Lack of Light Regulation of NADPH: Protochlorophyllide Oxido-Reductase mRNA in Cress Seedlings (*Lepidium sativum* L.)

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A cDNA clone for NADPH: protochlorophyllide oxidoreductase from barley was subcloned for production of antisense-mRNA. This enabled heterologous hybridization with RNA from cress seedlings (*Lepidium sativum* L.). The mRNA level for NADPH: protochlorophyllide oxidoreductase did not decrease in cress seedlings during irradiation with continuous far-red or white light up to 12 h. The amount of NADPH: protochlorophyllide oxidoreductase protein, identified by Western blot decreased 5-fold after continuous irradiation with white light for 12 h. Species differences for light regulation of RNA are discussed.

Introduction

The enzyme NADPH: protochlorophyllide oxidoreductase (Pchlide reductase, EC 1.6.99.1) has mainly been investigated in barley (*Hordeum vulgare*) plants (see [1] for earlier literature). The enzyme is accumulated together with its substrate Pchlide in the dark but disappears nearly completely in the light [2, 3]. Disappearance of the enzyme protein parallels the disappearance of its mRNA [4, 5] and a decrease in the rate of transcription of this gene [6]. During our investigations on light-regulated processes in cress seedlings [7] we became aware that light has no effect upon the mRNA level but only reduces the protein level of Pchlide reductase.

Materials and Methods

Basis for the preparation of a hybridization probe was the plasmid pHvDF 1 in *E. coli* HB 101 which contained an Pchlide reductase insert (538 bp) at the *Pst* I site of pBR 322 [8]. Since we had the information, that no positive signal had been found with mRNA from dicotyledonous plants after nick translation (K. Apel, personal commun.), we decided to prepare a probe of labeled anti-sense RNA (see Fig. 1).

For this purpose the plasmid was prepared according to [9] or [10] and subcloned in *E. coli* JM 101. The insert was excised from pHvDF 1 by *Pst* I digestion and ligated into the *Pst* I site of pBS (Stratagene, Heidelberg, F.R.G.). The orientation of the insert was verified by cleavage with *Bam*HI and *Bgl* II and by partial sequencing using *Taq* DNA Polymerase sequencing kit (US Biochemical Corp.). After linearization of the plasmid by *Bam* HI digestion, antisense transcripts of the Pchlide reductase gene fragment were prepared using T3 RNA polymerase (BRL) following the instructions provided by the manufacturers. The transcripts were radioactively labeled using α-[^{32}P]UTP at a specific radioactivity of 400 Ci/mmol.

The nitrocellulose filter (see Fig. 2) was hybridized with the labeled probe using 10^7 cpm in 10 ml of the hybridization solutions (5 × SSC, for 15 h) at 50 °C. The filter was washed in subsequent steps of increasing stringency: The most stringent wash was twice for 10 min at room temperature and once for 30 min at 65 °C in 2 × SSC, 0.1% SDS. The washed filters were exposed to Kodak XAR-5 films at −80 °C using intensifying screens (Kodak).

Results and Discussion

The clone pHvDF 1 contains a 538 bp cDNA insert for the Pchlide reductase of barley [8]. This cDNA covers the 3′-untranslated and part of the coding region of the corresponding mRNA. The insert was subcloned into a transcription vector in order to strengthen the hybridization activity with isolated RNA from garden cress. The scheme of Fig. 1 summarizes the subcloning and the preparation of the antisense-transcript of the Pchlide reductase gene fragment.

The Northern blot (Fig. 2) demonstrates that the antisense-mRNA probe hybridizes specifically to one RNA band of about 1.6 kbp. This is approximately the size expected for the mRNA of Pchlide reductase (1.7 kbp [8]). The blot shows furthermore that there seems to be no decrease in the mRNA level during irradiation with continuous far-red for up to 12 h. This irradiation is sufficient to induce a 5-fold increase of cab mRNA in the

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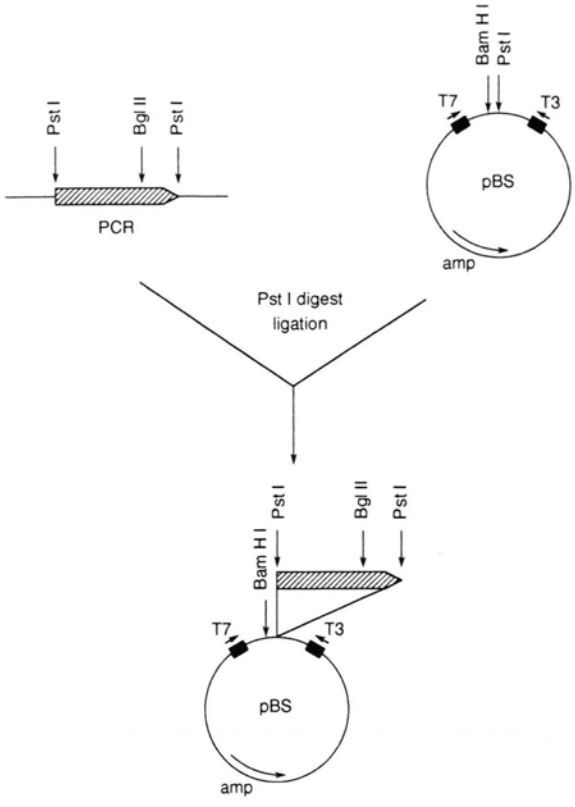

Fig. 1. Subcloning and preparation of antisense-RNA probe for NADPH:protochlorophyllide oxidoreductase. PCR = cDNA insert of clone pHvDF 1 containing 538 bp of the coding Pchlide reductase gene of barley. pBS = bluescript vector.

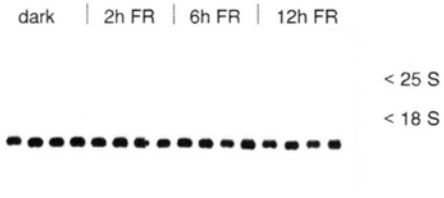

Fig. 2. Northern blot analysis of Pchlide reductase mRNA. Total RNA was isolated after [11] from cotyledons of cress plants grown for 4 days either in the dark or irradiated for the time indicated with continuous far-red. Equal amounts (20 µg) of total RNA were applied to each lane of the gel. After electrophoresis and blotting to nitrocellulose, the filter was hybridized with the labeled probe (see Fig. 1). Arrows indicate the position of 25S (3.9 kpb) and 18S (1.9 kbp) rRNA markers.

Table I. Slot blot analysis of mRNA for Pchlide reductase. Conditions as in Fig. 2. WL = white light; FR = far-red light.

Exp.	Treatment of seedlings	Relative activity after hybridization [%]
1	dark	91
2	dark	75
3	12 h WL	100
4	12 h WL	88
5	12 h FR	81

same plants (Kittsteiner and Rüdiger, unpublished results). A more quantitative analysis of Pchlide reductase mRNA pools was done by slot blot hybridization (Table I). There is no significant change in the mRNA level by either continuous far-red or white light irradiation of up to 12 h.

Contrary to the mRNA level, the level of the Pchlide reductase protein decreased during illumination. As shown in Fig. 3A, the protein can already be detected in a total protein extract from cotyledons by staining with colloidal gold. Identity of the protein was proven by immunostaining

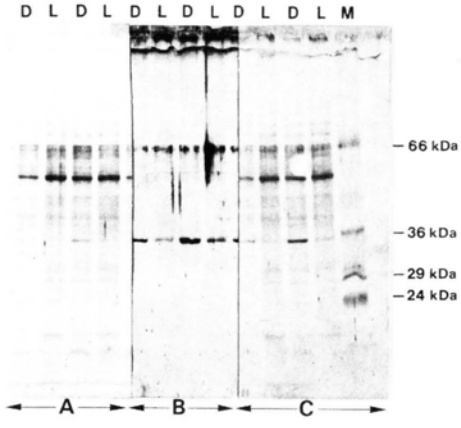

Fig. 3. Western blot analysis of Pchlide reductase protein according to [18]. Results of 2 experiments are shown. Cress cotyledons of plants either kept in the dark (D) or in white light for 12 h (L) were extracted with SDS-containing buffer by boiling for 5 min. Equal amounts (0.5 µl) of the extract were applied to each lane of the gel. After electrophoresis and electroblotting, the nitrocellulose was stained as follows: A: with colloidal gold according to [19] (= total proteins), B: with anti-Pchlide reductase antiserum (= Pchlide reductase), C: at first gold and then with the antiserum. M = molecular weight standards.

(Fig. 3B) and double staining with gold and immunoserum (Fig. 3C). It is evident from all 3 staining methods, that the amount of protein is less in the light than in the dark. Dilution series (not shown) revealed a 5-fold drop in the protein level upon irradiation of dark-grown seedlings with white light for 12 h.

After completion of this work, K. Apel (personal commun.) reported on a similar situation – decrease in protein level but lack of decrease in mRNA level of Pchlide Reductase – in *Arabidopsis thaliana*. This is a clear contrast to disappearance of the mRNA in barley seedlings [4, 5].

Another well investigated example of negative light regulation is that of phytochrome protein and mRNA. The decrease of its mRNA level is much more pronounced in some monocotyledons like oat [11] and rice [12] than in some dicotyledons like *Cucurbita* [13] or *Arabidopsis* [14]; phytochrome mRNA species from pea exhibit a medium light regulation [15, 16]. Findings on light regulation of RNA levels cannot be generalized without rigorous proof due to species differences.

Acknowledgements

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