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Expression of nuclear genes as affected by treatments acting on the plastids

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Abstract. In a preceding paper (Oelmüller and Mohr 1986, Planta **167**, 106–113) it was shown that in the cotyledons of the mustard (*Sinapis alba* L.) seedling the integrity of the plastid is a necessary prerequisite for phytochrome-controlled appearance of translatable mRNA for the nuclear-encoded small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase and the light-harvesting chlorophyll *a*/*b*-binding protein of photosystem II (LHCP). It was concluded that a signal from the plastid is essential for the expression of nuclear genes involved in plastidogenesis. The present study was undertaken to characterize this postulated signal. Chloramphenicol, an inhibitor of intraplastidic protein synthesis and Norflurazon, an inhibitor of carotenoid synthesis (to bring about photooxidative sensitivity of the plastids) were applied. We obtained the following major results. (i) After a brief period of photooxidative damage a rapid decrease of the above translatable mRNAs was observed. Conclusion: the signal is short-lived and thus required continually. (ii) Once the plastids became damaged by photooxidation, no recovery with regard to nuclear gene expression was observed after a transfer to non-damaging light conditions. Conclusion: even a brief period of damage suffices to prevent production of the signal. (iii) Chloramphenicol inhibited nuclear gene expression (SSU, LHCP) and plastidic development when applied during the early stages of plastidogenesis. Once a certain stage had been reached (between 36–48 h after sowing at 25° C) nuclear gene expression became remarkably insensitive toward inhibition of intraplastidic translation. Conclusion: a certain developmental stage of the plastid must be reached before the signal is released by the plastid. (iv) Under the growth conditions we adopted in our experiments the plastids in the mesophyll cells of mustard cotyledons developed essentially between 36 and 120 (−144) h after sowing. Only during this period could translatable mRNAs for SSU and LHCP be detected. Conclusion: the signal is released by the plastids only during this time span.

Key words: Nuclear gene expression – Photooxidation of chloroplast – Phytochrome – *Sinapis*.

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Abbreviations: CAP = Chloramphenicol (D-threo); cFR = continuous far-red light; FR = far-red light (3.5 $W \cdot m^{-2}$); GPD = glyceraldehyde-3-phosphate dehydrogenase; LHCP = light-harvesting chlorophyll *a*/*b*-binding protein of photosystem II; LSU = large subunit of RuBPCase; MDH = malate dehydrogenase; NF = Norflurazon; NIR = nitrite reductase; Pfr = physiologically active form of phytochrome; R = red light (6.8 $W \cdot m^{-2}$); RG9-light = long-wavelength far-red light (10 $W \cdot m^{-2}$); RuBPCase = ribulose-1,5-bisphosphate carboxylase; SSU = small subunit of RuBPCase; WL_s = strong white light (28 $W \cdot m^{-2}$); $\varphi_\lambda = \frac{[Pfr]\lambda}{[Ptot]}$ = photoequilibrium of phytochrome at wavelength λ

Introduction

In a preceding paper (Oelmüller and Mohr 1986) we have shown that the integrity of the plastid is a necessary prerequisite for the phytochrome-mediated appearance of translatable mRNA for the small subunit of ribulose-1,5-bisphosphate carboxylase (SSU) and light-harvesting chlorophyll *a*/*b*-binding protein of photosystem II (LHCP). It was concluded that a signal from the plastid is essential for the expression of nuclear genes involved in plastidogenesis.

This explains recent findings in connection with the expression of cloned SSU gene sequences in vivo: the expression of the inserted genes seems

to be related to the state of development of the chloroplasts (see Tobin and Silverthorne 1985, for review).

The present experiments were undertaken to further characterize the postulated signal. Our approach was based on the observations that in cotyledons of mustard (*Sinapis alba* L.) seedlings, plastids can be damaged rapidly by photooxidation in strong white light (WL_s) provided that synthesis of colored carotenoids was inhibited by herbicides such as Norflurazon (NF) while in far-red light (FR) – even though it strongly activates phytochrome – no photooxidative effects are observed even in the virtual absence of carotenoids (Frosch et al. 1979; Reiß et al. 1983). Photooxidative damage of the plastids prevents phytochrome-controlled appearance of nuclear-encoded translatable mRNAs of SSU and LHCP while synthesis of representative enzymes of cytosol, mitochondria and glyoxisomes – including phytochrome-induced proteins – was not impaired (Reiß et al. 1983; Oelmüller and Mohr 1986).

As a second tool, besides NF, we applied the water-soluble antibiotic chloramphenicol (CAP) to mustard seedlings. The drug is a potent inhibitor of protein synthesis on procaryotic-type ribosomes, including plastidic ribosomes, the synthesis of cytoplasmic proteins being relatively unaffected (Strzalka and Kwiatowska 1979). Within a certain range of concentrations (20–200 $\mu g \cdot ml^{-1}$), CAP even slightly increases the rate of phytochrome-mediated cytoplasmic processes such as anthocyanin synthesis (Wagner et al. 1967). In mediating this increase, CAP coacts multiplicatively with phytochrome. This indicates that the positive effect of CAP on cytoplasmic processes is independent of phytochrome action (see Mohr 1972). Moreover, inhibition of intraplastidic protein synthesis by CAP neither affects synthesis of plastid-bound proteins in the cytoplasm, nor their transport into plastids (Strzalka and Kwiatowska 1979).

Experiments will be described in which either NF or CAP was administered to the mustard seedling. The data obtained permit a physiological characterization of the plastidic signal which was postulated in the preceding report (Oelmüller and Mohr 1986) as a necessary prerequisite for phytochrome-controlled appearance of translatable mRNAs for SSU and LHCP.

In those cases where work with isolated nuclei has been feasible it has, so far, been found for SSU as well as LHCP that phytochrome (Pfr) acts on transcription rather than affecting the level of mRNAs posttranscriptionally (Silverthorne and Tobin 1985; Mösinger et al. 1985; Berry-Lowe and Meagher 1985). We assume that this applies to the present system as well. This assumption, however, is not essential for any conclusions to be drawn with regard to the properties of the above signal.

Material and methods

Seeds of white mustard (*Sinapis alba* L.) were obtained from Asgrow Company (Freiburg FRG) in 1979. They were selected and grown at 25° ± 0.5° C as described previously (Mohr 1966).

Treatments with the herbicide Norflurazon [NF, SAN 9789, 4-chloro-S-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3(2 H)-pyridazinone) and the antibiotic D-threo chloramphenicol (CAP) were performed as described previously (Frosch et al. 1979; Wagner et al. 1967).

The seedlings were grown in standardized far-red light (FR, 3.5 $W \cdot m^{-2}$; Mohr 1966). This light source was chosen because it permits strong phytochrome action but leads only to a small rate of protochlorophyll(ide)→chlorophyll(ide) photoconversion. Thus long-term phytochrome action can be studied without considerable chlorophyll accumulation (Mohr 1972).

For enzyme assays, a crude extract of 20 pairs of cotyledons was prepared with 1 g quartz sand and extraction buffer using mortar and pestle on ice. The enzyme assays were performed as described elsewhere:

Ribulose-1,5-bisphosphate carboxylase (RuBPCase; EC 4.1.1.39, Frosch et al. 1979).

Glyceraldehyde-3-phosphate dehydrogenases (GPD; NADP-dependent, EC 1.2.1.13; NAD-dependent, EC 1.2.1.12, Oelmüller and Mohr (1984).

Malate dehydrogenase (MDH; NAD-dependent, EC 1.2.1.37, Hock 1973).

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49, Löhr and Waller 1974).

β-amylase (EC 3.2.1.2, Sharma and Schopfer 1982).

Phenylalanine ammonia lyase (EC 4.3.1.5, Brödenfeldt and Mohr 1986).

Chalcone synthase (EC 2.3.1.74, Britsch and Grisebach 1985).

Nitrite reductase (NIR; EC 1.7.7.1, Rajasekhar and Mohr 1986).

Nitrate reductase (EC 1.6.6.1, Rajasekhar and Mohr 1986).

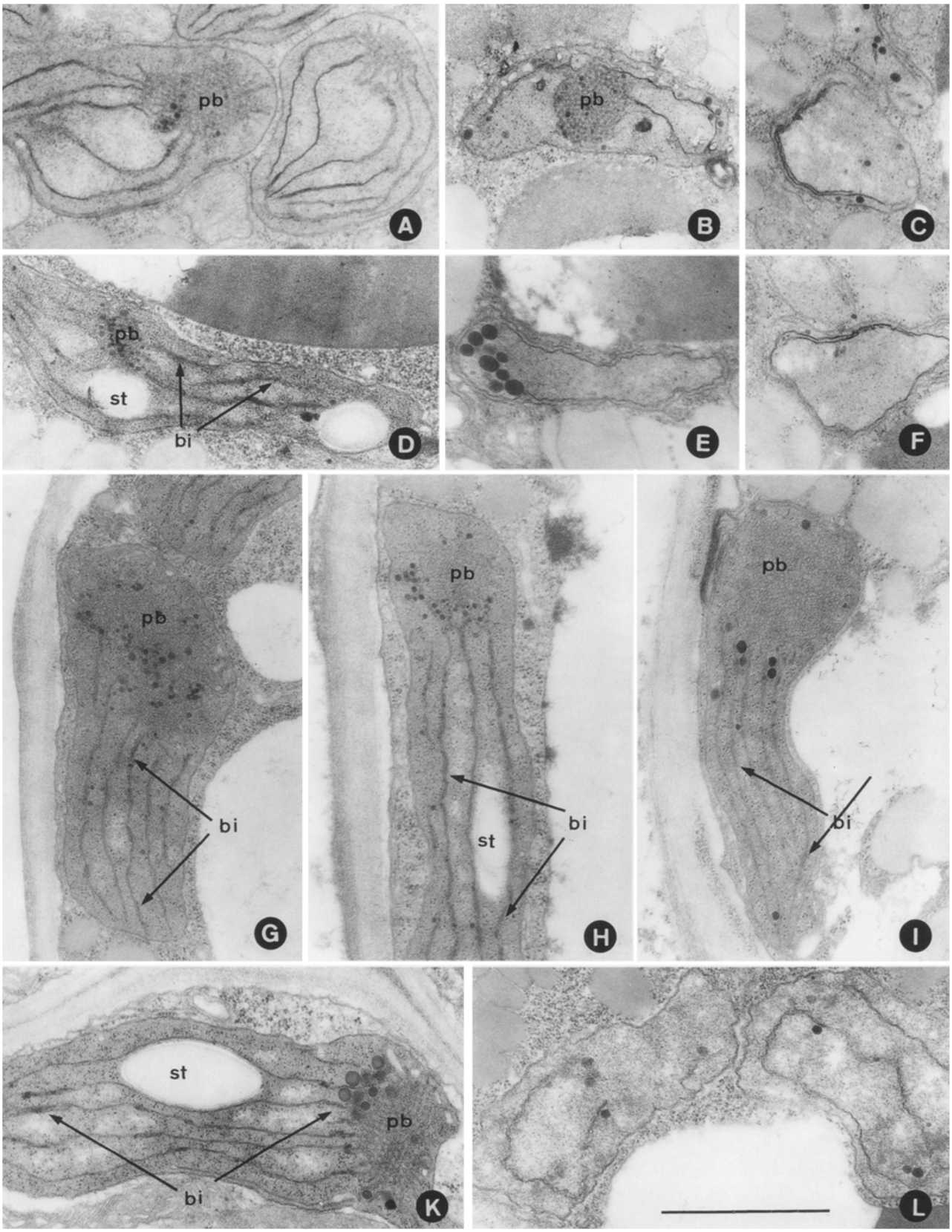
Total RNA was extracted as described by Link (1982) with slight modifications. After extraction with phenol-chloroform, nucleic acids were precipitated with isopropanol and washed twice with ethanol. Polysomal RNA (Fig. 10) was isolated as described by Gottmann and Schäfer (1982).

In-vitro translation of 10 μg of total RNA and 4 μg of polysomal RNA in a reticulocyte system, immunoprecipitation of the pSSU and pLHCP polypeptides with specific antisera raised in rabbits and in-vivo labelling of total protein (Fig. 10) have been described by Oelmüller and Mohr (1986). Quantification of the translation products was done according to Suissa (1983).

Electron microscopy was performed as described by Frosch et al. (1976).

Results

Application of CAP. When intraplastidic translation was inhibited by the application of CAP from the time of sowing onwards, development of the proplastid to an etioplast (in darkness) or to a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super-etioplast (in cFR, see Mohr 1972) was largely prevented (Fig. 1A–F). Correspondingly the levels of plastidic enzymes such as RuBPCase or NADP-GPD were strongly depressed (assay 60 h after sowing, Table 1), irrespective of whether the protein was in part plastid-encoded (large subunit of RuBPCase) or totally nuclear-encoded (NADP-GPD).

On the other hand, typical cytoplasmic enzymes were not adversely affected, including those which are strongly induced by phytochrome such as phenylalanine ammonia-lyase and chalcone synthase. The level of chalcone synthase was even considerably increased by the CAP treatment. The corresponding phenomenon was previously noticed in studies on anthocyanin accumulation (Wagner et al. 1967; Mohr 1972). The weak reduction of the level of malate dehydrogenase by CAP treatment must be attributed to the inhibition of a plastidic and-or mitochondrial isoenzyme (Sautter and Hock 1982).

Since macroscopic development of the mustard seedling was normal over the period investigated (up to 72 h after sowing), application of CAP seemed to be a useful tool to investigate the dependency of nuclear gene expression on intraplastidic translation.

As a major result, the pattern of translatable mRNA at 60 h after sowing was not significantly affected by the application of CAP (20 $\mu g \cdot ml^{-1}$) from the time of sowing (data not shown) except that the amounts of translatable $mRNA_{SSU}$ and $mRNA_{LHCP}$ were strongly reduced (Fig. 2). This result indicates that application of CAP not only leads to the expected inhibition of RuBPCase (LSU) formation but also to the disappearance of translatable $mRNA_{SSU}$ and $mRNA_{LHCP}$.

Quite unexpectedly, the strong inhibitory effect of CAP on the appearance of RuBPCase and NADP-GPD (see Table 1) was observed only when CAP was applied during the early phase of plastid development (Fig. 3). Later applications were ineffective in the case of NADP-GPD, while RuBPCase was still partly inhibited (presumably because of the impaired synthesis of LSU). On the basis of the data of Fig. 3, the kinetics of enzyme levels were determined between 36 and 72 h after sowing in cFR (Fig. 4). When CAP was applied 48 h after sowing the further rise in the levels of the plastidic enzymes NADP-GPD and nitrite reductase (NIR), and of the cytoplasmic enzymes NAD-GPD and NAD-MDH was not effected, while the rise of

Table 1. Activities of plastidic and extraplastidic enzymes isolated from cotyledons of 60-h-old mustard seedlings grown in cFR either on water or on a watery solution of chloramphenicol (CAP). Water controls = 100%. In the case of nitrate and nitrite reductases, seedlings were grown on 15 mM KNO_3 from sowing onwards. (With regard to nitrite reductase the residual enzyme activity was taken as the base line, see Rajasekhar and Mohr 1986)

Enzyme	CAP concentration [$\mu g \cdot ml^{-1}$]	enzyme activity [% of water control]
Plastidic		
RuBPCase	20	4
	40	2
	80	0
	200	0
NADP-GPD	20	28
	40	14
	80	12
	200	11
NIR	20	18
	40	0
	200	0
Extraplastidic		
Nitrate reductase[a]	20	16
	40	10
	200	8
Phenylalanine ammonia-lyase	20	119
Chalcone synthase	20	168
β-amylase	20	95
NAD-GPD	20	114
	40	120
	80	135
	200	147
glucose-6-phosphate dehydrogenase	20	93
	200	106
NAD-dependent malate dehydrogenase	20	107
	40	85
	80	83
	200	88

[a] The appearance of nitrate reductase is apparently regulated by the cell as if the enzyme were plastidic rather than cytosolic (see Rajasekhar and Mohr 1986)

Fig. 1. Sections through plastids from mesophyll cells of mustard seedling cotyledons after **A** 60 h dark, water control; **B** 60 h dark, 20 $\mu g \cdot ml^{-1}$ CAP; **C** 60 h dark, 200 $\mu g \cdot ml^{-1}$ CAP; **D** 60 h cFR water control; **E** 60 h cFR, 20 $\mu g \cdot ml^{-1}$ CAP; **F** 60 h cFR, 200 $\mu g \cdot ml^{-1}$ CAP; **G** 72 h cFR water control; **H** 48 h cFR + 24 h FR in the presence of 20 $\mu g \cdot ml^{-1}$ CAP; **I** 48 h cFR + 24 h FR in the presence of 200 $\mu g \cdot ml^{-1}$ CAP; **K** 60 h cFR, NF ($5 \cdot 10^{-6}$ M); **L** 60 h cFR + 1 h WL_s, NF ($5 \cdot 10^{-6}$ M). Note in **B, C, E, F, L** the poor state of thylakoids in contrast to the corresponding controls. *pb*, prolamellar body; *st*, starch; *bi*, bithylakoids (small grana). bar = 1 μm

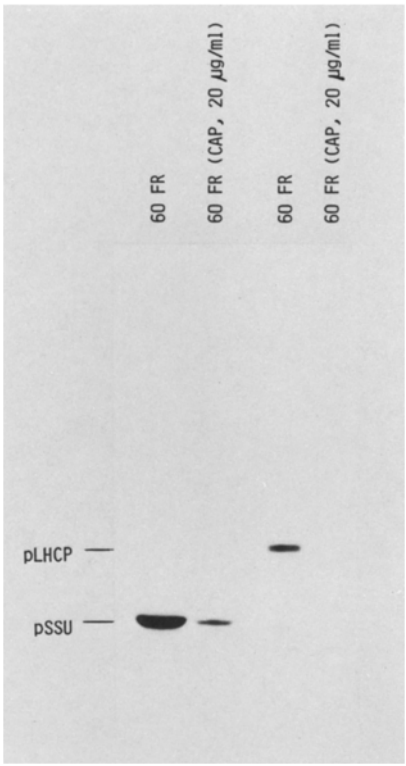

Fig. 2. Fluorograms of immunoprecipitates with SSU- and LHCP-antisera of the in-vitro translation products of total RNA from cotyledons of 60-h-old mustard seedlings grown in cFR in the absence or presence of CAP (20 $\mu g \cdot ml^{-1}$). *pLHCP*, precursor polypeptide of LHCP; *pSSU*, precursor polypeptide of SSU. For immunoprecipitation, equal amounts of radioactively labelled protein were used

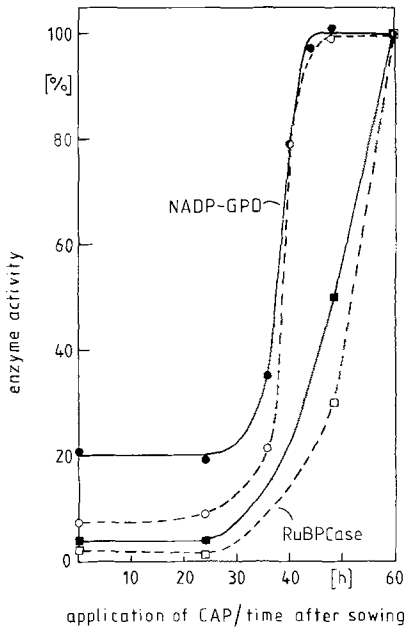

Fig. 3. The effect of the application of CAP (●, ■, 20 $\mu g \cdot ml^{-1}$; ○, □, 200 $\mu g \cdot ml^{-1}$) on the activities of NADP-GPD (●, ○) and RuBPCase (■, □) isolated from the cotyledons of 60-h-old mustard seedlings grown in cFR. The time point of CAP application is given on the abscissa. Percent enzyme activity means:

$$\left[\frac{\text{enzyme activity of CAP-treated seedlings}}{\text{enzyme activity of water controls}} \cdot 100\right]$$

RuBPCase was inhibited. The obvious conclusion that syntheses of nuclear-encoded plastidic GPD and NIR and their transport into the plastids are not impaired by inhibition of plastidic protein synthesis was confirmed by measurement of the levels of translatable mRNAs for SSU and LHCP under the same conditions. Table 2 shows that the amount of translatable $mRNA_{SSU}$ was not affected even by a treatment with 200 $\mu g \cdot ml^{-1}$ CAP which strongly inhibited the rise of RuBPCase activity (see Fig. 4). The amount of translatable $mRNA_{LHCP}$ was not affected by 20 $\mu g \cdot ml^{-1}$ CAP either. However, a relative decrease was seen with 200 $\mu g \cdot ml^{-1}$ CAP (Table 2).

The fine structure of plastids at 72 h after sowing was not affected by an application of CAP at 48 h (Fig. 1, G–I). This is in contrast to the conspicuous inhibition of plastid development when CAP was given from the time of sowing (Fig. 1, A–F).

It is concluded that plastid-bound nuclear gene expression becomes remarkably insensitive to an inhibition of plastidic protein synthesis once plastid development has reached a certain stage (48 h after sowing, Fig. 3). From that point onwards the appearance of translatable $mRNA_{SSU}$ is unimpaired by CAP, and the synthesis and translocation of plastidic GPD and NIR are unaffected.

These findings confirm previous results obtained with CAP-treated bean leaf disks that during inhibition of intraplastidic protein formation, synthesis of plastid-destined proteins in the cytoplasm, as well as their subsequent transport into plastids, can still proceed (Strzalka and Kwiatkowska 1979).

On the other hand, the higher dose of CAP (200 $\mu g \cdot ml^{-1}$) seemed to reduce the level of translatable $mRNA_{LHCP}$. This could be explained by a relatively small inhibition of transcription in combination with a faster turnover of translatable $mRNA_{LHCP}$ compared with $mRNA_{SSU}$. Circumstantial evidence obtained with aging cotyledons indicated in fact that the rate of decline of the level of translatable mRNA was considerably faster in the case of LHCP compared to SSU (Fig. 5).

Application of NF. The data obtained so far – insensitivity of plastid-bound nuclear gene expression to CAP, applied 48 h after sowing – were explained by the hypothesis that the plastidic signal

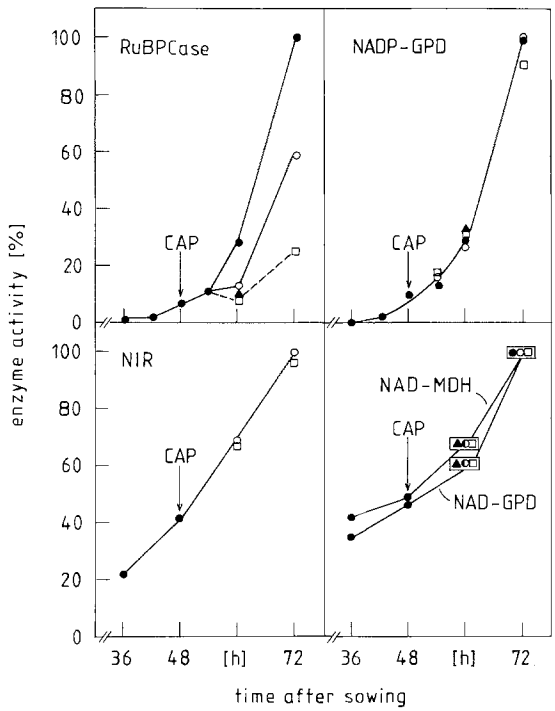

Fig. 4. Time courses of increase of several plastidic (RuBPCase, NADP-GPD, NIR) and extraplastidic (NAD-GPD, NAD-MDH) enzyme activities in the cotyledons of the mustard seedlings in cFR. Seedlings were either kept on water (●) or transferred to watery CAP-solutions at 48 h after sowing. ○, 20 μg·ml^{-1} CAP; □, 200 μg·ml^{-1} CAP; ▲, 1000 μg·ml^{-1} CAP. In the case of NIR, seedlings were grown in the presence of 15 mM KNO_3 from the time of sowing

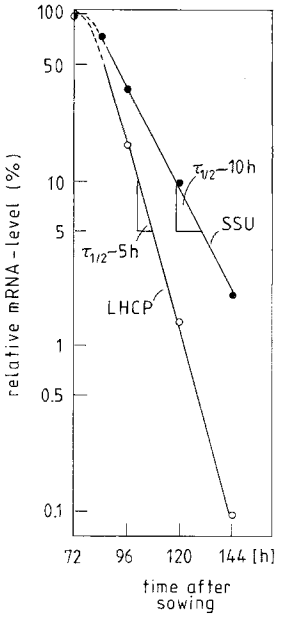

Fig. 5. Semilogarithmic plot of the amount of in vitro translatable mRNA for SSU and LHCP isolated from cotyledons of mustard seedlings grown continuously in cFR. At 72 h after sowing the highest amounts of translatable mRNA levels for LHCP and SSU were measured (100%). In recent experiments it was confirmed that beyond 72 h after sowing the inducibility of both translatable mRNAs by phytochrome declines rapidily (S. Schmidt et al., personal communication). In these long-term experiments (>3.5 d) mustard seedlings were grown on Hoagland nutrient solution (1/10 full strength) to avoid limitation by nutrients

Table 2. Amounts of translatable mRNA for SSU and LHCP contained in total RNA from cotyledons of 72-h-old mustard seedlings grown in cFR. At 48 h after sowing the seedlings were placed on watery CAP solutions instead of water. 100% refers to the water control at 72 h. Data are the means of four independent runs; SE estimated as approx. 10%

Treatment	mRNA (%)	
	SSU	LHCP
48 h cFR	42	23
72 h cFR (water control)	100	100
48 h cFR + 24 h cFR (20 μg·ml^{-1} CAP)	117	104
48 h cFR + 24 h cFR (200 μg·ml^{-1} CAP)	96	45

required for nuclear gene expression is no longer required once plastid development has reached a certain stage (at approx. 48 h). Norflurazon was applied to test the validity of this explanation. The experimental background of the approach was elaborated by Frosch et al. (1979): long-term far-red light (cFR) activates phytochrome but does not cause any damage to the plastids even in NF-

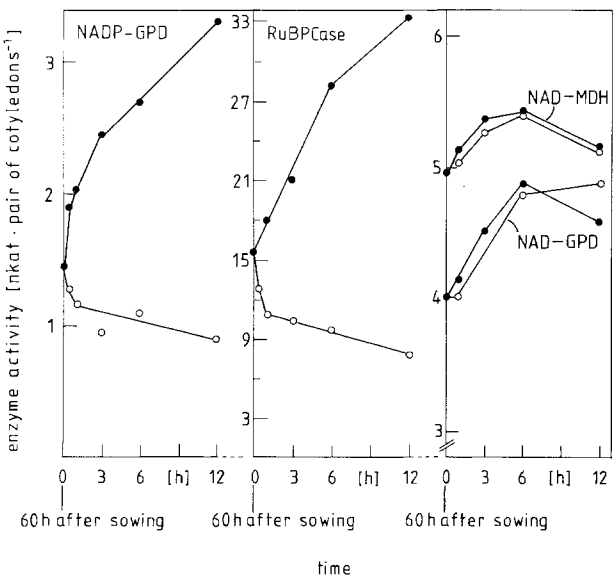

Fig. 6. Time courses of enzyme activities of plastidic (RuBPCase, NADP-GPD) and extraplastidic (NAD-MDH, NAD-GPD) enzymes in cotyledons of mustard seedlings treated with NF ($5 \cdot 10^{-6}$ M) from the time of sowing onwards. ●, NF treated, grown in cFR; ○, NF treated, kept in cFR until 60 h after sowing, then transferred to WL_s

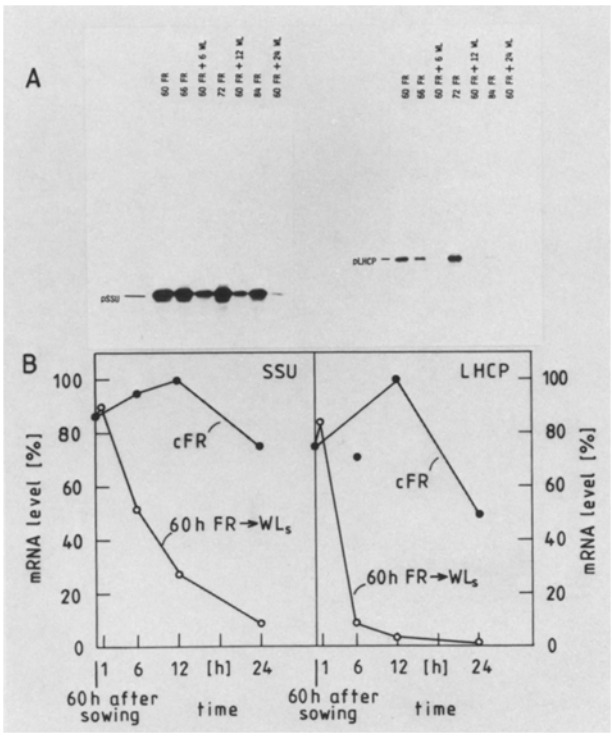

Fig. 7. Time courses of the amount of translatable mRNA for SSU and LHCP in the cotyledons of NF-treated mustard seedlings grown in cFR for 60 h. Seedlings were then kept either in FR (●) or transferred to WL_s (○). (**A**) Typical fluorograms of immunoprecipitates of the in vitro translation products of total RNA with SSU- and LHCP-antisera. (**B**) Relative amounts of translatable mRNAs based on three independent experiments. The amount determined with 72-h-old seedlings grown in cFR was used for reference (100%)

Table 3. Effects of pulses of R and long-wavelength FR (RG9) – given after 60 h cFR – on the levels of translatable mRNA for SSU and LHCP in darkness. Amount of translatable mRNA 60 h after sowing in cFR = 100%. $\varphi_R = 0.8$; $\varphi_{RG9} < 0.01$. Data from four independent runs; SE estimated as approx. 10%. D = darkness

Treatment	mRNA	
	SSU	LHCP
60 h cFR	100	100
60 h cFR +5 min R +12 h D	125	360
60 h cFR +5 min RG9-light +12 h D	85	240

treated seedlings depleted of carotenoids. The small rate of chlorophyll (Chl) accumulation in cFR is not affected by NF-treatment. Moreover, FR is so weakly absorbed by Chl that Chl-mediated photooxidations play no role even in strong

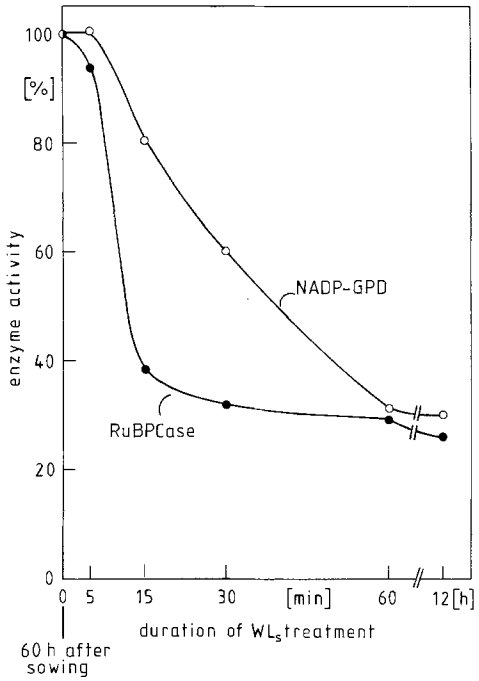

Fig. 8. Activity of RuBPCase (●) and NADP-GPD (○) in the cotyledons of 72-h-old mustard seedlings. Seedlings treated with NF were kept in cFR until 60 h after sowing and then placed in WL_s. The duration of the WL_s treatment is given on the abscissa. Until assay at 72 h after sowing, seedlings were kept again in FR. Enzyme activity isolated from 72-h-old seedlings kept continuously in cFR was chosen as a reference (100%)

cFR. On the other hand, when the same NF-treated seedlings are kept in WL_s – strongly absorbed by Chl – their plastids are largely destroyed as a consequence of Chl-mediated photooxidations (Reiß et al. 1983).

In agreement with these previous findings we observed that following a transfer from cFR to WL_s the levels of typical plastidic enzymes, RuBP-Case and NADP-GPD, decreased while cytoplasmic marker enzymes were unaffected (Fig. 6). The question is whether or not the amount of translatable mRNA of SSU and LHCP is affected by the photooxidative damage which obviously occurs to the plastids after a cFR→WL_s transfer.

Figure 7 shows that following the transfer from cFR to WL_s at 60 h after sowing a rapid decrease in the level of translatable mRNA for SSU and LHCP occurred, while in cFR the translatable mRNA levels increased until 72 h after sowing. The change of the mRNA levels between 60 and 72 h after sowing was indeed controlled by Pfr (Table 3) but even an almost complete return of Pfr to Pr by a pulse of long-wavelength FR ($\varphi_{RG9} <$ 0.01) at 60 h after sowing did not lead to a breakdown of the mRNA levels within 12 h (Table 3).

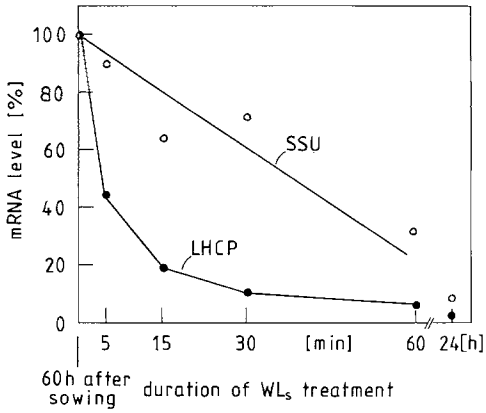

Fig. 9. Relative amounts of translatable mRNA for SSU (o) and LHCP (•) isolated from the cotyledons of 84-h-old NF-treated mustard seedlings, based on three independent experiments. Seedlings were kept in cFR for 60 h after sowing and then placed in WL_s. The duration of the WL_s treatment is indicated. Until assay at 84 h after sowing, seedlings were returned to FR. 100% = Amount of translatable mRNA isolated from the cotyledons of 84-h-old seedlings kept in cFR

Thus, the dramatic effect of WL_s on the mRNA levels (Fig. 7) did not occur via Pfr.

The data in Fig. 7 indicate that the level of translatable $mRNA_{LHCP}$ declines faster than the level of $mRNA_{SSU}$. This confirms the observation reported in Fig. 5.

Regarding the above question of whether or not the plastidic signal is continually required to allow expression of nuclear genes whose products are plastid-bound, Fig. 7 seems to provide the clear-cut answer "yes". The life-time of the plastidic signal must be short, and to maintain high levels of translatable mRNA intact plastids, which supply the signal continually, are required.

Can the plastids, once damaged by photooxidation, recover their potential to provide the plastidic signal required for nuclear gene expression? Seedlings treated with NF were kept in cFR until 60 h after sowing, then placed in WL_s for short terms and immediately returned to FR for another 12 h. Figure 8 shows that the RuBPCase level was indeed more sensitive towards short-term photooxidation than the GPD level but eventually – after 1 h WL_s treatment – both enzyme activities stayed at the same low level, and in both cases there was no recovery of enzyme level after the return to 12 h FR.

The same question was addressed at the level of translatable mRNAs (Fig. 9). One hour of WL_s treatment caused the mRNAs to disappear largely during the following 24 h even in the presence of cFR. The mRNAs responded differently: The level of translatable $mRNA_{LHCP}$ declined faster than the

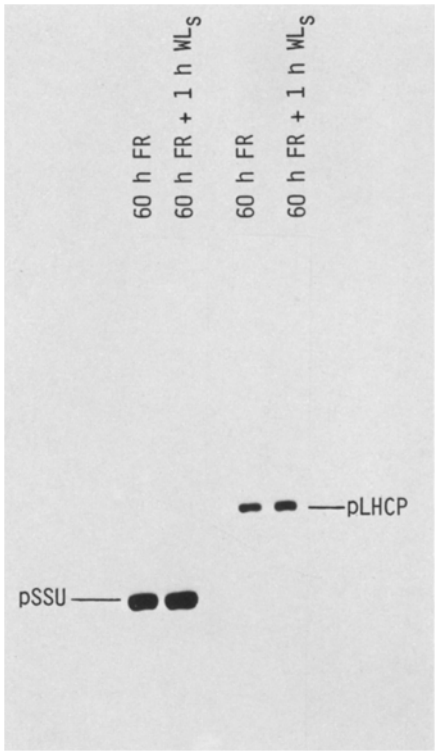

Fig. 10. Fluorograms of immunoprecipitates with SSU- and LHCP-specific antisera of the in-vitro translation products of polysomal RNA isolated from the cotyledons of NF ($5 \cdot 10^{-6}$ M)-treated mustard seedlings kept in cFR for 60 h or in cFR for 60 h followed by 1 h WL_s

level of translatable $mRNA_{SSU}$, in principal agreement with Figs. 5 and 7. In case of translatable $mRNA_{LHCP}$ even 5 min WL_s sufficed to cause an appreciable drop of the level, indicating a high sensitivity of maintenance of translatable mRNAs to slight disturbancies of the plastidic signal required for nuclear gene expression. On the other hand, there was no direct effect of 1 h WL_s on the level of translatable mRNAs for SSU and LHCP in the polysomal fraction (Fig. 10). This indicates that the photodamage exerted by 1 h WL_s did not affect the translatable mRNAs for SSU and LHCP per se.

By contrast, in situ labelling experiments of SSU indicated that 1 h W_s sufficed to damage the plastids so heavily that either the passage of proteins through the envelope or stabilization of the proteins inside the plastids was no longer possible. A [^{3}H]leucine solution was applied to the seedlings after 60 h cFR or after 60 h cFR plus 1 h WL_s treatment. The data show (Fig. 11) that labelled SSU (and LSU) could no longer be detected after 1 h WL_s even though translatable $mRNA_{SSU}$ was not affected by the 1 h WL_s treatment (see Fig. 10). Electronmicrographs of the plastids (Fig. 1, K + L)

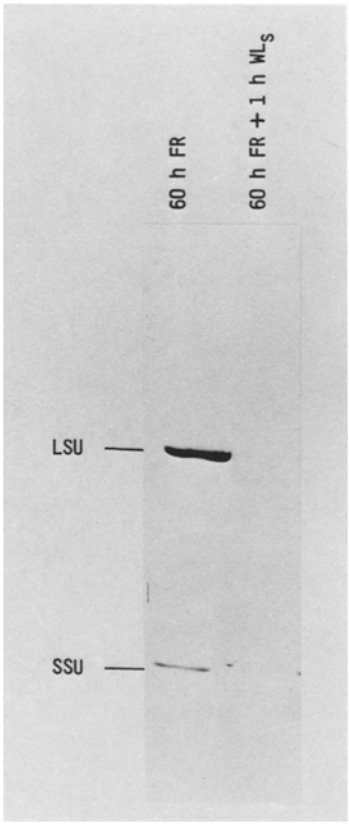

Fig. 11. Fluorogram of immunoprecipitates with antiserum raised against RuBPCase after in-vivo labelling of total protein in the cotyledons of mustard seedlings. Seedlings were treated with NF ($5 \cdot 10^{-6}$ M) from the time of sowing and kept 60 h in cFR, or 60 h in cFR and then transferred to WL_s for 1 h. Incubation with [^{3}H]leucine for 20 min was performed immediately after the light treatment. Equal amounts of labelled protein were used for immunoprecipitation

confirmed that 1 h WL_s treatment of cFR pretreated seedlings led to conspicuous damage of the organelle.

Discussion

From previous (Oelmüller and Mohr 1986) and the present data we conclude that a signal from intact plastids is needed to permit phytochrome-controlled appearance of translatable mRNAs in the case of those nuclear genes whose protein products become integral constituents of the plastid. The plastidic signal is required continually since its half-life is short (Fig. 9).

The plastidic signal could act either transcriptionally or post-transcriptionally. Only direct measurements of transcription can distinguish between the two possibilities (e.g. Silverthorne and Tobin 1984).

The plastidic signal is insensitive towards inhibition of intraplastidic translation by CAP. (Only at high concentration [200 $\mu g \cdot ml^{-1}$] was a certain drop observed in the level of translatable $mRNA_{LHCP}$.) However, if CAP is applied before 36 h after sowing the plastidic signal is dramatically decreased. This effect is correlated with an inhibition of plastid development. Apparently a certain developmental stage of the plastid must be reached – between 36 and 48 h after sowing – before the plastidic signal is released. Under our standardized growth conditions (25° C, see Mohr 1984, for details) the plastids in the mesophyll cells of mustard cotyledons develop essentially during the time span between 36 and 120 (144) h after sowing. Only during this period can translatable mRNAs for SSU and LHCP be detected (see Fig. 5). We conclude that the plastidic signal is released by the plastids only during this time span.

Circumstantial evidence in the literature emphasizes the importance of the state of the chloroplast for the expression of nuclear-encoded plastidic proteins (Taylor et al. 1984, Harpster et al. 1984). Vernet et al. (1982) reported that light-grown white callus derived from protoplasts of *Nicotiana sylvestris* does not contain RuBPCase activity and only low level of SSU-mRNA, while in green callus from the same protoplasts, RuBPCase activity and SSU-mRNA is present in abundance. More recently Eckes et al. (1985) – analyzing factors involved in organ specific gene expression – found that besides light, mature chloroplasts might be a pre-requisite for the expression of three nuclear-encoded DNA sequences. Börner et al. (1985) investigated the nuclear gene induced plastome mutant 'albostrians' of barley (*Hordeum vulgare*). This mutant lacks plastid ribosomes and is deficient in chlorophylls and carotenoids. This mutation not only prevents the synthesis of those proteins which are synthesized on chloroplast ribosomes, but also the accumulation of certain nuclear-encoded proteins such as SSU, LHCP, Calvin cycle enzymes and nitrate reductase, a cytoplasmic enzyme.

The nature of the postulated plastidic signal is unknown so far. The present data obtained with CAP and reports in print do not favor the idea that the 'signal' is of a protein nature.

(i) Feierabend (1979) and associates have shown that in cereal seedlings assembly of 70 S ribosomes was prevented by elevated growth temperatures (32–34° C). Under these conditions cytoplasmically synthesized plastidic proteins (such as SSU and nuclear-encoded subunits of the AT-Pase complex, Biekmann and Feierabend 1985) were found in substantial amounts. Our data (see

Tab. 2, Fig. 4, RuBPCase) are in agreement with these results and with the conclusion that intra- and extraplastidic synthesis of chloroplast proteins are not strictly coupled. Similarly, Allsop et al. (1981) demonstrated the lack of CAP inhibition of SSU labelling although LSU was strongly inhibited. These results show that cytoplasmic syntheses of SSU and of other plastidal proteins do not require the presence of 70 S translation products as a positive control signal.

(ii) Bradbeer et al. (1979) and Bradbeer (1981) – using two nuclear mutants of barley that develop defective plastidic DNA (Hagemann and Börner 1978) – concluded that cytoplasmic synthesis of plastidic polypeptides may be controlled by plastid synthesized RNA. However, experiments showing the passage of nucleic acids through both envelope membranes of the plastid are lacking (Douce and Joyard 1984).

In conclusion, our data support indeed the general statement forwarded by Douce and Joyard (1984) "that the balance observed between cell and chloroplast growth may be achieved by chloroplastic products exerting influence over the expression of nuclear genes which code for chloroplastic proteins".

However, the fact that not only the synthesis of plastidic proteins, but also the appearance of cytoplasmic nitrate reductase is prevented by a damage to the plastid (see Table 1, Börner et al. 1985) shows that the plastidic control over the appearance of nuclear-encoded proteins is not restricted to plastidic proteins proper, but probably involves those proteins as well which are functionally related to the plastid compartment, such as the enzymes of nitrate assimilation.

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