etiolated seedlings to the imbibition and germination of seeds. So we studied for the first time changes in the steady-state levels of r-proteins and mRNA for r-proteins in dry seeds and during the first 13 days of spinach plant development.

Different organisms employ distinct molecular strategies to ensure the coordinated synthesis of their r-proteins (for a recent review see [19]), and it is interesting to note that until now there is nothing known about the regulatory mechanisms operating in higher plants.

Changes in the steady-state levels of mRNA for 70S r-proteins and the LSU of Rubisco

The procedure of dot-blot titration we used in this study, enables us to follow the changes in the steady-state levels of individual RNA transcripts in a given amount of total RNA throughout the earliest stages of spinach plant development.

We can detect low levels of mRNA species for all genes studied in mature dry seeds and this result is in good agreement with the existence of preformed, stored mRNA in seeds permitting protein synthesis to start immediately at the earliest time of imbibition and germination [2, 4].

The objective of the present study was to identify possible regulatory mechanisms operating during the synthesis of components of the 70S ribosome. At day 5 of development, we observe a burst in accumulation of the transcripts of most of the genes studied (Figs. 2 and 3). Although ptDNA level increases, this increase is much less than the augmentation observed in the mRNA steady-state levels, suggesting that the plastome copy number only plays a minor role in this increase. Other factors influencing this augmentation could be an increased rate of transcription and/or a decreased rate of transcript degradation. It is interesting to note that at the same time a burst in accumulation of two transcripts for nuclear coded r-proteins (Fig. 3B, *rps*1 and "x") is also observed, indicating a coordination of the transcription in the two compartments. A similar coordinated transcription has been reported for the mRNAs of the plastid coded LSU and the nuclear coded SSU genes in pea [29].

Of the mRNAs studied, two show an increased accumulation at day 3 of development (Fig. 3B). These are the transcripts of the *rpl*23 and *rps*19 genes, which are clustered on the plastid DNA in a S10-like operon [22, 34, 35]. The whole cluster comprises the genes ***rpl*23**, *rpl*12, ***rps*19**, *rpl*22, ***rps*3**, ***rpl*16**, *rpl*14 and *rps*8 (genes in bold type have been studied in this paper). By S1 nuclease mapping it has recently been

shown in our laboratory that at least the genes *rpl*23, *rpl*2, *rps*19 and *rpl*22 are cotranscribed [35], but the transcription unit probably extends to further genes and the question arises upon the mechanism and the significance of the early accumulation of the mRNAs for the *rpl*23 and *rps*19 genes. Concerning the mechanism it could be either an increased stability of the transcripts [12, 33] for these genes and/or a differential gene transcription.

After day 6 of development the steady-state levels of all r-protein mRNAs seems to remain essentially constant. This result has to be interpreted carefully, because we measure here the abundance of individual mRNAs relative to total RNA, which is mainly rRNA, and changes in rRNA content surely influence the titration of individual mRNAs. But the result that the steady-state levels of mRNA for r-proteins remain stable during greening of our etiolated seedlings (after day 9 of development) is in agreement with two other reports. Posno *et al.* [23] showed that there are no differences in the steady-state levels of *rps*12 and *rps*7 transcripts between etiolated and greened *Spirodela* plants and Russel and Bogorad [26] reported that the pool of *rps*4 transcripts does not change significantly upon illumination of dark-grown maize seedlings.

The finding that the transcript level of the *rbc*L gene does not increase with light in spinach shows once more that the mechanism of regulation of this gene varies between different plant species: like in spinach there are in mustard [15, 16] and in maize [6] only minute or no differences between transcript levels in etiolated and illuminated plants, whereas in pea [10, 27, 28, 32] and *Spirodela* [23] an increasing steady-state level of LSU transcripts has been observed during greening of etiolated plants. In barley [12] *rbc*L transcript levels increase first in the light and then decline with increasing age of the seedlings.

Changes in the steady-state levels of r-proteins

In wheat endosperm of dry seeds, plastids have been observed, which appear to contain ribosomes [11]. So mature dry seeds have to contain the whole set of r-proteins. Despite that we cannot detect all r-proteins studied in dry seeds (Fig. 4A), indicating that the method of immunodetection used here is not sufficiently sensitive to detect small amounts of a protein. But the interesting conclusion we draw from our results is that the r-proteins are not present in equal amounts throughout the developmental period studied, but that there are significant differences in their steady-state levels and it seems that at least at the beginning of the development the pattern of r-proteins is dominated by unassembled and/or partially assembled r-proteins. This is in very good accord with recent results reported by Feierabend *et al.* [7]. They found that in extracts of rye leaves grown at 32 °C, a temperature which is non-permissive for plastid ribosome formation, 8 to 10 plastid r-proteins accumulate markedly as unassembled polypeptides. During chloroplast development Posno *et al.* [23] studied the expression of the *rps*12 and *rps*7 genes and they observe no significant changes in the steady-state levels of these proteins during the greening of etiolated *Spirodela* plants. They conclude that etioplasts already contain the complement of ribosomes necessary for the further development. Based on our results, we can confirm this hypothesis, but we can show further that important steps in the synthesis of components of the plastid ribosome are taking place during imbibition and germination of seeds.

The crucial question arising is about the biological significance of the sequential appearance of 70S r-proteins. The fluctuations we observe could reflect steps in the sequential assembly of the 70S ribosome. In *E. coli*, for example, *in vitro* reconstitution experiments [24] suggest that complexes consisting of r-proteins coded for by genes in the same operon are formed and incorporated as preformed complexes into the ribosome, instead of individual proteins, a mechanism which would obviously accelerate the assembly process. So it could be that the different steady-state levels we observe indicate that some r-proteins are not only present in the ribosomes, but also stored in preformed complexes, ready to be incorporated when there is an increased need for ribosomes. The ribosomal proteins detected very early in development (Fig. 4B) could also be primary RNA-binding proteins which are important for the early assembly of the ribosomal particles [40]. This may be the case at least for r-proteins CS-S10 and CS-S14

which have been identified as being primary 16S rRNA binding proteins in spinach [25].

For one r-protein studied here a correlation has been established with a r-protein gene, i.e. the *rps*19 gene codes for the spinach r-protein CS-S23 (cf. Table 2). So, in this case we can compare the developmental appearance of one r-protein with its proper mRNA. The mRNA shows a burst in accumulation at day 3 of development (Fig. 2), while we can detect the corresponding protein (CS-S23) at day 4 of development, indicating for this specific protein a transcriptional control.

Conclusion

The results presented in this paper show that the components of the 70S plastidic ribosome do not accumulate synchronously during spinach plant development and that different regulatory mechanisms are implicated in their synthesis.

1. The plastome copy number can only account for a very minor part in the drastic increase of mRNA levels for plastid coded r-proteins at day 5 of development. Other regulatory factors as an increased rate of transcription and/or a decreased rate of transcript degradation have to be responsible for this augmentation.

2. At least two genes, *rpl*23 and *rps*19, which form a transcription unit with some other r-protein genes, are regulated by an altered gene transcription and/or an increased stability of their transcripts.

3. The differential accumulation pattern of r-proteins and mRNA for r-proteins indicates that post-transcriptional regulation also plays an important role in plastid ribosome synthesis.

References

1. Aguettaz P, Seyer P, Pesey H, Lescure AM: Relations between the plastid gene dosage and the levels of 16S rRNA and *rbc*L gene transcripts during amyloplast to chloroplast change in mixotrophic spinach cell suspensions. Plant Mol Biol 8: 169–177 (1987).
2. Bewley JD: Protein and nucleic acid synthesis during seed germination and early seedling growth. In: Boulter D, Parthier B (eds) Nucleic Acids and Proteins in Plants I, pp. 559–591. Springer-Verlag, Berlin/Heidelberg/New York (1982).
3. Birnboim HC, Doly J: A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7: 1513–1523 (1979).
4. Barroto KE, Dure III L: The expression of chloroplast genes during cotton embryogenesis. Plant Mol Biol 7: 105–113 (1986).
5. Briat JF, Dron M, Loiseaux S, Mache R: Structure and transcription of the spinach chloroplast rDNA leader region. Nucleic Acids Res 10: 6865–6878 (1982).
6. Crossland LD, Rodermel SR, Bogorad L: Single gene for the large subunit of ribulose bisphosphate carboxylase in maize yields two differentially regulated mRNA. Proc Natl Acad Sci USA 81: 4060–4064 (1984).
7. Feierabend J, Schlüter W, Tebartz K: Unassembled polypeptides of the plastidic ribosomes in heat-treated 70S-ribosome-deficient rye leaves. Planta 174: 543–555 (1988).
8. Hallick RB, Bottomley W: Proposals for the naming of chloroplast genes. Plant Mol Biol Rep 1: 38–43 (1983).
9. Huynh TV, Young RA, Davis RW: Construction and screening cDNA libraries in λgt 10 and λgt 11. In: Glover DM (ed) DNA Cloning, vol. I, pp. 49–78. IRL Press, Oxford/Washington DC (1985).
10. Inamine G, Nash B, Weissbach H, Brot N: Light regulation of the synthesis of the large subunit of ribulose-1,5-bisphosphate carboxylase in pea: Evidence for translational regulation. Proc Natl Acad Sci USA 82: 5690–5694 (1985).
11. Kirk JTO, Tilney-Bassett RAE: The Plastids: Their Chemistry, Structure, Growth and Inheritance, 2nd ed. W. H. Freeman and Company, London/San Francisco (1978).
12. Klein RR, Mullet JE: Control of gene expression during higher plant chloroplast biogenesis. Protein synthesis and transcript levels of *psb*A, *psa*A-*psa*B, and *rbc*L in dark-grown and illuminated barley seedlings. J Biol Chem 262: 4341–4348 (1987).
13. Kuhlemeier C, Green PJ, Chua N-H: Regulation of gene expression in higher plants. Ann Rev Plant Physiol 38: 221–257 (1987).
14. Laemmli UK: Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature 227: 680–685 (1970).
15. Link G: Phytochrome control of plastid mRNA in mustard (*Sinapis alba* L.). Planta 154: 81–86 (1982).
16. Link G: Hybridization study of developmental plastid gene expression in mustard with cloned probes for most plastid DNA regions. Plant Mol Biol 3: 243–248 (1984).
17. Mache R, Dorne A-M, Marti-Battle R: Characterization of spinach plastid ribosomal proteins by two-dimensional gel electrophoresis. Mol Gen Genet 177: 333–338 (1980).
18. Mache R, Audren H, Bisanz-Seyer C, Dorne A-M, Massenet O, Rozier C, Thomas F: Biosynthesis of chloroplast ribosomal components. In Biggens J (ed) Progress in Photosynthesis Research, Vol. 4, pp. 547–551. Martinus Nijhoff Publishers, Dordrecht (1987).
19. Mager WH: Control of ribosomal protein gene expression.

Biochem Biophys Acta 949: 1–15 (1988).
20. Maniatis T, Fritsch EF, Sambrook J: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).
21. Nechushtai R, Nelson N: Biogenesis of photosystem I reaction center during greening of oat, bean and spinach leaves. Plant Mol Biol 4: 377–384 (1985).
22. Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesono K, Shiki Y, Takeuchi N, Chang Z, Hota S, Inokuchi H, Ozeki H: Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature 322: 572–574 (1986).
23. Posno M, Verweij WR, Dekker JC, de Waard PM, Groot GSP: The genes encoding chloroplast ribosomal proteins S7 and S12 are located in the inverted repeat of *Spirodela oligorhiza* chloroplast DNA. Curr Genet 11: 25–34 (1986).
24. Röhl R, Nierhaus KH: Assembly map of the large subunit (50S) of *Escherichia coli* ribosomes. Proc Natl Acad Sci USA 79: 729–733 (1982).
25. Rozier C, Mache R: Binding of 16S rRNA to chloroplast 30S ribosomal proteins blotted on nitrocellulose. Nucleic Acids Res 12: 7293–7304 (1984).
26. Russell D, Bogorad L: Transcription analysis of the maize chloroplast gene for the ribosomal protein S4. Nucleic Acids Res 15: 1853–1867 (1987).
27. Sasaki Y, Sakihama T, Kamikubo T, Shinozaki K: Phytochrome-mediated regulation of two mRNAs encoded by nuclei and chloroplasts of RuBisCO. Eur J Biochem 133: 617–620 (1983).
28. Sasaki Y, Nakamura Y, Matsuno R: Regulation of gene expression of ribulose bisphosphate carboxylase in greening pea leaves. Plant Mol Biol 8: 375–382 (1987).
29. Shinozaki K, Sasaki Y, Sakihama T, Kamikubo T: Coordinated light-induction of two mRNAs, encoded in nuclei and chloroplasts, of ribulose-1,5-bisphosphate carboxylase/oxygenase. FEBS Lett 144: 73–76 (1982).
30. Shinozaki K, Ohme M, Tanaka M, Wakasugi N, Hoyashida T, Matsubayashi N, Zaita J, Chunwongse J, Obokata J, Yamaguchi-Shinozaki K, Ohto C, Torazawa K, Meng BY, Sugita M, Deno H, Kamogashira T, Yamada K, Kusuda J, Takaiwa F, Keto A, Tohdoh N, Shimida H, Sugiura M: The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5: 2043–2049 (1986).
31. Sijben-Müller G, Hallick RB, Alt J, Westhoff P, Herrmann RG: Spinach plastid genes coding for initiation factor IF-1, r-protein S11 and RNA polymerase α-subunit. Nucleic Acids Res 14: 1029–1044 (1986).
32. Smith SM, Ellis RJ: Light-stimulated accumulation of transcripts of nuclear and chloroplast genes for RuBPcase. J Mol Appl Genet 1: 127–137 (1981).
33. Stern DB, Gruissem W: Control of plastid gene expression: 3′ inverted repeats act as mRNA processing and stabilizing elements but do not terminate transcription. Cell 51: 1145–1157 (1987).
34. Tanaka M, Wakasugi T, Sugita M, Shinozaki K, Sugiura M: Genes for the eight ribosomal proteins are clustered on the chloroplast genome of tobacco (*Nicotiana tabacum*): similarity to the S10 and *spc* operons of *E. coli*. Proc Natl Acad Sci USA 83: 6030–6034 (1986).
35. Thomas F, Massenet O, Dorne A-M, Briat J-F, Mache R: Expression of the *rpl*23, *rpl*2 and *rps*19 genes in spinach chloroplasts. Nucleic Acids Res 16: 2461–2472 (1988).
36. Thomas PS: Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc Natl Acad Sci USA 77: 5201–5205 (1980).
37. Vernon LP: Spectrophotometric determination of chlorophylls and pheophytins in plant extracts. Anal Biochem 32: 1144–1150 (1960).
38. Westhoff P, Nelson N, Bünemann H, Herrmann RG: Localization of genes for coupling factor subunits on the spinach plastid chromosome. Curr Genet 4: 109–120 (1981).
39. White BA, Bancroft FC: Cytoplasmic dot hybridization. J Biol Chem 257: 8569–8572 (1982).
40. Wittmann-Liebold B: Ribosomal proteins: their structure and evolution. In: Hardesty B, Kramer G (eds) Structure, Function and Genetics of Ribosomes, pp. 326–361. Springer-Verlag, Berlin/Heidelberg/New York (1986).
41. Zurawski G, Perrot B, Bottomley W, Whitfeld PR: The structure of the gene for the large subunit of Rubisco from spinach chloroplast DNA. Nucleic Acids Res 9: 3251–3270 (1981).
42. Zurawski G, Bohnert HJ, Whitfeld PR, Bottomley W: Nucleotide sequence of the gene for the Mr 32000 thylakoid membrane protein from *Nicotiana debneyi* predicts a totally conserved primary translation product of Mr 38950. Proc Natl Acad Sci USA 79: 7699–7703 (1982).
43. Zurawski G, Bottomley W, Whitfeld PR: Junctions of the large single copy region and the inverted repeats in *Spinacia oleracea* and *Nicotiana debneyi* chloroplast DNA: sequence of the genes for $tRNA^{His}$ and the ribosomal proteins S19 and L2. Nucleic Acids Res 12: 6547–6558 (1984).