Volume 246, number 1,2, 140–144 FEB 06960 March 1989

The 23 kDa polypeptide of the photosynthetic oxygen-evolving complex from mustard seedlings (*Sinapis alba* L.)

Nucleotide sequence of cDNA and evidence for phytochrome control of its mRNA abundance

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Received 25 January 1989

The nucleotide sequence of a cDNA from mustard shows 78% homology in deduced amino acid sequence for the mature protein compared to the sequence for the 23 kDa protein of the oxygen-evolving complex from spinach [(1987) FEBS Lett. 216, 234–240]. There is also a high degree of homology between the premature protein sequences concerning the hydrophobic domain and its distance from the suggested processing site. The accumulation of mRNA for the 23 kDa protein in mustard was stimulated by continuous far-red light and reversal experiments by means of red/far-red light pulse treatment show the involvement of phytochrome in controlling the mRNA abundance for the 23 kDa polypeptide in mustard. The accumulation of the mRNA can be inhibited in white light if the seedlings are treated with the herbicide Norflurazon.

Oxygen-evolving complex; Protein, 23 kDa; cDNA; Nucleotide sequence; Phytochrome control; (*Sinapis alba* L.)

1. INTRODUCTION

Green plants depend on photosynthesis for their energy supply. It is thus not surprising that many of their developmental processes are themselves controlled by light. In most cases, this control is not extended through chlorophyll or photosynthesis directly but through a number of photomorphogenetic photoreceptors, of which the best studied is the red/far-red light photoreversible pigment phytochrome [2].

On account of its immediate relevance for photosynthesis, once of the best studied developmental processes in plants is light-dependent greening whereby etioplasts from dark-grown plants develop into fully functional chloroplasts as a result of exposure to light [3].

In recent years, it has been possible to demonstrate that red light operating through phytochrome increases the levels of a number of mRNAs encoding polypeptides directly involved in photosynthesis and constituting part of a fully developed chloroplast. These include such diverse polypeptides as the small subunit of the key carbon-fixing enzyme ribulose-1,5-bisphosphate carboxylase [4], light-harvesting chlorophyll *a/b*-binding protein of photosystem (PS) II (LHCP) [4] and the 32 kDa D1 PS II reaction centre protein (QR or herbicide-binding protein) [5]. Light-dependent increases in the level of mRNAs encoding a number of other polypeptides involved in photosynthesis have been described but in these cases the involvement of phytochrome has not been demonstrated unequivocally [6]. For yet another group of polypeptides, phytochrome on light control of enzyme activities has been shown

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y07498

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

but evidence that these changes are due to increases in the amount of mRNA is still lacking [7]. We have recently constructed a cDNA library from mustard (*Sinapis alba* L.) seedlings and screened it by means of an antibody raised against the light-harvesting chlorophyll *a/b*-binding protein of mustard [8]. The antibody allowed the isolation of a single clone which could be identified on the basis of its sequence as encoding the 23 kDa polypeptide of the oxygen-evolving complex of PS II.

Two of those genes for which phytochrome control of transcript levels has been shown form part of the PS II complex (LHCP, D1). It thus seemed probable that phytochrome might control the levels of transcripts from other genes encoding PS II polypeptides. Furthermore, a study of phytochrome control of PS II polypeptide synthesis might be expected to provide insights into the coordination of control by an external factor (in this case light) of the synthesis of various components of a more complex structure.

Here, we present evidence that transcripts of the gene encoding the 23 kDa polypeptide of the oxygen-evolving complex of PS II are under the control of phytochrome.

2. MATERIALS AND METHODS

Mustard (*S. alba* L.) seeds obtained from Asgrow (Freiburg) (Harvest 1982) were sorted and sown as described [9].

Polysomal mRNA was extracted from mustard cotyledons, grown for 36 h in the dark and 12 h under red light [10] and poly(A)$^+$ mRNA purified by column chromatography with oligo(dT)-cellulose [11]. Using this poly(A)$^+$ mRNA, cDNA was synthesized and a λgtII cDNA library constructed according to [12,13].

The library was screened immunologically using antibodies raised against LHCP by Dr R. Oelmüller, Freiburg (details in [8]). Screening was carried out according to [14] using nitrocellulose filters.

DNA was sequenced by the dideoxy chain-termination method [15]. Northern and Southern analyses were carried out as in [16].

The procedure for dot-blot hybridization was as described by Bruns et al. [17] and total cellular RNA was extracted [18]. Herbicide treatments with Norflurazon were performed as described by Frosch et al. [19]. Light sources were as described in [20].

3. RESULTS AND DISCUSSION

Poly(A)$^+$ mRNA isolated from the cotyledons of mustard seedlings was used to synthesize cDNA [12]. This cDNA was inserted into the expression vector λgtII, which was again used to infect the host bacteria, *E. coli* strain Y1090 [13]. The cDNA library thus obtained was plated and screened for positive plaques. Approx. 100000 plaques were transferred to nitrocellulose and tested for positive reactions with antibody preparations [14]. This screening resulted in the isolation of a single positive clone which was replated and rescreened (not shown). Since the antibody preparation used in the screening process has been described as being raised against LHCP [8], a first attempt at confirming the identity of the clone was made by cross-hybridization experiments with two available authentic genomic LHCP clones. These consisted of a 3.3 kb fragment of the LHCP gene from *Arabidopsis thaliana* (described in [21]), which was a kind gift from Dr D.E. Meyerowitz and a genomic sequence from mustard isolated in our laboratory by Dr A. Batschauer. Surprisingly, Southern analysis revealed that, although the two authentic LHCP fragments hybridized strongly with one another, our new clone hybridized with neither. These results cast doubt upon the identity of our clone as a LHCP clone. To clarify the situation, the approx. 900 bp fragment was sequenced according to the dideoxy chain-termination method [15]. A search of the EMBL/GenBank database revealed only a single sequence having 73% or more homology to our sequence. This was the sequence encoding the 23 kDa protein of the oxygen-evolving complex of PS II in spinach (*Spinacia oleracea* L.) described in [1].

Fig.1 shows a comparison of the amino acid sequence derived from our cDNA sequence with that derived from the spinach cDNA sequence [1]. The sequences showed 78% homology (i.e. identical amino acids) and of the remaining 22%, 90% represented conservative exchanges. Both our amino acid sequence and that of spinach showed only weak homology with that described for the LHCP of *Arabidopsis* [21]. It thus seems reasonable to assume that our clone also represents the 23 kDa polypeptide of the PS II oxygen-evolving complex.

Further characterization of the clone is shown in fig.2, which depicts the Northern analysis of the clone, i.e. its hybridization with denatured poly(A)$^+$ mRNA isolated from mustard cotyledons (lane A). In Norflurazon-treated seedlings, which

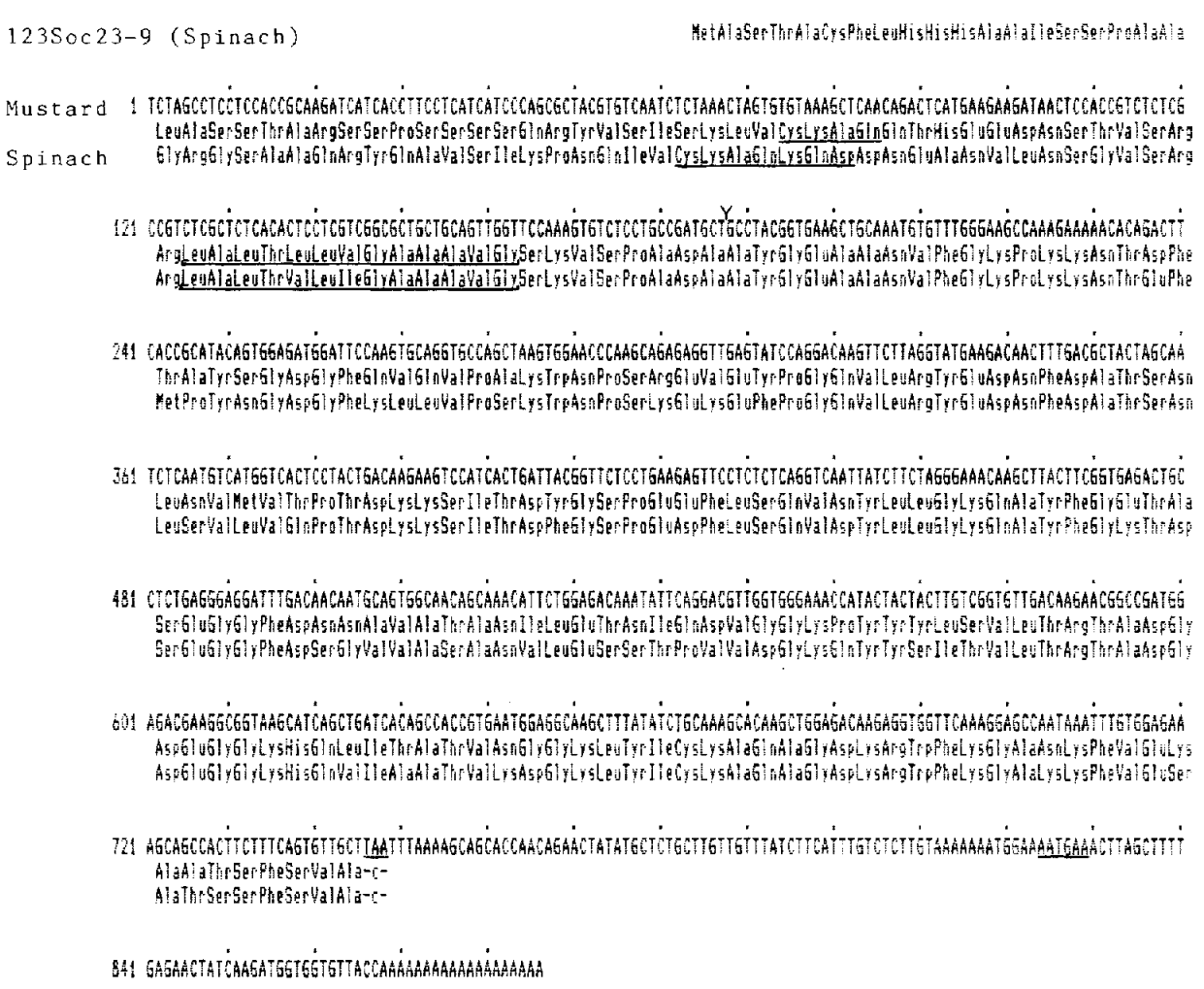

Fig.1. Nucleotide sequence and deduced amino acid sequence of the cDNA for the 23 kDa polypeptide of the oxygen-evolving complex from mustard. The amino acid sequence for the 23 kDa protein from spinach [1] is aligned with the mustard sequence. The deduced amino acid sequence from mustard cDNA shows 83% homology to the hydrophobic domain (underlined) suggested in spinach and the same processing site (Y) [1]. Stop codon, putative polyadenylation signal and amino acids involved in β-sheet structures as described [1] are underlined. The amino acid sequences of spinach and mustard are 70% (premature protein) and 78% (mature protein) homologous to each other, respectively.

were put under strong white light for 48 h no corresponding mRNA is detectable (lane B). This implies that for expression of the mRNA in the nucleus, intactness of the chloroplasts is necessary. Fig.3 represents the corresponding Southern analysis utilizing high-molecular-mass DNA isolated from mustard and digested with various restriction endonucleases. This analysis revealed the possibility that the 23 kDa protein is coded for by a small multigene family (3 genes, unpublished). This contrasts with the results of Tittgen et al. [22] for spinach where it appeared that only a single gene encoded the 23 kDa protein. Investigation of the light dependence for the appearance of the corresponding mRNA was carried out by dot-blot hybridization experiments. 40 mustard seedling cotyledons were collected every 6 h after sowing and total mRNA was extracted [18]. Identical amounts of mRNA were then denatured and different series of dilutions applied to a

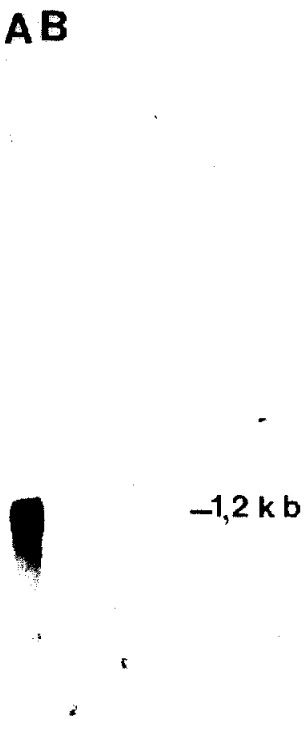

Fig.2. Northern blot analysis of mustard 23 kDa polypeptide mRNA. Seedlings were grown in white light in the presence (B) or absence (A) of the herbicide Norflurazon. Poly(A)$^+$ mRNA was isolated and analysed with the nick-translated cDNA probe from the 23 kDa protein.

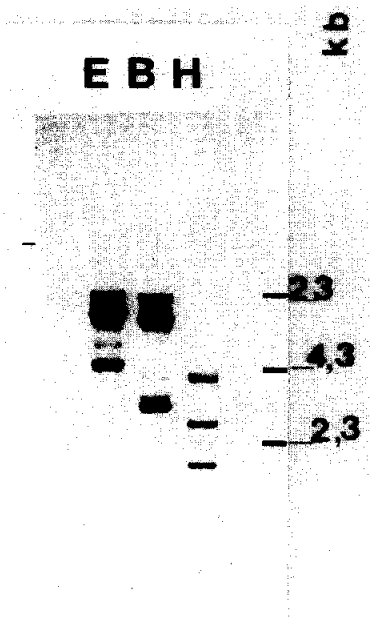

Fig.3. Hybridization of nick-translated 23 kDa polypeptide gene probe to *Bam*HI (B), *Eco*RI (E), and *Hin*dIII (H) restriction digests of mustard DNA.

nitrocellulose filter [23]. After baking and prehybridization, hybridization with 23 kDa polypeptide cDNA labelled by nick translation was determined. Hybridization signals were visualized by autoradiography and quantified by counting the dots. Fig.4 shows the changes occurring in the amounts of 23 kDa polypeptide mRNA during development of mustard cotyledons under various light regimes. Up to 30 h after sowing no mRNA accumulation can be detected under any circumstances. After 30 h, the amount of mRNA from seedlings kept under continuous red light increases rapidly, reaching a maximum after approx. 66 h. mRNA from seedlings kept in darkness also begins to accumulate after 30 h but the rate of accumulation is much lower and the greatest amount measured (after 72 h) is only 20% of that found under continuous red light. Continuous far-red light is also effective in inducing mRNA accumulation. As in dark-grown seedlings, the highest amount could be detected at 72 h and represented 43% of that found after 66 h under continuous red light.

The stimulatory effect of light on accumulation of 23 kDa polypeptide mRNA, in particular the effectiveness of far-red light, which is not absorbed by chlorophyll, suggested the involvement of

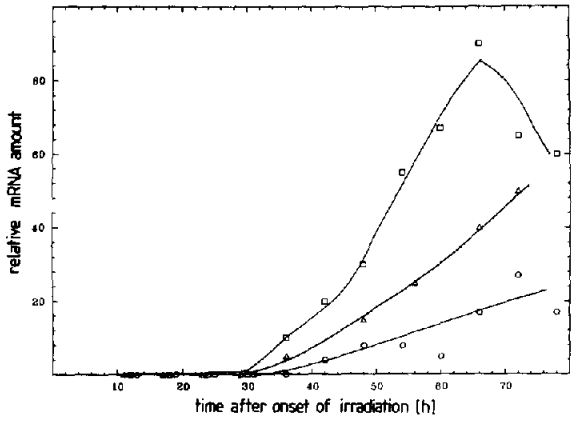

Fig.4. Time course of mRNA accumulation of the 23 kDa protein in mustard seedling cotyledons grown under continuous red (□), far-red (△) light and darkness (○).

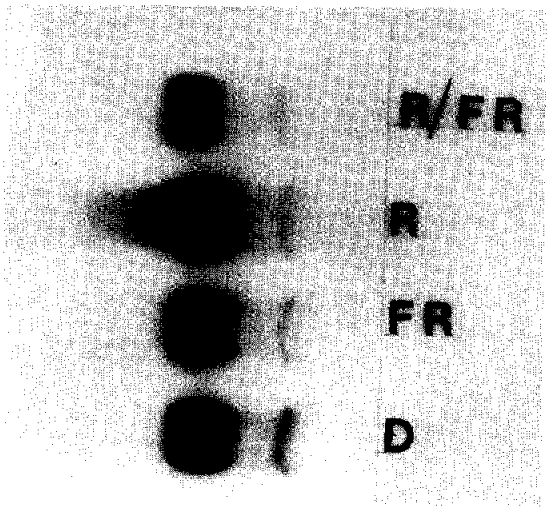

Fig.5. Effects of red and far-red light pulses on the 23 kDa protein mRNA level. Mustard seedlings were grown for 48 h in the dark and then subjected to different light regimes (5 min) as indicated at the top of each lane. After the light treatment, seedlings were returned for 12 h in darkness prior to harvesting cotyledons for mRNA extraction and Northern analysis.

phytochrome as the responsible photoreceptor. To test the involvement of phytochrome a red light pulse (establishing the maximal level of about 80% of phytochrome in the active Pfr form) was immediately followed by a reverting long-wavelength far-red light pulse (converting almost all Pfr back to Pr). Such reversion experiments are shown in fig.5. This reversion of the inductive red light pulse by the far-red light pulse indicates that the accumulation of mRNA encoding the 23 kDa polypeptide is under phytochrome control. Therefore, similarly to other peptides of the PS II complex, a temporal developmental pattern, a dependence on the 'plastidic factor' and on phytochrome could be demonstrated for accumulation of the mRNA encoding the 23 kDa polypeptide of the oxygen-evolving complex.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (SFB 206) and the Konrad Adenauer Stiftung.

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