Regulatory factors involved in gene expression (subunits of ribulose-1,5-bisphosphate carboxylase) in mustard (Sinapis alba L.) cotyledons

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Abstract. Phytochrome-controlled appearance of ribulose-1,5-bisphosphate carboxylase (RuBPCase) and its subunits (large subunit LSU, small subunit SSU) was studied in the cotyledons of the mustard (Sinapis alba L.) seedling. The main results were as follows: (i) Control of RuBPCase appearance by phytochrome is a modulation of a process which is turned on by an endogenous factor between 30 and 33 h after sowing (25°C). Only 12 h later the process begins to respond to phytochrome. (ii) The rise in the level of RuBPCase is the consequence of a strictly coordinated synthesis de novo of the subunits. (iii) While the levels of translatable mRNA for SSU are compatible with the rate of SSU synthesis the relatively high LSU mRNA levels are not reflected in the rates of in-vivo LSU or RuBPCase syntheses. (iv) Gene expression is also abolished in the case of nuclear-encoded SSU if intraplastidic translation and concomitant plastidogenesis is inhibited by chloramphenicol, pointing to a "plastidic factor" as an indispensable prerequisite for expression of the SSU gene(s). (v) Regarding the control mechanism for SSU gene expression, three factors seem to be involved: an endogenous factor which turns on gene expression, phytochrome which modulates gene expression, and the plastidic factor which is an indispensable prerequisite for the appearance of translatable SSU mRNA.

Key words: Gene expression – Phytochrome – Plastidic factor – Ribulose-1,5-bisphosphate carboxylase – Sinapis.

Introduction

Plastidogenesis in higher plants is characterized by its light dependency: the appearance of many plastidic proteins is controlled by light via phytochrome (Mohr 1984; Tobin and Silverthorne 1985). Moreover, plastid development depends on the coordinated expression of nuclear and plastidic genes. In recent efforts to understand the control mechanisms, RuBPCase, the enzyme responsible for CO2 fixation in photosynthesis, has attracted particular interest (see Inamino et al. 1985 for references to pertinent literature). The enzyme RuBPCase is a multimeric protein comprised of eight large subunits (LSU) and eight small subunits (SSU) (Baker et al. 1975). The enzyme is localized in the plastid, but the plastid genome encodes only the LSU. The genetic information of the SSU is nuclear-coded. The SSU is synthesized in the plastid where it combines with the LSU to form the active enzyme (see Inamino et al. 1985 for references to pertinent literature).

Previous studies using pea seedlings have shown that the steady-state level of mRNA coding for the SSU of RuBPCase increases strongly upon exposure of the plants to light (Smith and Ellis 1981; Thompson et al. 1983; Coruzzi et al. 1984). This appears to be due to a specific increase in
transcription since the copy number of the SSU gene(s) in the nucleus was not significantly affected by light (Sasaki et al. 1986). Run-off transcription experiments using isolated pea or *Lemma* nuclei (Gallagher and Ellis 1982; Silverthorne and Tobin 1984) indicate that light increases the steady-state levels of SSU mRNA by increasing the transcription of the SSU gene(s).

The level of LSU mRNA also increases after exposure of pea plants to light, but in this case there appears to be a correlation between the rate of increase in LSU mRNA and an increase in plastid genome copy number (Thompson et al. 1983; Sasaki et al. 1984, 1986).

In mustard seedling cotyledons, accumulation of RuBPCase and NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase (NADP-GPD) is known to be controlled by phytochrome (Brüning et al. 1975). The young mustard seedling does not synthesize RuBPCase or NADP-GPD, and only traces of seed-borne enzyme activity can be detected before 36 h after sowing at 25°C. An important finding in the present context was that for the appearance of both enzymes it does not matter whether the light which activates phytochrome is given from the time of sowing, from 24 h after sowing, or only from 36 h after sowing onwards (Brüning et al. 1975). This was interpreted to indicate that the mustard seedling is not "competent" to respond to phytochrome by synthesis of NADP-GPD or RuBPCase before 36 h after sowing (25°C) (Mohr 1983). Other enzymes in the mustard cotyledons, not related to photosynthesis, can be induced by phytochrome much earlier in the course of development. There is obviously a temporal pattern of inducibility of enzymes by the far-red-absorbing form of phytochrome (Pfr). The specification of this "temporal pattern of competence to Pfr" is not influenced by phytochrome (Mohr 1983).

In a previous paper (Oelmüller and Mohr 1984) we have shown that an increase in the activity of NADP-GPD becomes detectable at the same time in dark-grown material as in the seedling kept in continuous far-red light (cFR) (i.e. kept continuously, from the time of sowing, under the strong action of phytochrome). During the first 6 h after the onset of enzyme synthesis the increase of NADP-GPD activity in FR is approx. 2.7 times the increase in the dark. Thus, the light effect is multiplicative, indicating a modulation of gene expression by phytochrome rather than an induction proper? The rate of NADP-GPD accumulation decreases in darkness beyond 48 h while it steeply increases in the light. This indicates that accumulation of NADP-GPD crucially depends on phytochrome action from 48 h onwards.

In the present paper we describe the appearance of RuBPCase and its subunits in the cotyledons of the mustard seedling. The following questions are addressed: (1) Is control of RuBPCase synthesis by phytochrome a modulation or an induction proper? In the case of modulation, are the time courses of appearance of RuBPCase in light and darkness multiplicatively related as described previously for NADP-GPD (Oelmüller and Mohr 1984)? (2) Are measurements of the pertinent mRNA levels compatible with the rates of synthesis of the subunits? (In pea the situation with LSU mRNA is not clear, see Inamine et al. 1985.) (3) Is accumulation of RuBPCase the result of a coordinated synthesis of LSU and SSU, including temporal coordination with regard to responsiveness to phytochrome? (4) Is gene expression abolished in the case of nuclear-encoded LSU if intraplastidic translation is inhibited and plastidogenesis impaired by the application of chloramphenicol (CAP)?

**Material and methods**

Seeds of white mustard (*Sinapis alba* L.) were obtained from Asgrow Company (Freiburg-Ebnet, FRG) in 1979. Selection of the seeds, germination and growth conditions (25±0.5°C) were as described previously (Mohr 1966).

For light treatment, a standard far-red light source (3.5 W· m⁻², Mohr 1966) was used. This kind of light does not cause significant protochlorophyll(ide) → chlorophyll(ide) photoconversion, while phytochrome-mediated responses are strongly potentiated (Mohr 1972).

Ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39) activity was assayed at pH 8.2 by measuring the acid-stable radioactivity produced in the reaction between ribulose-1,5-bisphosphate and NaH¹⁴CO₃ at 25°C according to Frosch et al. (1979).

For in-vivo labelling of total protein, 20 pairs of cotyledons were shaken immediately after removal from the seedling in 2 cm³ of a radioactive [¹⁴H]leucine solution (15·10¹⁵ Bq per pair of cotyledons) for 30 min in green safelight, the cotyledons were washed four times with a large excess of distilled water and frozen in liquid nitrogen. The cotyledons were ground with 1 g quartz sand, 0.5 g Dowex 1 X 2 and 4 cm³ of extraction buffer (100 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris)-HCl, pH 7.4, 150 mM NaCl, 5 mM ethylene diaminetetraacetic acid (EDTA) and the homogenate clarified by centrifugation (20 min, 39,000 g). A 100-mm³ aliquot of the supernatant was used to determine the radioactivity incorporated into the protein fraction by collecting the triehloroacetic-acid precipitate on GF/C Whatman filters (Whatman, Springfield Mill, Kent, UK).

For immunoprecipitation of LSU and SSU, 100 mm³ of the supernatant was incubated with 50 mm³ of RuBPCase antiserum, incubated for 1 h at 25°C and at 4°C overnight. *Staphylococcus aureus* cells (50 mm³) were used to achieve the precipitation of the antibody-antigen complex. Washing of the precipitate, separation of the immunoprecipitated polypeptides by