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Circadian rhythm and phytochrome control of LHC-I gene transcription

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A small amount of translatable mRNA for a nuclear-coded precursor apoprotein of the LHC-I (light-harvesting complex of photosystem I) with a molecular mass of 26 kDa is present in etiolated bean leaves. The expression of this protein is phytochrome-controlled and follows circadian oscillations, for the appearance of which a red-light pulse is sufficient. The rhythmical oscillations persist for many hours in the dark following the red-light pulse or in continuous white light. The similarity of this rhythm in the light-induced accumulation of LHC-I mRNA with that of LHC-II mRNA [(1989) Plant Physiol. 90, 665] suggests that the same oscillator may govern the expression of all chloroplast protein genes regulated by light.

Light-harvesting complex; Photosystem I; Phytochrome; Circadian rhythm; Gene expression; Transcription, control of; mRNA

1. INTRODUCTION

Many physiological processes in higher plants are under the control of an endogenous circadian rhythm. In the last few years, diurnal fluctuations have been demonstrated in the steady state and translatable levels, as well as in the transcription rates, of several transcripts in plants grown under alternating 12 h light/12 h dark cycles: cabI, cabII, rbcS, rbcL, pSAD, psbA, psbB, psaA, OEEI [1–7]. The fluctuations persisted upon transferring the plants to constant light or dark, indicating control by an endogenous circadian clock of gene expression.

In our laboratory we have shown [8] that oscillations in the translatable and steady state levels of the LHC-II transcripts appear when etiolated bean leaves are transferred to the dark after a 2 min red light pulse (RL), or to continuous white light (CWL) or to the dark (D) after preexposure to CWL. The oscillations persisted for at least 70 h and had a period of about 24 h. These results indicate that no preadaptation of the plants to the 12 h light/12 h dark cycles is required for the oscillations of the LHC-II mRNA to appear, and that a 2 min RL pulse is sufficient to induce the fluctuations. In addition, we found [8] that the oscillator governing the fluctuations is synchronized by phytochrome. Rhythmical oscillations in the cabII and rbcS transcript levels have been also demonstrated recently in plants grown under CWL by Spiller et al. [4] and Nagy et al. [5].

In the present work, we study the light-induced accumulation of the translatable LHC-I mRNA, in an effort to see whether the fluctuations in the mRNA level under constant light conditions are a common phenomenon for all LHC proteins.

Little is known about the regulation of synthesis of the LHC-I apoproteins. It has been shown that, like the situation with the LHC-II apoprotein, they are nuclear-coded [3,9,10] and their 20 kDa subunit is synthesized in the cytoplasm as a higher molecular weight precursor [3]. The accumulation of both the 20 kDa polypeptide and its translatable mRNA have been also found to fluctuate in light/dark synchronized cells [3], while the diurnal fluctuations in the steady state levels and the transcription rates of cabI transcripts during

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growth of plants in 12 h light/12 h dark conditions, were found to persist after transfer of the plants to CWL or D [6].

We report here that a small amount of translatable LHC-I mRNA is present in etiolated bean leaves, that the light-induced increase in the amount of the translatable LHC-I mRNA is phytochrome-controlled, and that, similarly to the LHC-II mRNA, entrainment to light/dark cycles is not required for the light-induced rhythm in LHC-II mRNA level to be observed.

2. MATERIALS AND METHODS

Leaves of 5-day etiolated *Phaseolus vulgaris* plants (red kidney var.), after removal of one of their cotyledons, were placed in covered Petri dishes on moist filter paper and were exposed as before [8] (a) to a red light (RL) for 2 min (2 W/m^2) and/or far red light (F) for 5 min (3 W/m^2), and then kept in the dark (D), or (b) to CWL (8 W/m^2). At various time intervals leaves were removed from the remaining cotyledon and immersed in liquid nitrogen.

The poly(A) mRNA was prepared from leaves according to [11] and translated on a wheat germ cell-free system as in [8,12]. Aliquots of translation products, containing equal trichloroacetic acid-precipitable radioactivity, were used for immunoprecipitation as in [8] by an antibody raised against barley CPIa [13]. The immunoprecipitates were analyzed by SDS-slab-PAGE and detected by fluorography as in [14].

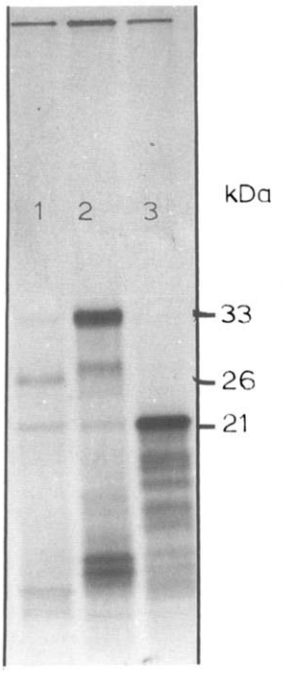

Fig.1. SDS-PAGE separation of immunoprecipitates from total in vitro translation products of bean poly(A) mRNAs with barley anti-CPIa (1), and pea anti-LHC-II (2), and anti-SSU (3). Aliquots of equal TCA-precipitable counts were used for immunoprecipitation, and the immunoprecipitates were loaded on the gel.

3. RESULTS

The antibody raised against the CPIa complex of barley, used in this study, has been shown to immunoprecipitate the 64–66 kDa polypeptides originating in the CPI-photosystem I P_{700}-core protein, and the 4 polypeptides (21–24 kDa) of the LHC-I antenna [13].

When this antibody preparation was added to the total in vitro translation products of the poly(A) mRNAs, isolated from the bean leaves, 3 polypeptides (33, 26 and 21 kDa) were mainly immunoprecipitated (fig.1). Of these, the most prominent band was that of the 26 kDa polypeptide. Taking into account the finding that the 20 kDa polypeptide of the LHC-I complex is synthesized in the cytoplasm, as a 24 kDa precursor [3], it is very likely that the 26 kDa immunoprecipitation product corresponds to the precursor of the 20–21 kDa LHC-I apoprotein. It should be noted that in bean the 21 kDa polypeptide is the main Chl-binding polypeptide of LHC-I [15].

The 33 kDa polypeptide is probably the LHC-II precursor apoprotein, since it has similar electrophoretic mobility on polyacrylamide gels (see fig.1) and is known to cross-react with this antibody preparation [13]. However, the possibility that it may also be the precursor protein of another polypeptide out of the 3 remaining of the LHC-I complex cannot be disregarded.

Finally, the 21 kDa polypeptide migrates exactly at the same position as the SSU precursor protein (see fig.1). We cannot, therefore, say whether this polypeptide belongs also to the LHC-I complex, or whether it is the SSU precursor coprecipitating with the 21 kDa LHC-I apoprotein via non-specific binding or cross-reaction. It has to be noted, however, that a large amount of translatable SSU mRNA is accumulated in the etiolated bean [8] as compared to the amount of the mRNAs coding for other proteins, which usually contaminates the immunoprecipitates from in vitro translation products.

We cannot explain why 4 polypeptides in the molecular mass range of 21–24 kDa are immunoprecipitated by the CPIa antibody from barley thylakoids, whereas only one out of them predominates in the immunoprecipitates produced by the same antibody preparation from the total in vitro translation products of bean poly(A) mRNAs. This may indicate that either some of the LHC-I apoproteins of bean are antigenically different from those of barley, or, most probably, that the 20/21 kDa mature polypeptide is the predominant one in the LHC-I complex of bean, as also suggested from earlier findings [15,16]. The possibility, finally, that the 26 kDa precursor protein is the only nuclear-coded LHC-I apoprotein in bean, in contrast to the other 3 LHC-I polypeptides which may be coded by the chloroplast genome, cannot be overruled.

As shown in figs 2 and 3, a small amount of the mRNA coding for the 26 kDa polypeptide (LHC-I mRNA) is present in bean leaves grown in the dark for 7 days. A 2 min RL pulse applied to 5-day-old etiolated bean leaves induces an increase in the amount of the LHC-I mRNA during the subsequent dark incubation for 48 h, as compared to the corresponding amount in non-illuminated plants, grown in the dark for the same period of time. A 5 min FR pulse, immediately following the RL pulse, prevents this increase. This indicates, therefore, that the light-induced increase in the amount of the translatable LHC-I mRNA is phytochrome controlled (fig.2).

The amount of the LHC-I mRNA accumulated in the 5 day old bean illuminated by the 2 min RL pulse and then transferred to the dark seems to oscillate. Thus, whereas the LHC-I mRNA level is low 36 h after the pulse, it increases during the following 12 h in the dark to reach a maximum level at 48 h. Thereafter, a minimum appears at about 60 h after the RL pulse, increasing again 12 h later. The amount of the translatable LHC-I mRNA, therefore, follows a rhythmical pattern with a period of about 24 h (fig.2).

Similar oscillations in the light-induced LHC-I mRNA accumulation appear when 5 day old etiolated bean leaves are transferred to continuous white light (fig.3). As shown, the relatively large amount of LHC-I mRNA accumulated initially (during the first 6 h of continuous illumination) is reduced 6 h later (12 h after the onset of illumina-

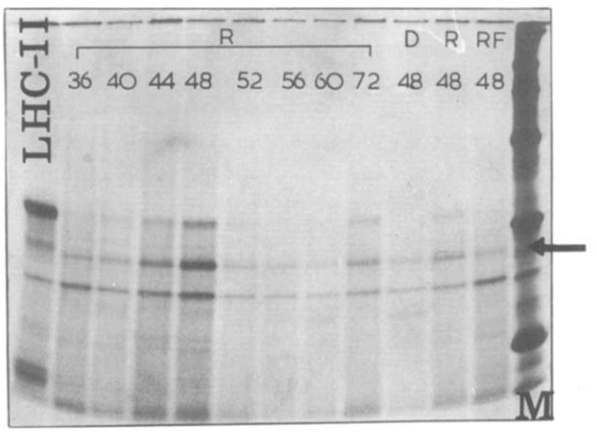

Fig.2. SDS-PAGE separation of immunoprecipitates from total in vitro translation products of poly(A) mRNAs with anti-CPIa. Poly(A) mRNAs were isolated from etiolated bean leaves exposed to a 2 min RL pulse (R), or to a 2 min RL pulse followed by a 5 min FR light (RF), and then transferred to the dark for 36 (36) to 72 h (72). Immunoprecipitation and SDS-PAGE as in fig.1. D, 5-day etiolated plant that remained in the dark for an additional 48 h period; LHC-II, immunoprecipitate with anti-LHC-II; M, methylated ^{14}C-molecular weight marker proteins. The arrow shows the 26 kDa precursor protein of LHC-I.

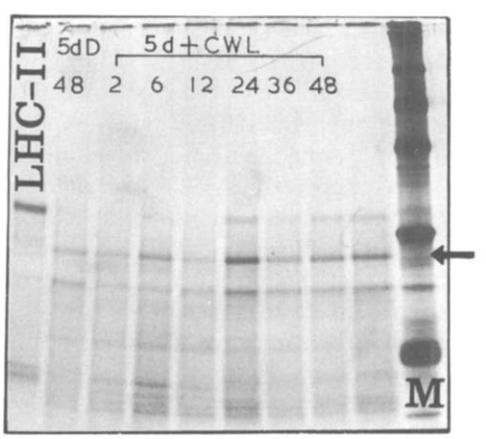

Fig.3. SDS-PAGE separation of immunoprecipitates with anti-CPIa from total in vitro translation products of poly(A) mRNAs isolated from 5 day old etiolated bean leaves exposed to CWL for 2–48 h (2–48), or to the dark (D) for 48 additional hours. Aliquots of translation products containing equal trichloroacetic acid-precipitable counts were used for immunoprecipitation and SDS-PAGE. LHC-II, immunoprecipitate with anti-LHC-II; M, methylated ^{14}C-marked protein. The arrow shows the 26 kDa precursor protein of LHC-I.

tion), it increases again to reach a high level 24 h after the onset of illumination and it again reduced during the following 12 h. The oscillations continue up to about 72 h that we have tested.

It is clear, therefore, that the LHC-I mRNA oscillates following an endogenous circadian rhythm, in a way similar to that of the LHC-II apoprotein [8].

4. DISCUSSION

The results shown above indicate that similarly to the situation found with LHC-II mRNA, the light-induced accumulation of the LHC-I mRNA is phytochrome-controlled, and fluctuates when etiolated bean leaves are transferred to CWL, or to darkness following a 2 min RL pulse. Previous entrainment of the plants to regular alternation of day–night conditions, therefore, is not required for the fluctuations to appear, suggesting that the mechanism constituting the biological clock is endogenous. Furthermore, a 2 min RL pulse seems sufficient for the appearance of the rhythm, the day/night alternation being probably required to 'correct' the clock. The similarity of the fluctuations in the light-induced accumulation of LHC-I mRNA to that of LHC-II, suggests that the same oscillator governs the expression of all chloroplast protein genes regulated by light. Taking into account our earlier findings that the oscillator governing the rhythm in LHC-II gene transcription is synchronized by phytochrome, we assume that a similar situation prevails in LHC-I transcription. It is difficult, however, to say how the phytochrome photoreceptor protein is involved in the synchronization of the clock, nor what is the physiological signal and transduction chain induced by phytochrome resulting in this synchronization. The elucidation of this mechanism must await further experimentation.

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