Comparison of the effects of exogenous native phytochrome and in-vivo irradiation on in-vitro transcription in isolated nuclei from barley (Hordeum vulgare)

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Abstract. In barley seedlings the transcription of genes coding for the light-harvesting chlorophyll a/b protein (LHCP) is stimulated and the transcription of genes coding for the NADPH-protoporphyrilide oxidoreductase (reductase) is repressed by light working via the phytochrome system. This phytochrome-mediated control of gene expression has been studied by monitoring in-vitro transcription in isolated nuclei. Two different experimental approaches have been used to elucidate the function of phytochrome (Pfr) during the transduction of the light signal. Concentrations of phytochrome were varied experimentally either by illuminating intact plants or macerated plant material prior to the isolation of nuclei or by adding purified phytochrome (Pfr) in its native 124-kDa form to the isolated nuclei. Our results indicate that there are at least two different steps involved in the phytochrome control of specific gene expression. (i) There is a rapid and transient change in the transcription rate which is saturated by very low levels of Pfr. (ii) There is a change in the duration and the maximum range of the transient change; this step requires relatively high Pfr concentrations and thus reacts very sensitively and rapidly to changes in Pfr levels as induced by secondary irradiations. This second step, but not the first one, could be triggered by the addition of purified oat phytochrome to a reconstituted nuclear system. This effect of purified phytochrome could only be shown if nuclei isolated from red-light (R)-irradiated seedlings were used. It was thus possible to simulate the effect of an in-vivo-applied second R pulse by the addition of Pfr to nuclei isolated from R-preirradiated plants.

Key words: Hordeum (transcription) – Light-harvesting chlorophyll a/b protein – NADPH-protoporphyrilide oxidoreductase – Phytochrome and transcription – Transcription (run-off).

Introduction

Phytochrome, a chromoprotein consisting of a tetrapyrrolic chromophore and a 124-kDa apoprotein is now well established as one of the main photoreceptors controlling photomorphogenesis in plants (Shropshire and Mohr 1983). It has been shown (Gallagher and Ellis 1982; Stiekema et al. 1983; Apel et al. 1983; Kaufman et al. 1984) that the rate of transcription of specific genes could play a dominant part within the reaction chain which is responsible for their expression. Previously (Mösinger et al. 1985) we have demonstrated that in nuclei isolated from in-vivo-preirradiated barley seedlings, the in-vitro transcription rates of genes coding for two plastid proteins is controlled by phytochrome. Within the same population of nuclei the transcription rate of the mRNA coding for the light-harvesting chlorophyll a/b-binding protein (LHCP) is strongly increased by light, whereas the transcription of the mRNA coding for the NADPH-dependent protoporphyrilide oxidoreductase (reductase) is reduced. The kinetics of both responses indicate that these changes are transient. Neither the mechanism of this control nor the signal transduction chain connecting the phytochrome system and the control loci of transcription have so far been investigated directly.

Abbreviations: D = darkness; FR = long-wavelength far-red light; LHCP = light-harvesting chlorophyll a/b protein; Pfr,124 = phytochrome in its red- or far-red-absorbing form (the number indicates the apparent molecular weight); R = red light; reductase = NADPH-protoporphyrilide oxidoreductase
In the present work we have analysed more extensively the effect of phytochrome \( (P_{fr}) \) on the rate of transcription of genes coding for the LHCP and the reductase in isolated nuclei of barley seedlings. The concentrations of \( P_{fr} \) were manipulated experimentally either by illuminating intact plants or macerated plant material prior to the isolation of nuclei or by adding purified degraded phytochrome \( (P_{dr}) \) to the isolated nuclei.

Our results indicate that there are at least two different steps involved in the phytochrome control of specific gene expression. One of these steps could be simulated in a reconstituted nuclear system by adding purified phytochrome in the \( P_{fr} \) form.

Material and methods

Growth of plants. Barley \( (Hordeum vulgare \, L. \, cv. \, Carina) \) plants were grown in absolute darkness on moist vermiculite at 25°C for 5 d. Light sources were: white light, fluorescent tubes 3000 lx; red light, 660 nm and far-red light, 760 nm (fluence rate 7 W·m\(^{-2}\); each) with modified Leitz Prado 500 W projectors (Leitz, Wetzlar, FRG); for further specifications see Heim and Schäfer (1984).

Isolation of nuclei. Nuclei were isolated by the method of Willmitzer and Wagner (1981), modified as described in Mösinger and Schäfer (1984). For the 2.5-h-long maceration of the tissue, which is performed at 25°C just prior to homogenization in order to obtain a partial degradation of the cell walls, the following enzymes were used: Rohament CT as cellulase (3 mg·ml\(^{-1}\)) and Rohament P as pectinase (1.5 mg·ml\(^{-1}\)) from Röhm, Darmstadt, FRG. The vacuum infiltration of the enzyme solution into the tissue was standardized to four 1-min infiltrations per assay. Under these conditions the yield of nuclei was about 3·10\(^{10}\)·4·10\(^{6}\) g\(^{-1}\) weight.

In-vitro transcription. In-vitro transcription was performed as described in Mösinger et al. (1985). An aliquot of 2·10\(^{11}\) nuclei was used per assay with 7.5·M\(_{eq}\) \( x^3\text{P} \text{UTP} \) (UTP; specific radioactivity 15 TBq·mmol\(^{-1}\)).

Isolation of RNA transcripts, preparation of plasmid DNA, and dot blot hybridization were carried out according to Mösinger et al. (1985). A clone coding for a B1 hordein (Rasmussen et al. 1983) and pBR 322 were included as controls in all hybridization experiments.

If phytochrome or other reagents were used, these components were added to the nuclei after resuspension at 0°C in nuclei resuspension buffer 2 (NRB 2; Mösinger and Schäfer 1984) together with the ribonuclease inhibitor. Within 15 min the assay was started by adding \( \text{[32-P]} \text{UTP} \) and transferring the preparations to 30°C.

Phytochrome purification. Rye phytochrome was isolated following a procedure described by Kerscher and Nowitzki (1982), using 5-d-dark-grown seedlings. Oat phytochrome was prepared according to the method described by Vierstra and Quail (1983). Quality and purity of both phytochrome preparations were monitored by spectroscopic measurements and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

All experiments with purified phytochrome were performed in essentially the same way: nuclei preparations which had been stored at \(-80°C\) since their isolation were thawed, pelleted, and resuspended in nuclei resuspension buffer 2 (see Mösinger and Schäfer 1984). Phytochrome was added under green safelight and the mixture incubated on ice for 15 min in the dark. In-vitro transcription was started by the addition of labelled UTP and the other three nucleotide triphosphates. All steps including the transcription assays, which were stopped by addition of deoxyribonuclease, were performed under green safelight. The assays were processed and hybridizations were performed as described above, except that the total labeled RNA from each in-vitro transcription assay was used to challenge the filters.

Results

Effects of irradiation of intact plants and homogenates on transcription of specific genes

The effects of in-vivo irradiations on in-vitro transcription were first analyzed in detail in order to lay a foundation for the subsequent in-vitro-reconstitution experiments.

Induction kinetics. Plants were grown in the dark for 5 d and then exposed to a 1-min R pulse or a 5-min FR treatment and returned to darkness. After the periods of time indicated in Figs. 1 and 2 the entire shoots were cut into short pieces, treated with pectinase and cellulase for 2.5 h at 25°C and nuclei were subsequently isolated as described in Mösinger et al. (1985). In-vitro transcription was done under conditions described in Material and methods and the \( \text{[32-P]} \)-pulse-labelled RNA extracted from the assay mixtures. Equal amounts of these labelled RNA probes, calculated from total incorporation measurements, were hybridized to nitrocellulose strips on which a large excess of the specific complementary DNA probes (clone pHvLF2 encoding LHCP and clone pHvDF1 encoding reductase, see Gollmer and Apel 1983 and Apel et al. 1983) were fixed as dots. The amount of hybridization per dot was quantified by liquid scintillation counting.

Three hours after an initial R pulse, the transcription rate of LHCP mRNA reached its maximum at a level about 12 times above the dark control. Even during the initial 30 min, a fourfold increase could be detected (Fig. 1).

Within the initial 90 min, a FR treatment produced the same response as a R pulse, indicating that this rapid first response is saturated at even very low amounts of \( P_{fr} \), as established by a saturating FR pulse (less than 0.1% of the total cellular phytochrome, Schäfer et al. 1983). Ninety minutes after induction, in-vitro transcription activity of nuclei from FR-treated plants started to decline,