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Ribosome-deficient plastids affect transcription of light-induced nuclear genes: genetic evidence for a plastid-derived signal

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Abstract. Transcription of ten nuclear genes was analysed in the *albostrians* mutant of barley (*Hordeum vulgare* L.). The lack of plastid ribosomes in white seedlings of this mutant results in a complex alteration of nuclear gene expression at the transcriptional level. We found a strong reduction in the accumulation of mRNAs transcribed from nuclear genes encoding chloroplast enzymes involved in the Calvin cycle, the chlorophyll a/b binding protein, and the cytosolic enzyme nitrate reductase. In contrast, the levels of transcripts of the genes encoding the cytosolic glycolytic enzymes glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase were slightly enhanced. Accumulation of chalcone synthase mRNA even reaches much higher levels in white than in green leaves. Ribosome-deficient plastids were combined by crossing with a nuclear genotype heterozygous for the *albostrians* allele. Analysis of transcript levels in F_1 plants having the same nuclear genotype and differing only with respect to their content of normally developed chloroplasts versus undifferentiated mutant plastids, provided strong genetic evidence for the plastid being the origin of a signal (chain) involved in regulation of nuclear gene expression. Results of run-on transcription in isolated nuclei demonstrated that the plastid signal acts at the level of transcription; it does not interfere with gene regulation in general. Mechanisms triggering nuclear gene expression in response to light operate in white mutant leaves: the very low levels of mRNAs derived from nuclear genes encoding chloroplast proteins and the strongly enhanced level of chalcone synthase mRNA were both light inducible. Also the negative regulation of leaf thionin gene expression by light is observed in white *albostrians* seedlings. Furthermore, in spite of its low absolute level, the circadian rhythm in the abundance of the *cab* mRNA is still detectable in plastid ribosome-deficient seedlings. Thus, functional plastid protein biosynthesis and photosynthesis are not preconditions for the circadian oscillations in the level of mRNA transcribed from this gene (family).

Key words: Barley (*Hordeum vulgare*) – Nuclear gene regulation – Plastid ribosome deficiency – Circadian rhythm

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Introduction

Besides the eukaryotic nuclear genome, plant cells harbour plastid and mitochondrial genomes which have many prokaryotic features owing to their endosymbiontic origins (cf. Gray 1992). During evolution, the majority of genes were lost from the original endosymbiont and, in part, translocated to the nucleus. As a consequence of this gene transfer, some basic functions of the organelles, such as propagation, and DNA replication depend exclusively on genetic information in the nuclear DNA. However, photosynthesis and respiration require close interaction between gene products encoded in the nuclear DNA and in the genome of the respective organelle. Therefore, coordinate regulation of nuclear genes present in only one or a few copies per cell and organellar genes present in several hundreds or even thousands of copies, is a basic necessity for photosynthesis, respiration and protein synthesis in both mitochondria and chloroplasts.

Most data concerning the expression of nuclear-encoded chloroplast proteins have been accumulated for the *cab* and *rbcS* gene families, which encode the light-harvesting complex chlorophyll a/b-binding protein of photosystem II (LHCP) and the small subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), respectively. Molecular processes at the transcriptional and translational levels, including proteolytic degradation of un-assembled subunits, appear to be responsible for the stoichiometric expression of *rbcS* and *rbcL* genes which reside in nuclear and chloroplast DNA, respectively (Winter and Feierabend 1990). Regulatory mechanisms acting at the transcriptional level seem to be more impor-

tant for the light-regulated expression of nuclear genes than of chloroplast genes. Post-transcriptional events have been reported to be especially important in the regulation of chloroplast gene expression (Deng and Gruissem 1987; Mullet and Klein 1987; Klein and Mullet 1990; Schrubar et al. 1990).

It has been observed that expression of the *rbcS* and *cab* gene families was either repressed or not activated in albina mutants of *Hordeum vulgare* bearing undifferentiated plastids (Börner et al. 1976; Batschauer et al. 1986; Hess et al. 1991). This observation was made also in leaf cells of plants having photooxidatively damaged plastids due either to treatment with bleaching herbicides (Oelmüller and Mohr 1986; Ernst and Schefbeck 1988; Burgess and Taylor 1987) or to mutations that block the biosynthesis of protective pigments (Mayfield and Taylor 1984; Giuliano and Scolnik 1988). Furthermore in experiments using transgenic plants (e.g. Herrera-Estrella et al. 1984; Simpson et al. 1986; Stockhaus et al. 1989), a strong correlation was observed between the presence of green, differentiated chloroplasts and the expression of reporter genes controlled by promoters of nuclear-encoded chloroplast proteins. Based on these results, a control mechanism was postulated that triggers the expression of nuclear genes encoding chloroplast proteins in accordance with the developmental status of the plastids (for reviews see Börner 1986; Taylor 1989; Rajasekhar 1991; Susek and Chory 1992). The nature of the postulated plastid signal or factor and its mode of action on nuclear gene expression are still obscure.

Convincing evidence for the plastid as the origin of the signal (chain) has not been presented so far. The results obtained with transgenic plants could reflect cell-type specificity controlled by the nucleus rather than the plastid. Photooxidative damage is thought to be restricted to the chloroplast compartment in norflurazone-bleached plants or carotenoid-deficient mutants, and will presumably eliminate any plastid signal. It is, however, difficult to rule out the possibility that labile cytoplasmic compounds are affected as well.

The barley mutant *albostrians* lacks plastid ribosomes in cells of white tissue (Börner et al. 1976; Knoth and Hagemann 1977). This phenotype is caused by the recessive nuclear mutation *albostrians* (*as;* Hagemann and Scholz 1962). The white tissue containing ribosome-less plastids shows drastically reduced levels of nuclear-encoded chloroplast enzymes of the Calvin cycle (Bradbeer et al. 1979; Boldt et al. 1992) and proteins of the thylakoids (Börner et al. 1976). This was shown for the *cab* and *rbcS* gene families to be a consequence of the reduced accumulation of their transcripts (Hess et al. 1991). White and green leaves have the same nuclear genotype *(asas)* and differ only with respect to the occurrence of either normal chloroplasts or undifferentiated plastids. Therefore it was postulated that a factor deriving from the plastids (and not the nucleus) determines the different levels of nuclear gene expression in white and green leaves (Bradbeer and Börner 1978; Hagemann and Börner 1978; Bradbeer et al. 1979). However, the primary effect of the *as* allele is not known and the data could also be explained if the recessive *as* allele were to show different activities in cells of white and green leaves.

In this paper we have made the following observations. Firstly, mRNAs transcribed from several nuclear genes accumulate in white and green leaves of the *albostrians* mutant to very different levels, as demonstrated by Northern blot analysis. Secondly, transcriptional regulation by light and by circadian rhythm occurs independently of the plastid signal. Thirdly, using run-on transcription assays, we show that the postulated plastid effect can already be observed at the level of transcription and, finally, by analysis of green and white *Asas* seedlings the origin of the postulated signal (chain) is shown to be actually in the plastid/chloroplast.

Materials and methods

Plant material. The barley mutant line *albostrians* (*Hordeum vulgare* cv. Haisa) contains a recessive allele *(albostrians, as)*, which induces in the homozygous condition ribosome-less white plastids. The offspring of selfed *asas* plants consist of approximately 10% green, 10% white and 80% green-white striped seedlings because only part of the plastid population is affected by the action of the *as* allele. Green (or etiolated yellow) leaves have the normal wild-type phenotype and were used as a control, as were the striped leaves, which have intermediate characteristics depending on the respective amount of green or white tissue.

Seedlings were raised in moist vermiculite at 23° C in a dark room or under a light/dark regime of 16 h/8 h with an illumination of 32 W/m^2. Plants were harvested 6 days after sowing. White *albostrians* plants were carefully checked to exclude contamination with small areas of green tissues. Etiolated seedlings were harvested under a green safety light into liquid nitrogen and subsequently sorted under normal illumination into etiolated yellow wild-type, striped, and white mutant plantlets. All plants of this line have the *asas* genotype, which induces this albina phenotype and ribosome deficiency. Entirely white shoots developed occasionally on green or striped plants of the *albostrians* line. These white shoots developed fertile flowers (Börner 1985) and were used in crossing experiments. Green heterozygous plants having one wild-type allele (*Asas* plants) are unable to induce ribosome-less plastids and remain stably green.

For crossing experiments, barley plants were cultivated under greenhouse conditions. Selfing was prevented by mechanical removal of the anthers from the female partner, followed by enclosure of the ears of both parental plants together in a plastic bag. Crossings were carried out in the following reciprocal combinations: *asas* (white) × *AsAs* (green) and *AsAs* (green) × *asas* (white). Plants of the cv. Frigga were used as green wild type (*AsAs*).

Northern hybridization. Total RNA was isolated according to Paulsen and Bogorad (1988). Identical absolute amounts of RNA were separated on 1.5% agarose-formaldehyde gels, transferred to nylon membrane by

Table 1. Probes used to determine the level of mRNA accumulation in Northern hybridizations or run on experiments respectively

Plasmid	Encoded protein	Origin	Probe fragment	Reference
R6.1	Phosphoribulokinase	Wheat	*Eco*RI, 1.2 kb	Raines et al. 1989
P20	Cytosolic phosphoglycerate kinase	Wheat	*Eco*RV, 0.7 kb	Longstaff et al. 1989
P7.1	Chloroplast phosphoglycerate kinase	Wheat	*Eco*RI, 1.3 kb	Longstaff et al. 1989
F16.1	Chloroplast fructose-1,6-bisphosphatase	Wheat	*Eco*RI, 1.2 kb	Lloyd et al. 1991
pZm57	Chloroplast glyceraldehyde phosphate dehydrogenase	Maize	*Eco*RI, 1.4 kb	Brinkmann et al. 1987
pZm9	Cytosolic glyceraldehyde phosphate dehydrogenase	Maize	*Eco*RI, 1.8 kb	Brinkmann et al. 1987
pbNRp10	Nitrate reductase	Barley	*Eco*RI/*Hin*dIII, 1.1 kb	Cheng et al. 1986
pCHs11	Chalcone synthase	Barley	*Eco*RI, 1.5 kb	Rohde et al. 1991
pHvDF2	Thionin	Barley	*Pst*I, 0.4+0.3 kb	Bohlmann and Apel 1987
p541	Chlorophyll a/b binding protein of photosystem II.	Wheat	*Eco*RI/*Pst*I, 1.7 kb	Lamppa et al. 1985
pC222	25S ribosomal RNA	Barley	*Pst*I, 0.45 kb	Forde et al. 1981

capillary blotting and hybridized as described previously (Hess et al. 1992). Table 1 summarizes the gene probes used in this work. Plasmids were cleaved with the appropriate restriction enzymes, the probe fragments were separated by agarose gel electrophoresis and isolated using Spinbind DNA extraction units (FMC Bioproducts Rockville, Me., USA).

Isolation of nuclei and run on transcription. Nuclei were isolated as described by Lu et al. (1990). Run-on experiments were carried out according to Fejes et al. (1990). Labelled RNA was isolated as described by Mösinger et al. (1985), ethanol-precipitated twice, re-dissolved in prewarmed hybridization buffer (Hess et al. 1992) and added to the prehybridized filter membranes in a total volume of 0.8 ml using 10 ml screw-cap glass centrifuge tubes in a hybridization oven (Bachofer, Reutlingen, FRG). DNA fragments containing part of the coding regions used in nuclear run-on experiments were dot-blotted according to standard methods (Sambrook et al. 1989) using the Minifold dot-blot apparatus (Schleicher and Schuell, Dassel, FRG).

Results

Transcript levels for selected nuclear genes

We studied transcript accumulation from several nuclear genes that encode enzymes of the Calvin cycle or their cytosolic isoenzymes. The genes coding for chloroplast phosphoribulokinase (PRK), fructose-1,6-bisphosphatase (Fbpase), phosphoglycerate kinase (cpPGK) and glyceraldehyde-3-phosphate dehydrogenase (cpGAPDH) showed similar patterns of mRNA accumulation. Levels of these mRNAs were strongly reduced in both etiolated and light-grown *albostrians* plants compared to wild-type plants. Nevertheless, induction by light is clearly detectable (Fig. 1A). A comparison of the mRNA levels for cpGAPDH and cytGAPDH, the glycolytic cytosolic isoenzyme, reveals a remarkable difference: the extremely low amount of cpGAPDH mRNA contrasts with a distinctly higher level of cytGAPDH mRNA in white than in green leaves. The same finding was observed for

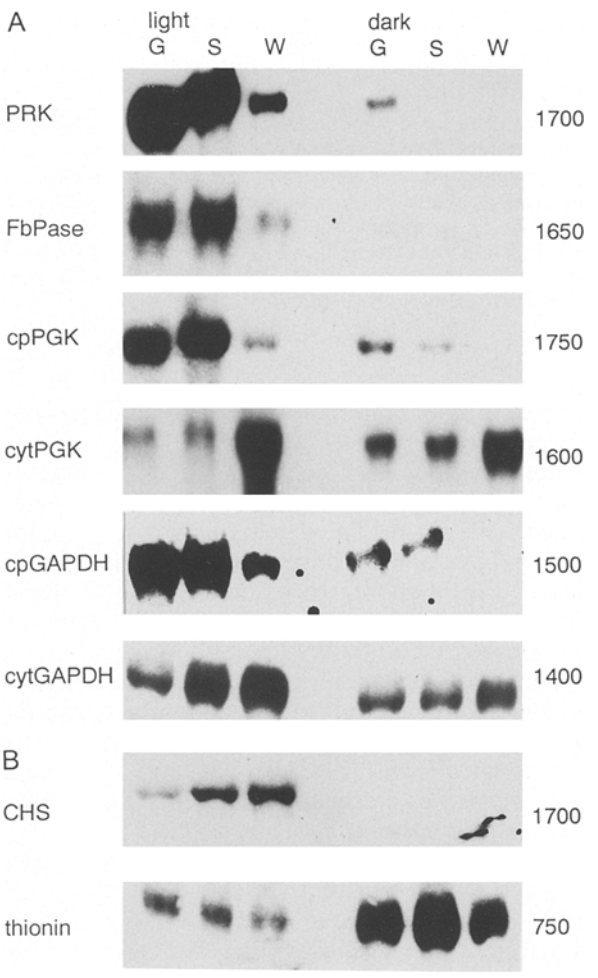

Fig. 1. Steady-state level of mRNA for **A** phosphoribulokinase (PRK), fructose-1,6-bisphosphatase (FbPase), chloroplast and cytosolic phosphoglycerate kinase (PGK), chloroplast and cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH), **B** chalcone synthase (CHS) and barley leaf thionin. *Left* panel: Total RNA was isolated from green (G), striped (S) and white (W) *albostrians* leaves grown in 16/8 h light/dark regime. *Right* panel: Same set of preparations from etiolated seedlings. Approximate size of transcripts in bp is indicated at the right margin as estimated from RNA length standards (Gibco-BRL)

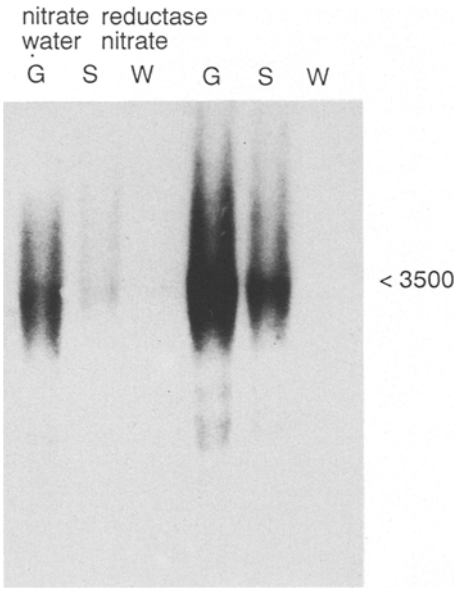

Fig. 2. No mRNA for the apoprotein gene of NADH-dependent nitrate reductase is detectable in RNA-preparations from white *albostrians* seedlings. RNA was isolated from light-grown seedlings either watered with distilled water only or to which 50 mM KNO_3 were added 24 h prior to harvest. The effect of nitrate induction is detectable in RNA from green (G) wild-type and striped (S) chimeric control plants, but not in RNA from white (W) *albostrians* plants. Transcript size in kb is indicated at the right

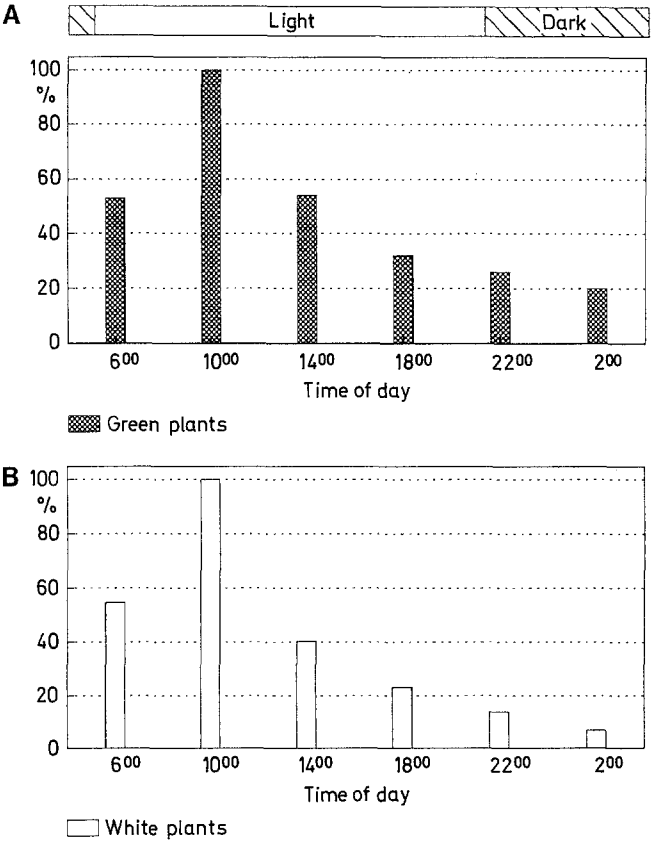

Fig. 3. Circadian rhythm of *cab* mRNA accumulation is detectable in **A** green and **B** white mutant seedlings, despite the low absolute level in white plants. Total RNA was extracted at the indicated time points throughout the day. After Northern hybridization, filters were stripped and re-hybridized with an rDNA probe to normalize for RNA loading. Measurements were made in duplicate and the autoradiogramms were scanned with a densitometer. The indicated numbers are related to the strongest respective signal in either **A** or **B**, set to 100%

another pair of isoenzymes, the chloroplast and cytosolic phosphoglycerate kinases (Fig. 1A).

The nuclear genes for a leaf thionin and for chalcone synthase (CHS), respectively, were included in this study as examples of light-regulated genes having no function in photosynthesis and encoding proteins located in the cytoplasm. Furthermore, the thionin gene is light-repressed, whereas the gene encoding CHS is activated by light. In the case of barley leaf thionin, we detected a slight reduction in level of its mRNA in white leaves. A pronounced reduction in the amount of thionin mRNA was observed in all types of light-grown compared to etiolated leaves (Fig. 1B). The level of chalcone synthase mRNA was drastically enhanced in light-grown white leaves (Fig. 1B).

Using a DNA probe homologous to the barley gene encoding the apoprotein of NADH-specific nitrate reductase, it was not possible to detect this mRNA species in RNA from white seedlings (Fig. 2). A response to nitrate was not detectable in white mutant leaves, whereas green and chimeric striped barley seedlings showed a distinctly enhanced level of nitrate reductase mRNA after induction with KNO_3 (Fig. 2).

A well-known feature of *cab* gene regulation is the circadian rhythm of transcript abundance (e.g. Nagy et al. 1988; Paulsen and Bogorad 1988; Giuliano et al. 1988; Adamska et al. 1991). Figure 3 shows that this characteristic oscillation of *cab* mRNA content is also detectable in white *albostrians* seedlings and does not differ significantly in form from that seen in green seedlings.

Run-on transcription

Run-on transcription experiments using isolated nuclei were carried out, in order to differentiate between effects on mRNA stability and on nuclear transcription in *albostrians* seedlings. A reduction in *cab* gene expression and a remarkable induction of *chs* expression are clearly detectable in white *albostrians* nuclei at the transcriptional level (Fig. 4). Strong hybridization signals were obtained with the immobilized *cab* gene sequence only when transcripts from control nuclei were used; conversely, intense hybridization to the *chs* probe was seen with transcripts from nuclei of white leaves. Labelled run-on transcripts from control plants hybridized only weakly with the chalcone synthase probe. Hybridization to the selected gene probes was specific, as indicated by a lack of hybridization to filter-bound pBR322 or bacteriophage λ DNA (Fig. 4).

Genetic evidence for a plastid-derived signal

The mutant line *albostrians* of barley (*H. vulgare* cv. Haisa) contains a recessive allele *(albostrians, as)*, which

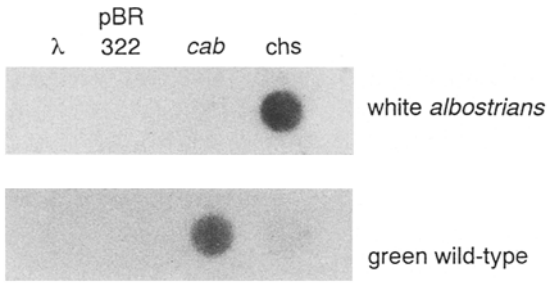

Fig. 4. Run on transcription experiment: DNA fragments containing part of the coding regions of *cab*1 and chalcone synthase *chs* were dot-blotted together with restricted DNA of bacteriophage lambda (λ) and plasmid pBR322 as controls. Hybridization of labelled transcripts resulting from incorporation of ^{32}P[UTP] in isolated nuclei of white *albostrians* seedlings or green control plants to the immobilized gene probes is evident

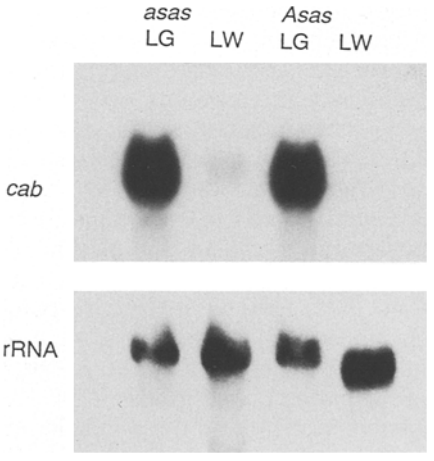

Fig. 5. RNA isolated from light-grown green (LG) and white plants (LW), either homozygous for the *albostrians* allele (genotype *asas*) or from crossings with the cv. Frigga *(AsAs)* and therefore heterozygous for the *albostrians* allele (genotype *Asas*). The RNA was hybridized with a 1.7 kb *Eco*RI/*Pst*I DNA fragment containing part of the wheat *cab*1 gene cDNA (Lamppa et al. 1985). As a control the same filter was hybridized subsequently with a probe for 25S rRNA (Forde et al. 1981), which is not affected by the mutation

induces in the homozygous condition ribosome-less white plastids in part of the progeny (see the Materials and methods). Once induced the albina phenotype is strictly maternally inherited (Hagemann and Scholz 1962; Knoth and Hagemann 1977). In this study crosses were performed to investigate whether the low transcript levels of nuclear genes are caused directly by the nuclear *albostrians* mutation or induced by a signal originating from the white ribosome-deficient plastids.

Crosses between a green, wild-type (genotype *AsAs*) female parent and white plants of the *albostrians* mutant line (genotype *asas*) as male parent resulted in 100% green F_1 plants (genotype *Asas*). Green heterozygous plants having one wild-type allele are unable to induce ribosome-less plastids and remain green. In contrast, crosses between a white female parent of the mutant line (genotype *asas*) and green wild type (genotype *AsAs*) as male parent produced an F_1 generation consisting of 100% white seedlings (genotype *Asas*). This result confirms the maternal inheritance of leaf colour reported previously by Hagemann and Scholz (1962) and supports the conclusion that the *as* allele induces inheritable alterations in the plastids.

The progeny of these crosses made it possible to compare the effect of green versus white plastids on nuclear gene transcription in plant material possessing identical nuclear genotypes with the dominant wild-type allele *As* and to rule out potential effects of the recessive *albostrians* allele. Total RNA was isolated from F_1 *Asas* seedlings and hybridized with a *cab* gene probe for comparison to RNA from *asas* plantlets. We obtained identical results independently of the nuclear background. Substantial amounts of mRNA were accumulated in green, but never in white seedlings, whereas 25S rRNA (taken as an example of an unaffected nuclear transcript) was present in normal amounts in both seedling types (Fig. 5).

Discussion

Previous studies on chloroplast enzymes in the *albostrians* mutant led to the conclusion that a plastid signal or factor is involved in the regulation of certain nuclear genes (Bradbeer and Börner 1978; Hagemann and Börner 1978; Bradbeer et al. 1979). Very low levels of *cab* and *rbcS* mRNAs in this mutant were more recently ascribed to the absence of a plastid factor that triggers expression of these genes depending on the differentiation state of the plastids (Hess et al. 1991). The main argument in favour of a plastid origin of the supposed signal is that white and green seedlings have the same nuclear genotype *(asas)* and differ from each other only with respect to the type of plastid (undifferentiated mutant plastid or normal chloroplast; cf. Hagemann and Scholz 1962). However, it might be argued that the activity of the nuclear *as* allele may differ between green and white leaf tissue and first causes the repression of nuclear-encoded chloroplast proteins, subsequently resulting in rudimentary plastids. In this case, it might be assumed, that the *"albostrians"* gene is a regulatory gene and could encode, for example, a transcription factor. In order to test this possibility, we included the analysis of both green and white *Asas* seedlings in our investigations. The mutant *as* allele shows no activity in this material because it is recessive to the wild-type allele (Hagemann and Scholz 1962). We found the same drastic reduction in *cab* mRNA in white *Asas* leaves as in white leaves with the *asas* genotype. This finding provides strong genetic evidence for the above assumption that it is actually the plastid/chloroplast which causes the observed effects on nuclear gene expression.

We studied the transcription and accumulation of transcripts from a variety of nuclear genes in order to obtain more information on the effects of the plastid signal. Based on their transcript levels in white leaves, the genes investigated can be classified into four groups:

(1) Light-induced genes that encode proteins typical for photosynthetically active chloroplasts (LHCP, PGK,

PRK, Fbpase, GAPDH) and the non-chloroplast enzyme nitrate reductase are severly repressed in white *albostrians* leaves. These data confirm and extend data on light-regulated and tissue-specific expression of these genes obtained in other systems (Raines et al. 1989; Oelmüller et al. 1988; Mohr et al. 1992; Shih and Goodman 1988; Lloyd et al. 1991).

(2) Genes for glycolytic enzymes show slightly enhanced transcript levels in white mutant leaves (cytPGK and cytGAPDH).

(3) Genes encoding negatively light-regulated cytosolic proteins exhibit slight decreases in mRNA level; examples include thionin (this work), phytochrome (Hess et al. 1991) or protochlorophyllide oxidoreductase (Hess et al. 1992).

(4) The light-induced gene for chalcone synthase shows super-induction in white *albostrians* leaves. A similar enhancement of steady-state levels of *chs* mRNAs is also known to occur in Norflurazon-bleached leaves (e.g. Ehmann and Schäfer 1988).

Thus our Northern data reveal complex alterations in nuclear gene expression in white leaves of the *albostrians* mutant.

A high expression level of chalcone synthase, a key enzyme in flavonoid biosynthesis, might be explained as a secondary effect of the albina phenotype causing (light) stress conditions. The somewhat enhanced levels of cytosolic PGK and GAPDH mRNAs could be explained similarly (Yang et al. 1993); alternatively, it may reflect an increase in the demand for energy provided by the glycolytic pathway in a mutant lacking photosynthesis. Comparison with data on actual enzyme levels suggests that translational mechanisms are also involved in regulating glycolytic activity. White plants contain about 130% of the cytPGK (Boldt et al. 1992), but only about 50% of the cytGAPDH activity of wild-type plants (Bradbeer and Börner 1978; Bradbeer et al. 1979).

The fact that transcript levels of nuclear genes such as actin or 25S rRNA are unaffected (Hess et al. 1991, 1992) or even elevated (cytPGK, cytGAPDH, CHS, this paper) demonstrates that there is no general impairment of nuclear gene transcription in plastid ribosome-deficient seedlings. Instead, our data indicate that a subset of nuclear genes are either not activated or specifically repressed if plastid factors signal that only rudimentary or immature organelles are present. In addition to genes for Calvin cycle enzymes (this work), genes encoding components of photosystem II (Batschauer et al. 1986; Mayfield and Taylor 1987; Stockhaus et al. 1989; Hess et al. 1991) belong to this regulatory group. However certain genes not coding for chloroplast proteins are obviously also affected, including nitrate reductase (Börner et al. 1986; Oelmüller et al. 1988; Mohr et al. 1992; this work), peroxisomal enzymes (Schwartz et al. 1992; Boldt et al., unpublished) and, probably, mitochondrial enzymes involved in glycine decarboxylation (Kim and Oliver 1990). Such a regulatory mechanism which monitors the development of photosynthetic competence seems reasonable in the latter two examples, because genes for photorespiratory proteins are concerned. On the other hand, it is still not clear why nitrate reductase is in this group (Mohr et al. 1992). That specifically photosynthetic genes, but not all nuclear genes coding for chloroplast proteins, are controlled by the plastid factor(s) is evident from the fact that several genes encoding enzymes of chlorophyll biosynthesis (Hess et al. 1992) or proteins involved in chloroplast DNA replication are expressed in white *albostrians* plants at normal or enhanced levels (Hess et al. 1993).

The plastid signal does obviously not interfere with the light induction of transcription. A comparison of lanes containing RNA from light- and dark-grown white *albostrians* leaves in Fig. 1 shows that, despite the very low mRNA level, light induction of transcription takes place in the case of genes encoding Calvin cycle enzymes. This is in agreement with data on the expression of *cab* and *rbc*S genes in this mutant (Hess et al. 1991). Furthermore, genes not affected by the plastid factor show their normal positive *(chs)* or negative light response (thionin). These observations are in agreement with the previous finding that phytochrome A is present in considerable amounts and that the gene encoding it is negatively regulated by light in white leaves of *albostrians* barley (Hess et al. 1991). The circadian rhythm of accumulation of *cab* mRNA was also clearly detectable in white *albostrians* leaves (Fig. 3). This result indicates that neither photosynthetic activity nor plastid translation products are required for the circadian modulation of gene expression. These observations suggest that the plastid signal does not interfere with the circadian rhythm and light induction of gene activity.

Although our results clearly demonstrate an involvement of the plastid in regulating transcription of certain nuclear genes, the possibility cannot be ruled out that a nuclear gene product can itself monitor the developmental stage of the plastid. In this case, the plastid would play only a passive role in nuclear gene activation or repression and the "plastid factor" would simply be the developmental or, in a catabolite activation-like process, the metabolic stage per se. Similarly, Rajasekhar (1991) suggested that an assumed inability of undifferentiated plastids to import nuclear gene-encoded proteins leading to an accumulation of precursors could act as the plastid signal. However, ribosome-deficient plastids are able to import proteins as demonstrated by their ability to replicate their DNA (Knoth and Hagemann 1977; Scott et al. 1982; Herrmann and Feierabend 1980; Hess et al. 1993) and by data from studies on protein transport (Strzalka et al. 1983).

More probably, the plastid actually produces a factor or signal which could well be the first link in a signal chain. The nature of the plastid product, however, remains elusive. Originally, plastid RNA was suggested to be the factor (Bradbeer et al. 1979). Chloroplast transcription has been proposed as an essential prerequisite for expression of *rbcS* and *cab* genes (Rapp and Mullet 1991). Although transcription of plastid genes was detected in the *albostrians* mutant (Hess et al. 1993), the steady-state level of chloroplast $tRNA^{Glu}$ is extremely low in this mutant (Hess et al. 1992). Apart from its function in translation, this tRNA is essential for the first step. in chlorophyll biosynthesis (Schön et al. 1986).

Thus the lack of $tRNA^{Glu}$ leads to a block at the first step of chlorophyll biosynthesis in ribosome-free plastids of *albostrians* barley. Therefore intermediates of this pathway are either not accumulated or accumulated to an extremely low extent. Intermediates of chlorophyll biosynthesis are clearly interesting candidates for the plastid signal (cf. Batschauer et al. 1986; Johanningmeier 1988; Hess et al. 1992; Susek and Chory 1992).

The plastid signal chain affects nuclear gene expression at the transcriptional level, as is obvious from our studies on run-on transcription in isolated nuclei and from data reported by several other authors (Batschauer et al. 1986; Burgess and Taylor 1988; Ernst and Schefbeck 1988; Giuliano and Scolnik 1988). The rapid decrease in certain nuclear transcripts after photooxidative destruction of chloroplasts suggests that the plastid signal is necessary for gene activation rather than repression (Oelmüller et al. 1988). The last link in such a signal chain originating from the plastid should be a nuclear transcription factor. There is currently no evidence either for a specific protein involved in this regulation or for a general transcription factor modified under plastid/chloroplast control. The identification of such a transcription factor will be a tempting task in order to elucidate the genetic interaction between plastids and nucleus.

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