

## Developmental and light-dependent cues interact to establish steady-state levels of transcripts for photosynthesis-related genes (*psbA*, *psbD*, *psaA* and *rbcL*) in rice (*Oryza sativa* L.)

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Received: 16 August 1993/11 September 1993

**Abstract.** The steady-state transcript levels for *psbA*, *psbD*, *psaA* and *rbcL* are low in dark-grown rice seedlings as compared to those grown in light. Following seed germination, they accumulate in an age-dependent manner, in dark as well as light, reaching a maximal level on the 7th or 8th day, before a slow decline sets in. But transcripts for *psbA* and *psbD* continue to maintain relatively high levels even after 10 days of growth in light. Exposure of 5-day-old dark-grown seedlings to light results in an approximately 25–60-fold increase in transcripts during a period of 72 h, followed by a decrease. An analysis of data from both lines of investigation reveals that the developmental programme increases the transcript levels for *psbA*, *psbD*, *psaA* and *rbcL* by about 10-, 2.3-, 7.0- and 8.0-fold, respectively, between 5–8 days after germination and it is independent of light. At the same time, exposure of the seedlings to light during this period further enhances transcript levels by 5-, 11.4-, 6.6- and 7.8-fold, respectively. Thus, both developmental and light-dependent cues contribute to establish steady-state levels of transcripts for the chloroplast genes investigated.

**Key words:** *Oryza sativa* – Chloroplast genes – Developmental regulation – Light regulation – Gene expression

### Introduction

While the role of light in chloroplast biogenesis is indisputable, the extent of its effect on chloroplast gene expression has remained controversial due to the lack of adequate knowledge about the mode of action and the relative contribution of the various steps which are influenced by light (Mullet 1988; Gruissem 1989; Herrmann et al. 1991; Link 1991; Rochaix 1992; Tyagi et al. 1993). Thus, whereas in barley, exposure of dark-grown seedlings to light causes little increase in steady-state mRNA levels of chloroplast photosynthesis-related genes (Mullet and Klein 1987), in

spinach, maize, sorghum, wheat and mothbean a several-fold increase has been observed (Herrmann et al. 1985; Rodermel and Bogorad 1985; Deng and Gruissem 1987; Schrubar et al. 1991; Kawaguchi et al. 1992; Kelkar et al. 1993). This situation is reflected also in studies on transcription rates. While some investigations indicate that transcription rates are not appreciably influenced (Deng and Gruissem 1987; Mullet and Klein 1987; Krupinska and Apel 1989), others report that they increase significantly (Klein and Mullet 1990; Klein 1991; Schrubar et al. 1991; Kawaguchi et al. 1992; Rapp et al. 1992), particularly on exposure to light. Studies to determine the effective wavelength of light in barley revealed that high-fluence blue light is most effective in the induction of *psbD-psbC* transcript accumulation, but red or far-red light was found to be ineffective (Gamble and Mullet 1989). A proper evaluation of the light effect is difficult as mRNAs for several chloroplast genes accumulate in a temporal/spatial manner, independent of light (Thompson et al. 1983; Dietrich et al. 1987; Hughes et al. 1987; Baumgartner et al. 1989; Kelkar et al. 1993). It is, therefore, natural to assume that interaction of *both* developmental and light-dependent cues would ultimately establish the degree of gene expression as well as the state of differentiation of chloroplasts. This assumption is strongly substantiated by recent studies on photomorphogenic mutants of *Arabidopsis* (Chory 1992; Wei and Deng 1992; Hou et al. 1993).

We have recently undertaken studies on the chloroplast genome of *indica* rice and also on the organ-specific expression of photosynthesis-related genes (Kapoor et al. 1991, 1993). Earlier, the chloroplast genome of *japonica* rice has been completely sequenced and a transcription map prepared (Hiratsuka et al. 1989; Kanno and Hirai 1993). The only other study on the regulation of gene expression in rice is that of Chen et al. (1992). They have shown that transcript levels of the *psaA-psaB-rps14* operon are not significantly influenced by light and that a control is probably exerted at a translational or post-translational level. In contrast to this, the present study reveals that mRNAs for several chloroplast genes accumulate both

in a development- and a light-dependent manner. The increase is 25–60-fold following the illumination of 5-day-old dark-grown seedlings. Efforts have also been made to determine the relative contribution of developmental and light-dependent signals in establishing steady-state transcript levels.

## Materials and methods

**Plant growth conditions.** Seedlings of *O. sativa* L. subsp. *indica* cv Pusa 169 were raised on wet cotton soaked in salts of MS medium (Murashige and Skoog 1962) after surface-sterilization of seeds with 0.1% HgCl<sub>2</sub> for 10 min. To study the developmental expression of chloroplast genes, seedlings were grown in the dark in a B.O.D. incubator (Scientific Equipment Works, India), or in light (9 W m<sup>-2</sup>, from mercury vapour lamps) in a plant growth chamber (VEPHQ 511350, Heraeus Vötsch GmbH, Germany), under controlled environmental conditions (temperature 28 ± 1 °C, humidity 90–100%) for up to 10 days. For studies on light-dependent gene expression, seedlings were grown for 5 days in the dark and then exposed to light (as above) for specified durations.

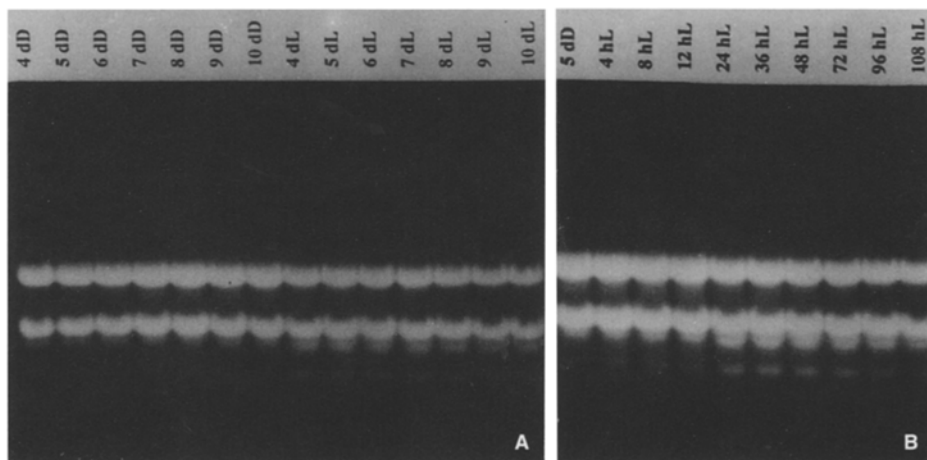
**Northern analysis.** Total cellular RNA was isolated from leaf material by using the procedure developed by Logemann et al. (1987). Twenty micrograms of denatured RNA samples were resolved on formaldehyde-agarose gels (see Ausubel et al. 1989). For qualitative analysis, the gel was stained with ethidium bromide (2 µg/ml) and visualized on a UV transilluminator (Ausubel et al. 1989). The uniform intensity of ribosomal RNA bands was taken both as a criterion to exclude differential RNA degradation in different samples and evidence for equal loading of RNA in different lanes (Fig. 1). From another gel containing similar samples, RNA was blotted onto a Hybond-C (nitrocellulose) filter (Amersham International Inc., UK) according to the manufacturer's specifications. Prehybridization was carried out in plastic bags containing a 200 µl/cm<sup>2</sup> prehybridization solution comprising 50% formamide, 5 SSC, Denhardt's solution (1 mg/ml each of polyvinylpyrrolidone, bovine serum albumin and Ficoll 400), 50 mM of sodium phosphate buffer (pH 6.5) and 250 µg/ml of sonicated and denatured herring sperm DNA, on an incubator shaker (Labline Instruments Inc., USA) at 50 rpm and 42 °C, for 24 h. For hybridization, heterologous DNA probes from spinach, specific to thylakoid protein genes (see Kapoor et al. 1991) or the ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit gene (Zurawski et al. 1981), were labelled using a multiprime DNA labelling system (Amersham International Inc., UK) and (α-<sup>32</sup>P)-deoxyCTP (BRIT, India) following the manufacturers specifications. After 24 h of hybridization, filters were washed twice with 2 SSC and 0.1% SDS at room temperature for 15 min, followed by

two washes with the same solution at 50 °C for 10 min each. The filters were wrapped in Cling-film and exposed to X-ray films (Konica, Japan), in a cassette containing an intensifying screen, at -70 °C. The relative abundance of the transcripts for various genes was quantified from the autoradiograph using a LASER densitometer (2202 Ultrosan, LKB, Sweden). For plotting these values, the maximum value for each transcript is considered as 100 and relative values for other samples are depicted accordingly. In the case of *psbD*, combined values of multiple transcripts are given.

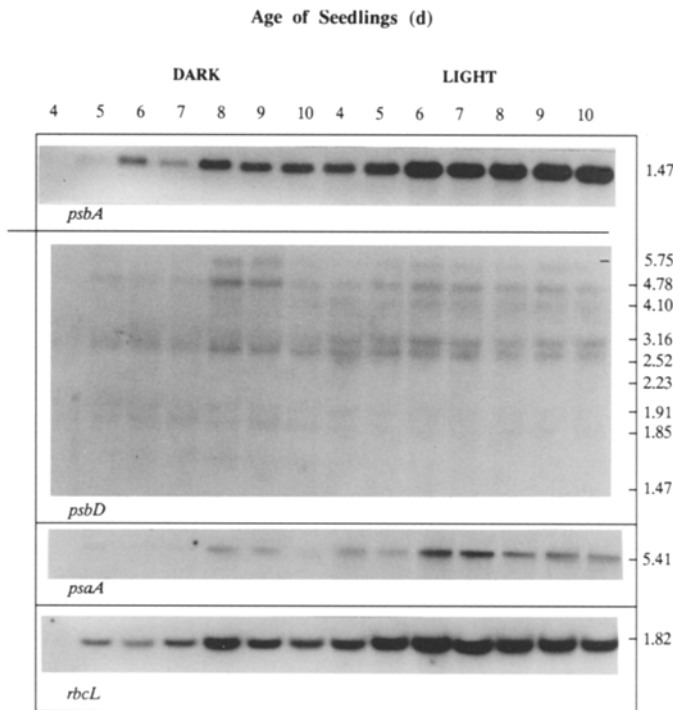
## Results and discussion

The representative genes investigated are *psbA* and *psbD* coding for the PS II reaction center polypeptides D<sub>1</sub> and D<sub>2</sub>, *psaA* coding for the PS I reaction center P<sub>700</sub> chlorophyll *a* apoprotein A, and *rbcL* coding for the large subunit of the stromal enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase. As shown in Figs. 2 and 4, gene-specific probes hybridize to either a single transcript (1.47 kb, *psbA*; 5.41 kb, *psaA*; 1.82 kb, *rbcL*) or to multiple transcripts (5.75, 4.78, 4.10, 3.16, 2.52, 2.23, 1.91, 1.85 and 1.47 kb, *psbD*) which may arise due either to post-transcriptional processing of a polycistronic mRNA or to multiple promoter activity (Berends et al. 1987; Woodbury et al. 1988; Yao et al. 1989; see also Tyagi et al. 1993). The sizes of the transcripts are similar to those reported for *japonica* rice (Chen et al. 1992; Kanno and Hirai 1993). But for the *psbD* region, Kanno and Hirai (1993) found only four transcripts, instead of the nine observed by us, which may be due to the lower sensitivity of the non-radioactive hybridization system employed by them. Since *psbD* is part of the *psbD-psbC* operon, a gene-specific probe for *psbC* also hybridizes with similar transcripts. In addition, a new transcript (1.71 kb) appears and the intensity of 1.91-kb transcript increases several fold (Kapoor et al. 1993). However, it remains to be confirmed that these changes are the result of an additional promoter activity as shown in pea (Woodbury et al. 1988) and tobacco (Yao et al. 1989).

Since light is responsible for differentiation of chloroplasts from proplastids as well as from etioplasts and, further, since development of etioplasts from proplastids in different species proceeds at different rates (Thompson et al. 1983; Newcomb 1990; Schrubar et al. 1991; Kelkar et



**Fig. 1. A, B.** Quality of RNA samples as determined by staining agarose gels with ethidium bromide. Ribosomal RNAs appear as prominent bands. **A** RNA from leaves of dark (*D*) – and light (*L*) – grown seedlings for a varying number of days (*d*). **B** RNA from leaves of seedlings exposed to light durations shown at the top

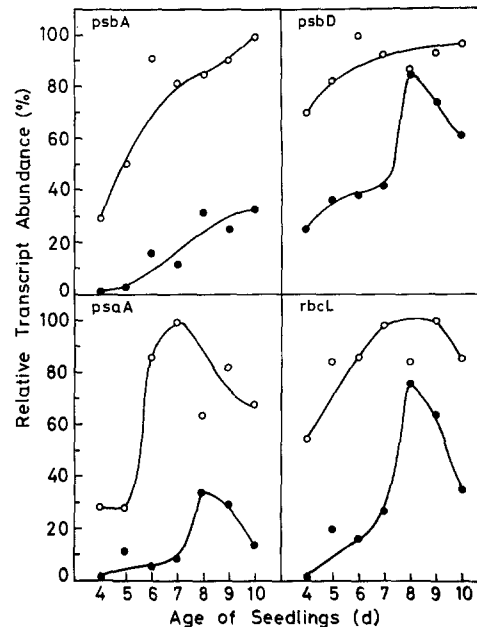


**Fig. 2.** Age-dependent (temporal) accumulation of transcripts for various chloroplast-encoded photosynthesis-related genes in rice seedlings grown in dark or light. The size (kb) of transcript(s) is given on the right

al. 1993), we have investigated the effect of development as well as light on steady-state transcript levels during chloroplast biogenesis in rice. For this purpose, transcript levels were measured in 4–10-day-old seedlings grown in continuous dark or light. Alternatively, 5-day-old dark-grown seedlings were exposed to varying durations of light and the kinetics of mRNA accumulation evaluated.

#### *Temporal accumulation of transcripts in dark and light depends on the age of the seedlings*

During a period of 4–10-day development in continuous dark or light, the transcript abundance for all the genes (*psbA*, *psbD*, *psaA* and *rbcL*) was found to be influenced by the age of the seedlings (Figs. 2 and 3). In 4-day-old dark-grown seedlings, levels of all the transcripts were less than 1.5% of the maximum values attained in case of light-grown seedlings, except for *psbD* for which up to 25% levels were already established in the dark. With continued development in the dark, the levels increased as a function of plant age and reached their maxima on the 8th day. This increase might be required to develop the “competence” of the plastids in “anticipation” of the forthcoming light-dependent log phase of development (see Mohr 1984; Link 1988). On the other hand, during development in light, levels of transcripts for all the genes, even on the 4th day, were already almost as high as the maximum possible in the dark despite the increased age, i.e., on the 8th day. Continued growth in light further increased the levels in a gene-specific manner. After reaching maximum levels during



**Fig. 3.** Quantitative representation of the transcript levels for various genes, shown in Fig. 2. RNA was from seedlings grown in dark (closed circle) or light (open circle)

the “build-up” phase, transcripts for *psaA* and *rbcL* gradually declined – nonetheless, the levels remained higher than those achieved in the dark at any stage of seedling development. In contrast, transcripts for *psbA* and *psbD* (in light only) were maintained at a high level. This might be necessary to fulfill the requirements of protein synthesis for polypeptides D<sub>1</sub> and D<sub>2</sub>, which have high turn-over rates in light (Vermaas and Ikeuchi 1991). As far as individual transcripts for *psbD* are concerned, most were found to be low in the dark, as compared to light, and showed little age-dependent variation except for two (5.75 and 4.78 kb) which reached almost the same level as in light on the 8th day, reflecting a strong age-dependent control on their expression in the dark (Fig. 2).

In mustard, similar studies on *psaA*, *petA*, *petB* and *atpA* (Dietrich et al. 1987) showed a pattern of initial increase up to 3 days of germination followed by a decline in transcript levels, in both light and dark. On the other hand, in barley, transcripts for *psaA* and *rbcL* had possibly reached their maximum levels by 4.5 days as extended growth in the dark resulted in a steep decline (Mullet and Klein 1987). As far as age-dependent expression of *psbA* is concerned, variable observations have been made in mustard (Hughes et al. 1987) and barley (Mullet and Klein 1987). The situation in light-grown seedlings of rice is somewhat similar to that in mustard as the transcript levels continue to rise. But, in the dark, the results in rice are different from those in other species. While in rice the transcript levels continue to increase with age, in other species they decline after an initial increase. In *indica* rice, the time taken for achieving the maximum levels for most of the transcripts, i.e., 8 days, might indicate a slower rate of development in comparison to that in barley or mustard. This is also apparent by the slow rate of leaf expansion. Therefore, while the 8-day-old stage in rice may represent an

advanced stage of etioplast differentiation, the 4-day-old seedlings might represent a very early state (nearer to the proplastid stage) of plastid biogenesis, as reflected by very low levels of transcripts.

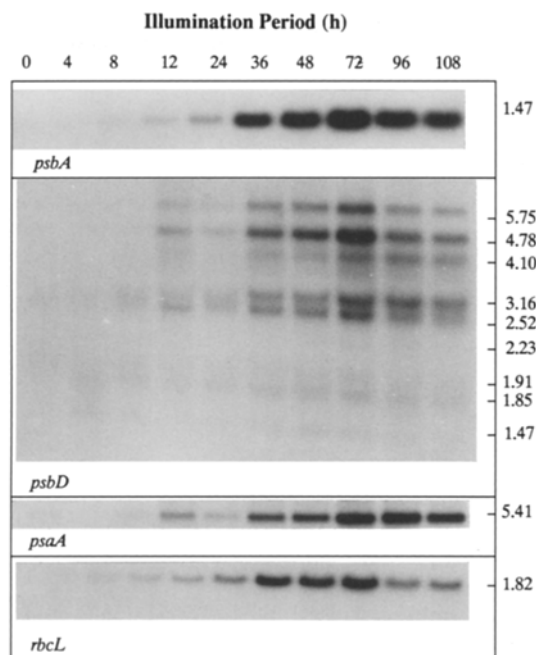
*Light-induced increase in transcripts closely follows the pattern of age-dependent expression*

After dark-to-light transition, the transcript levels rise slowly with increasing durations of light exposure up to 72 h (Figs. 4 and 5). Subsequently, i.e., within the next 36 h, transcripts show a 20–50% decline in a gene-specific manner. Similar expression patterns have also been reported in other plants including maize, barley and wheat (Rodermel and Bogorad 1985; Mullet and Klein 1987; Kawaguchi et al. 1992). However, the observations on the expression of genes in *indica* rice differ from other investigations in the following respects: (1) the relative increase (i.e., 25–60-fold) in the transcript levels is very high as compared to other systems; in *Sorghum*, however, the increase is somewhat comparable to that in rice and the same is true for *Vigna aconitifolia* if an early stage of etioplast differentiation is selected for studying light effects (Schrubar et al. 1991; Kelkar et al. 1993), and (2) transcripts of rice chloroplast genes take a longer period (72 h) to reach the maximum levels as compared to the plant species mentioned above, in which maximum levels for most of the transcripts are attained within 24–36 h of illumination. This could also be the reason for the failure of other workers to observe the light-stimulation of the *psaA-psaB-rps14* transcript after 24 h of illumination of 11-day-old dark-grown *japonica* rice (Chen et al. 1992). Obviously, in rice, the developmental process is slow, which can be exploited for understanding the finer points of regulation during the initial phases of chloroplast biogenesis.

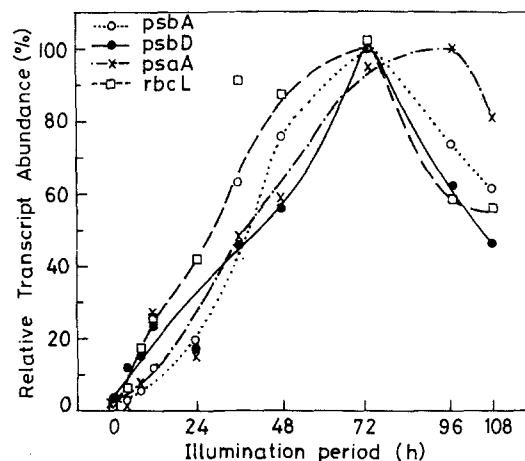
Differences with respect to the accumulation patterns of *psbD* transcripts are also worth mentioning. Extensive analysis of expression of this particular operon in barley has revealed that all dark-specific transcripts decline and are barely detectable after 72 h of exposure, while two new transcripts become prominent after longer hours of illumination (Gamble et al. 1988). In rice, however, five major transcripts – of 5.75, 4.78, 4.10, 3.16 and 2.52 kb – accumulate at a very high level up to 72 h of illumination followed by a marginal decline. The remaining four transcripts do not change significantly on exposure to light. A similar increase of almost all of the transcripts following illumination has been reported in *Sorghum* (Schrubar et al. 1991). Also, in *V. aconitifolia* most of the transcripts are maintained even with a longer period of illumination (Kelkar et al. 1993). Taken together, the above data show that light plays a significant role during chloroplast biogenesis by promoting high transcript abundance.

*Relative contribution of development and light*

Ideally, to study the effect of any factor on a phenomenon all other parameters should be kept constant. However, due to experimental limitations, it was not possible to keep the



**Fig. 4.** Kinetics of light-dependent accumulation of transcripts for various chloroplast-encoded photosynthesis-related genes. The size (kb) of transcript/(s) is given on the right. O represents the control RNA isolated from 5-day-old dark-grown seedlings



**Fig. 5.** Quantitative representation of the transcript levels for the various genes shown in Fig. 4

“age factor” constant with increasing durations of light exposure in the course of the present investigation. Therefore, it was thought that the exclusive influence of light on the expression levels would become clearer if observed in the background of the data on age-dependent development in the dark, as discussed earlier.

The *psbA* transcript – a major light-enhanced transcript species – reaches its maximum levels (50-fold higher) after 72 h of illumination of 5-day-old dark-grown seedlings (Fig. 5, Table 1 B). In terms of development, this stage corresponds to that of the 8-day-old dark-grown seedlings when only a 10-fold increase in the transcript levels – compared to the 5-day-old dark-grown seedlings – was observed (Table 1 A). Therefore, light alone causes an in-

**Table 1.** Relative contribution of development (temporal) and light in expression of photosynthesis-related genes in rice. A, ratio of transcript levels in 8-day-old seedlings versus 5-day-old seedlings, grown in the dark. B, ratio of transcript levels in 5-day-old dark-grown seedlings exposed to light for 72 h (3 days) versus 5-day-old dark-grown seedlings. C, ratio of B versus A

Gene	A	B	C
<i>psbA</i>	10.0	50.0	5.0
<i>psbD</i>	2.3	26.2	11.4
<i>psaA</i>	7.0	46.0	6.6
<i>rbcL</i>	8.0	62.0	7.8

crease of only 5-fold over the corresponding levels in the dark-grown plants of the same age (Table 1C). Likewise, an effective light-dependent increase of approximately 11.4-, 6.6- and 7.8-fold has been determined for *psbD*, *psaA*, and *rbcL*, respectively (Table 1).

To conclude, it is clear that both the developmental programme and light interact to establish the final steady-state levels of photosynthesis-related chloroplast genes – a quantitation of which has been made for rice during the course of this investigation. The role of such an interaction has become strikingly evident from photomorphogenic mutants of *Arabidopsis* where a release from dark-dependent developmental control results in expression of chloroplast genes even in the dark (Chory 1992; Wei and Deng 1992). It is, therefore, likely that, basically, light negates the suppressive effect of developmental control, which in itself is age-dependent (and species-specific), and eventually affects the full potential of expression of chloroplast genes. One must, therefore, attempt to unravel the mechanisms of transduction of signals from light and development in order to understand the differential expression of chloroplast genes. At the same time, the precise level of action of these signals in gene expression needs to be determined.

**Acknowledgements.** We are grateful to Professor R. G. Herrmann, Munich, for kindly providing us with the clones containing gene-specific probes used in this study. This research was funded by the Department of Science and Technology (equipment facility) and University Grants Commission, India. A fellowship from CSIR to S. K. is also acknowledged.

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Communicated by J.-D. Rochaix