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Photoregulation of Chloroplast Development: Transcriptional, Translational and Post-Translational Controls? [and Discussion]

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Photoregulation of chloroplast development: transcriptional, translational and post-translational controls?

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Chloroplast development involves the nucleus, the cytoplasm and the chloroplast of plant cells. This may be illustrated by reference to the two most abundant proteins of the chloroplast: (i) the soluble CO_2-fixing enzyme ribulose 1,5-bisphosphate carboxylase–oxygenase, whose large subunit (LSU) is encoded in chloroplast DNA and synthesized on chloroplast ribosomes and whose small subunit (SSU) is encoded in nuclear DNA, synthesized on cytoplasmic ribosomes in precursor form and transported into chloroplasts, and (ii) the thylakoid-bound light-harvesting chlorophyll *a*/*b* complex, whose pigment components are synthesized in the chloroplast and whose apoproteins resemble the SSU in site of coding and site of synthesis. We have examined the extent to which biosynthetic events in the nucleocytoplasmic compartments are coordinated with those inside the chloroplast during the de-etiolation of pea seedlings. We have examined the levels of LSU, SSU and the light-harvesting chlorophyll *a*/*b* protein (LHCP) by using a highly specific radioimmune assay. The steady-state levels of the corresponding mRNAs have been determined using specific cloned DNA probes. With the SSU, the mRNA and protein levels are near the limit of detection in dark-grown plants but increase markedly under continuous white light, with a lag of about 24 h. The protein appears to be under simple phytochrome control at the level of the steady-state concentration of its mRNA. The LSU also appears to be regulated through the steady-state concentration of its mRNA but in this case the mRNA is not under simple phytochrome control. The LHCP mRNA is readily detectable in dark-grown plants and accumulates further under illumination in a phytochrome-mediated manner. However, the LHCP itself (like chlorophyll) is not detectable in dark-grown plants and accumulates to high levels only under continuous illumination, with a lag of about 6 h. Post-translational control is particularly important in the accumulation of the LHCP: continuous chlorophyll synthesis is required for the stabilization of the protein within the thylakoid membrane, at least during the early stages of chloroplast development.

Introduction

In most angiosperms, chloroplast development goes to completion only in the light (Thomson & Whatley 1980). Two photoreceptors have been implicated unambiguously in this phenomenon. They are phytochrome (Mohr 1977), which exists in two forms, P_r and P_{fr}, absorbing principally red and far-red light respectively, and protochlorophyllide (Boardman *et al.* 1978) which absorbs principally in the blue and red regions of the spectrum. There is also evidence for a third photoreceptor that is responsive to blue light (Senger 1982).

Protochlorophyllide regulates the synthesis of chlorophyll and the accumulation of chlorophyll-binding proteins. Like all the other steps in the biosynthesis of chlorophyll, the reduction of protochlorophyllide to chlorophyllide by the NADPH-linked enzyme protochlorophyllide reductase (PCR) occurs in the chloroplast. In most angiosperms, but not in all (Adamson &

Hiller 1981), this enzyme is light-dependent. The conversion of the ternary complex NADPH–PCR–protochlorophyllide to NADP–PCR–chlorophyllide requires the absorption of a photon by the protochlorophyllide molecule (Griffiths 1978). No other step in the chlorophyll biosynthetic pathway, including the esterification of chlorophyllide to chlorophyll *a* and the oxidation of the latter to chlorophyll *b*, is directly light-dependent. Nevertheless, the light requirement of the PCR reaction means that continuous chlorophyll synthesis requires essentially continuous illumination. Continuous accumulation of certain chlorophyll-binding proteins also requires continuous illumination (Bennett 1983).

TABLE 1. CHLOROPLAST PROTEINS WHOSE mRNAs ARE KNOWN TO BE REGULATED BY PHYTOCHROME

protein	site of synthesis	effect of red light pulse on mRNA level	references
32 kDa thylakoid protein	chloroplast	increase	1
small subunit of RuBP carboxylase–oxygenase	cytoplasm	increase	2
light-harvesting Chl *a*/*b* protein	cytoplasm	increase	2, 3
protochlorophyllide reductase	cytoplasm	decrease	4

References: 1, Link (1982); 2, Tobin (1981*a*); 3, Apel (1979); 4, Apel (1981).

Phytochrome regulates a wider range of events in chloroplast development than protochlorophyllide but the details of its mechanism of action are less well understood. However, it is clear that phytochrome controls the level of certain mRNAs involved in chloroplast development. The first direct evidence for the regulation of leaf mRNA levels by light was provided by Tobin & Klein (1975), who used *in vitro* cell-free translation systems to show that light increased the levels of the mRNAs for certain unidentified proteins. Subsequently, the two most abundant leaf mRNAs under phytochrome control have been identified as two chloroplast polypeptides: (i) the small subunit (SSU) of the soluble CO_2-fixing enzyme ribulose 1,5-bisphosphate (RuBP) carboxylase–oxygenase (Tobin 1981*a*) and (ii) the light-harvesting chlorophyll *a*/*b* protein (LHCP), the most abundant chlorophyll-binding protein of the thylakoid membrane (Apel & Kloppstech 1978; Apel 1979; Tobin 1981*a*).

The levels of several mRNAs for chloroplast proteins have now been shown to be controlled by phytochrome (table 1). In each case, the involvement of phytochrome was established by the classical red–far-red reversibility test. Because P_r is converted to P_{fr} by red light and P_{fr} is converted back to P_r by far-red light (Mohr 1977), any physiological response dependent on P_{fr} will, in principle, be elicited by red light but not by far-red light; indeed, far-red light, if administered sufficiently quickly after red light will prevent the expression of the response to red light. With the exception of the mRNA for PCR, a pulse of red light was found to increase the levels of the mRNAs listed in table 1; the level of PCR mRNA was decreased by a pulse of red light.

Three of the mRNAs listed in table 1 (those for LHCP, SSU and PCR) are found in the polyadenylated mRNA fraction and are presumed to be cytoplasmic mRNAs that have been transcribed from nuclear genes. For LHCP mRNA and SSU mRNA, there is direct evidence for the nuclear location of the corresponding genes (Gallagher & Ellis 1982) and for translation of the mRNAs on cytoplasmic ribosomes (Ellis 1981). One of the mRNAs listed in table 1, the mRNA coding for a 32 kDa thylakoid protein, is encoded (Bedbrook *et al.* 1978) and

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translated (Eaglesham & Ellis 1974) in the chloroplast. Thus, unlike protochlorophyllide, which exerts its effects entirely within the chloroplast, phytochrome regulates aspects of chloroplast development occurring in the nucleus, the cytoplasm and the chloroplast itself.

Another difference between the two photoreceptors is that whereas the effects of the excitation of protochlorophyllide on chlorophyll synthesis persist in darkness only for as long as it takes to convert chlorophyllide to chlorophyll *a* and chlorophyll *b* (a matter of minutes), the effects of the formation of P_{fr} from P_r by a brief pulse of red light on the levels of certain mRNAs may persist (and indeed may not be apparent) for many hours. This difference is due

TABLE 2. SUMMARY OF PROCEDURE USED TO ASSAY SINGLE CHLOROPLAST PROTEINS IN TOTAL LEAF EXTRACTS

(BSA, bovine serum albumin; protein A, immunoglobulin G-binding protein from *Staphylococcus aureus*. For details see Vaessen *et al.* (1981).)

(1) extract leaves with sodium dodecyl sulphate
(2) perform sodium dodecyl sulphate polyacrylamide gel electrophoresis of proteins
(3) transfer proteins to nitrocellulose (NC) by electrophoresis
(4) soak NC with BSA
(5) soak NC with antibody
(6) wash NC
(7) soak NC with ^{125}I-labelled protein A
(8) wash NC
(9) radioautograph

to the stoichiometric involvement of chlorophyllide in chlorophyll synthesis compared with the essentially catalytic role of P_{fr}. One consequence of this difference is that whereas the maximal protochlorophyllide-mediated effects of light on chlorophyll synthesis are seen only under essentially continuous illumination, the phytochrome-mediated effects of light are often nearly maximal under intermittent brief pulses of light separated by long dark periods (e.g. 2 min of white light every 2 h). Indeed, Apel (1979) has shown that the rate of accumulation of translatable polyadenylated LHCP mRNA is the same for several hours whether the barley plants are exposed to continuous illumination with white light or exposed to a single 15 s pulse of red light followed by darkness. However, after about 8 h the level of LHCP mRNA begins to fall in the plants returned to darkness but continues to rise under continuous illumination.

We have addressed ourselves to two major questions that arise from the above results. Firstly, how is the synthesis of the LHCP coordinated with that of chlorophyll *a* and chlorophyll *b*, when the apoprotein is synthesized in the nucleocytoplasmic compartment under the control of phytochrome and the pigments are synthesized in the chloroplast under the control of protochlorophyllide? (Note that in posing the question in this way, we are not forgetting that the maximal flux through the chlorophyll biosynthetic pathway under light that is saturating for protochlorophyllide reduction is regulated by phytochrome; see Mohr (1977).)

Secondly, in the synthesis of RuBP carboxylase–oxygenase, how is the synthesis of the SSU, which is encoded in the nucleus and synthesized in the cytoplasm (Ellis 1981), coordinated with that of the large subunit (LSU), which is encoded and synthesized in the chloroplast (Blair & Ellis 1973; Coen *et al.* 1977)? Does phytochrome regulate the synthesis of the LSU? To answer these questions, we have determined the levels of LHCP, LSU and SSU and the levels of their respective mRNAs in pea plants grown under various light regimes. The proteins have been detected by the procedure outlined in table 2, based on the immunological method of

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Vaessen *et al.* (1981). The levels of the mRNAs have been determined by hybridization of ^{32}P-labelled cloned DNA probes to leaf mRNA transferred by blotting from agarose gels to nitrocellulose sheets or deposited directly as dots onto nitrocellulose sheets under a slight vacuum.

The accumulation of chloroplast proteins and their messenger RNA molecules during greening

When etiolated pea seedlings 8 days old are transferred to continuous white light (photon fluence rate 100 μmol m^{-2} s^{-1}), chlorophyll accumulation is initially slow but accelerates until, after about 48 h, it is very rapid (figure 1). Whereas chlorophyll *a* is formed from the beginning

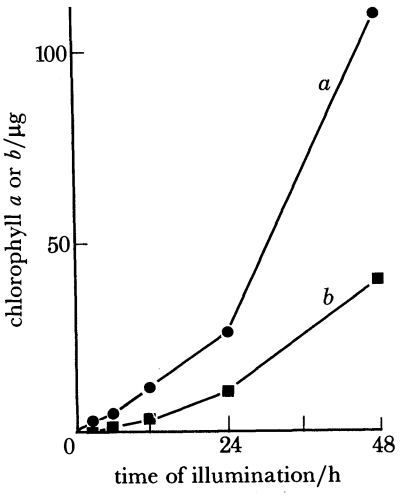

Figure 1. Chlorophyll *a* and chlorophyll *b* contents of apical buds during de-etiolation of pea seedlings 8 days old, under continuous white light. Results expressed per bud.

of the light period, chlorophyll *b* is detectable only after about 6 h. Because most of the chlorophyll *b* and about one-third of the chlorophyll *a* of the photosynthetic membrane occur in the form of the light-harvesting chlorophyll *a*/*b* complex (Bennett 1983), it would be expected that the accumulation of the apoprotein of this complex, i.e. LHCP, would follow a similar time course to that of chlorophyll *b*. That this is so is shown in figure 2, where the LHCP, LSU and SSU contents of the apical buds of pea seedlings are shown during the first 48 h of de-etiolation. Note that all three polypeptides are at or below the limits of detection in plants grown in the dark for 8 or 10 days. The LHCP becomes detectable after about 6 h of illumination, whereas the SSU becomes detectable only after 24 h. The LSU is also detectable after about 6 h but, unlike the LHCP, the LSU remains at very low levels until the SSU begins to accumulate. These results indicate that the kinetics of accumulation of LHCP, LSU and SSU are different.

What is the mechanism that enables the LHCP to accumulate to comparatively high levels before the subunits of RuBP carboxylase–oxygenase? The prior appearance of the LHCP has already been observed in expanding barley leaves by Viro & Kloppstech (1980). As with other monocots, the expanding leaves of barley display at any one time a gradient of chloroplast

development along the blade, with the least mature chloroplasts at the base and the most mature at the tip. The relative abundance of LHCP compared with RuBP carboxylase–oxygenase is higher at the base of the leaf than at the tip. Viro & Kloppstech (1980) have shown that this result can probably be explained in terms of the levels of the corresponding mRNAs. Thus the LHCP mRNA was more abundant than the SSU mRNA near the base of the leaf but less abundant towards the tip. The mRNAs were assayed by *in vitro* translation–immunoprecipitation.

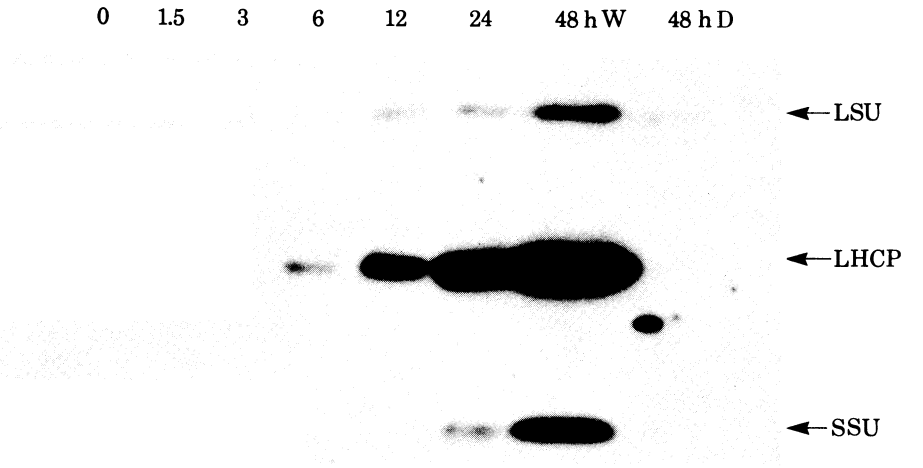

FIGURE 2. Accumulation of LSU, SSU and LHCP during de-etiolation of pea seedlings. Apical buds were harvested after 8 or 10 days of etiolation, or after 8 days of etiolation followed by 1.5–48 h of exposure to white light (W) (100 μmol m^{-2} s^{-1}). Samples corresponding to the sodium dodecyl sulphate extract of one-fiftieth of an apical bud were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis and analysed by the procedure outlined in table 2. The radioautogram shows the position and relative concentrations on the nitrocellulose sheet of the ternary complexes involving LSU (or SSU or LHCP), antibody and ^{125}I-labelled protein A.

We have observed an equivalent *temporal* change in the relative abundance of LHCP mRNA and SSU mRNA during the de-etiolation of pea seedlings. Figure 3 shows the results obtained when total RNA (20 μg) from the apical buds of pea seedlings was fractionated by agarose gel electrophoresis, transferred by blotting to sheets of nitrocellose and then hybridized with ^{32}P-labelled copy DNA (cDNA) probes complementary to SSU mRNA and LHCP mRNA. Each probe became hybridized to a single region of the filter where the corresponding mRNA was located. The level of SSU mRNA increases steadily from an initial value (in etiolated plants) that is at or below the level of detection to a very high level after 6 days of illumination. Control plants maintained in darkness fail to accumulate detectable levels of the mRNA over the same period. In contrast, the LHCP mRNA is readily detectable in dark-grown plants and accumulates further on illumination.

The results in figure 3 provide a direct visual comparison between the proportional abundances of SSU mRNA and LHCP mRNA in different RNA samples. It is clear from these results that the relative abundance of LHCP mRNA remains relatively constant between 2 and 6 days after the start of illumination, whereas the relative abundance of SSU mRNA increases steadily during this period. Although these data establish that the ratio of SSU mRNA to LHCP mRNA increases during de-etiolation, they should not be interpreted to indicate that LHCP

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mRNA accumulation is restricted to the first 2 days of illumination. The data in figure 3 underestimate the *absolute* increase in LHCP mRNA and SSU mRNA during de-etiolation because the total RNA content of the leaves begins to increase between 24 and 48 h after the start of illumination (data not shown).

The presence of LHCP mRNA and the absence of SSU mRNA in dark-grown plants together provide an explanation for the fact that the LHCP appears more rapidly than SSU during

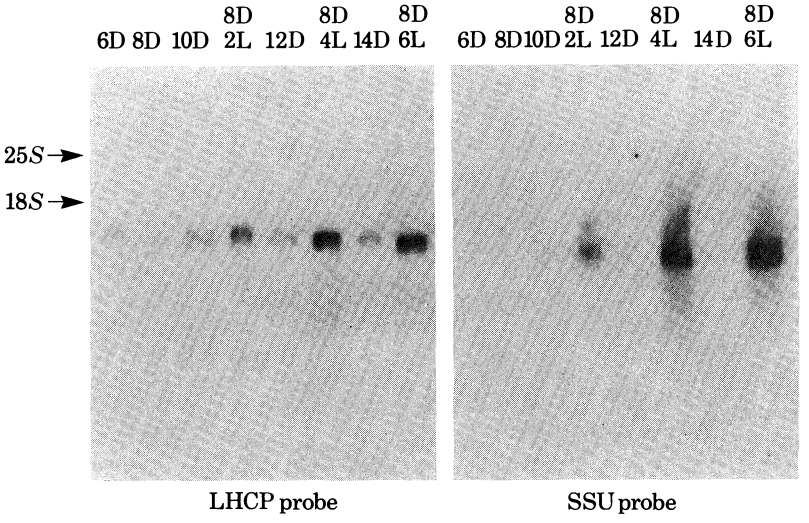

FIGURE 3. Light-dependent accumulation of SSU mRNA and light-stimulated accumulation of LHCP mRNA during de-etiolation of pea seedlings. Peas were grown in the dark (D) for the times indicated (in days), or transferred into continuous illumination with white light (L) after 8 days. Total RNA was extracted from the apical buds and 20 μg of each sample was fractionated in agarose-formamide gels. The RNA was blotted onto nitrocellulose sheets and hybridized with ^{32}P-labelled nick-translated SSU plasmid (pSSU 160) or LHCP plasmid (pFab 31) (radioactive count of each was 10^7 min^{-1}). See Gallagher & Ellis (1982) for a description of the origins of the clones. The nitrocellulose sheets were washed, dried and radioautographed.

de-etiolation (figure 2). However, the presence of LHCP mRNA in etiolated peas warrants some comment. Although it confirms the result obtained by Cuming & Bennett (1981) when LHCP mRNA was assayed by *in vitro* translation of polyadenylated mRNA followed by immunoprecipitation, it runs contrary to the results of Apel & Kloppstech (1978) and Apel (1979), who could not detect LHCP mRNA in barley leaves by translation *in vitro* and immunoprecpitation. This difference should alert us to the possibility that different plants may show somewhat different patterns of photoregulation of gene expression.

A CRITICAL ASSESSMENT OF MESSENGER RNA ASSAYS

In the assay of mRNA levels, RNA blots such as those shown in figure 3 are superior to *in vitro* translation–immunoprecipitation assays in several respects. Firstly, RNA blots are performed on total RNA and permit the detection of total mRNA levels, whereas translation assays have in the past usually been performed only on polyadenylated mRNA. Secondly, RNA blots provide a built-in check on the degree of mRNA breakdown in the samples. Thirdly, RNA blots do not suffer from the criticism sometimes made of translation assays (but never to our knowledge substantiated for plant mRNA) that translation assays will not detect mRNA

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sequences that have been chemically modified *in vivo* to render them untranslatable. However, RNA blots have several disadvantages: firstly, it is tedious to perform replicated assays on large numbers of samples; secondly, transfer from agarose gel to nitrocellulose sheet is rarely quantitative; and thirdly, in our hands at least, small to moderate differences in the levels of a mRNA between samples cannot be measured reliably. RNA blots are best suited to the study of large differences in mRNA levels and are the best means of determining whether a DNA probe is hybridizing to a single RNA species.

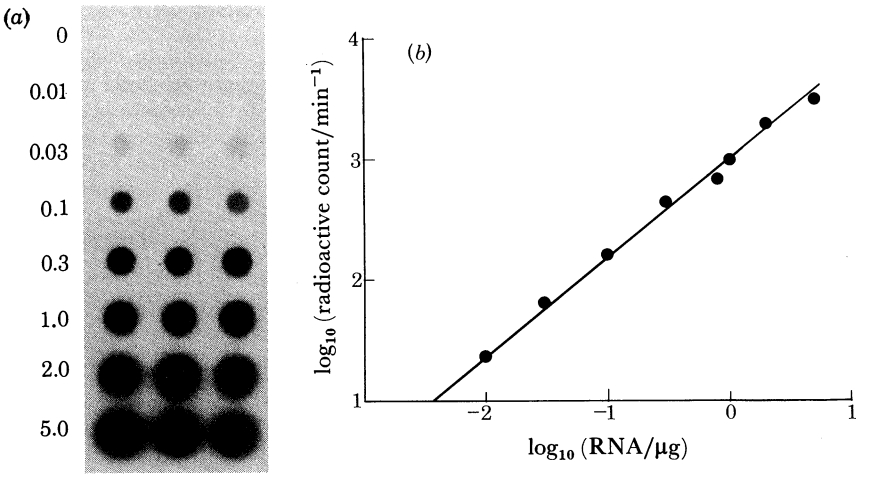

FIGURE 4. Assay of specific mRNAs by dot blotting. Total leaf RNA from plants exposed to 48 h of continuous white light was applied in the indicated quantities in 2.25 M NaCl, 0.225 M trisodium citrate, pH 7.0, to a nitrocellulose sheet held under a slight vacuum in a Perspex manifold. The LHCP mRNA content of each RNA dot was determined by hybridization of ^{32}P-labelled nick-translated LHCP probe (see description of table 3 for details). (*a*) Radioautogram of ^{32}P-labelled hybrids, showing triplication. Total RNA applied (micrograms) is shown on the left. (*b*) Double-logarithmic plot of ^{32}P label in hybrids as a function of total RNA content loaded per dot.

In view of the disadvantages of *in vitro* translation–immunoprecipitation assays and RNA blots, we have had recourse to RNA dot–blots. In this procedure, total RNA in 2.25 M NaCl, 0.225 M trisodium citrate, pH 7.0, is loaded in triplicate on to a nitrocellulose sheet by means of a manifold that applies a slight vacuum to the underside of the sheet. After the RNA is baked onto the sheet, the latter is immersed in a solution containing ^{32}P-labelled DNA probe. After hybridization and washing, the filter is radioautographed to obtain a visual record on the degree of hybridization of the probe to each dot of RNA. The dots are then cut from the nitrocellulose sheet and counted for ^{32}P by scintillation spectrometry to obtain a numerical record of the degree of DNA–RNA hybridization. Figure 4 shows both types of record obtained with a series of concentrations of an RNA sample extracted from pea seedlings that had been de-etiolated under continuous white light for 48 h. The probe is cDNA complementary to LHCP mRNA. The double-logarithmic plot of the numerical data is linear over the entire concentration range from 0.01 to 5 μg of total RNA per dot. Replication is excellent. Routinely, we load 0.8 μg of RNA per dot in these assays. This ensures that high sensitivity is achieved for samples containing little of the mRNA of interest without the risk of overloading the dot with RNA.

Does phytochrome regulate the levels of mRNAs for chloroplast proteins?

We have used the dot–blot assay to determine whether the light-dependent increases in LHCP mRNA and SSU mRNA during de-etiolation of peas (figure 3) are under phytochrome control (table 3). The test for phytochrome involvement is the classical red–far-red reversibility test referred to above.

Table 3. Photoregulation of mRNAs for SSU and LHCP

(Pea plants were grown in darkness for 8 days and then (*a*) transferred to white light 100 μmol m^{-2} s^{-1}) for 48 h, (*b*) maintained in darkness for 48 h, or (*c*) irradiated as described with red light (15 μmol m^{-2} s^{-1}) or far-red light (8 μmol m^{-2} s^{-1}), or both, and then returned to darkness for 48 h. Triplicate samples of total RNA (0.8 μg) of apical buds were applied to nitrocellulose sheets in 2.25 M NaCl, 0.225 M trisodium citrate, pH 7.0, under slight vacuum. Filters were baked at 80 °C for 2 h *in vacuo* and hybridized with ^{32}P-labelled cDNA probes for either SSU or LHCP mRNAs at 42 °C for 48 h. After being washed at 60 °C in 15 mM NaCl, 1.5 mM trisodium citrate, pH 7.0, the filters were radioautographed and the individual dots excised for scintillation spectrometry. The results show radioactive count per minute ± standard error of triplicates.)

	hybridization of cDNA probes			
	SSU		LHCP	
light treatment	^{32}P	percentage	^{32}P	percentage
48 h white	2211 ± 76	100	695 ± 42	100
48 h dark	29 ± 2	1.3	128 ± 5	18
15 min red, 48 h dark	215 ± 4	9.7	403 ± 2	58
15 min far-red, 48 h dark	85 ± 1	3.8	239 ± 16	34
15 min red, 15 min far-red, 48 h dark	78 ± 4	3.5	223 ± 1	32

For SSU mRNA, there is only a very low signal for the RNA extracted from dark-grown peas but the signal for RNA extracted from plants exposed to 15 min of red light followed by 48 h of darkness is much higher (and about 10% of that for plants exposed to continuous white light for 48 h). In contrast, a 15 min pulse of far-red light, either by itself or immediately after a 15 min pulse of red light, results in relatively little production of SSU mRNA. Thus the level of SSU mRNA in peas shows classical far-red light reversibility of red light induction and is concluded to be under phytochrome control. This agrees with the conclusion reached by Tobin (1981*a*) for the SSU mRNA of *Lemna gibba*.

Smith & Ellis (1981) found a substantial difference in the levels of hybridizable LSU mRNA between light-grown and dark-grown peas, and Shinozaki *et al.* (1982) observed a large increase in the level of translatable LSU mRNA during de-etiolation of pea plants. We have found (data not shown) that LSU mRNA is readily detectable in etiolated plants and that its concentration increases markedly in response to continuous white light and to a lesser extent in response to a brief pulse of red or far-red light. As yet we are unable to say whether red or far-red light is the more effective in inducing LSU mRNA. For LSU mRNA of *Sinapis alba*, Link (1982) provides data indicating that far-red light induces a higher level of the mRNA than red light. Considerably more work is required before the photoregulation of LSU mRNA levels can be understood, but there are preliminary indications that at least in white mustard (Link 1982) the steady-state level of this mRNA is not under simple phytochrome control. It is possible that phytochrome is not involved directly in the transcription of the genes for LSU in the chloroplast. Note that the presence of LSU mRNA in etiolated pea accords with the synthesis of LSU by isolated intact etioplasts of pea (Siddell & Ellis 1975).

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Table 3 confirms the result presented earlier (figure 3) (see also Cuming & Bennett 1981) to the effect that the level of LHCP mRNA found in etiolated peas is comparatively large (about 18% of the value found for plants exposed to continuous white light for 48 h). When etiolated peas are exposed to a 15 min pulse of red light and then returned to darkness for 48 h, the level of LHCP mRNA increases substantially above the dark level and approaches that obtained under continuous illumination. The inductive effect of red light is significantly reduced by an immediately subsequent pulse of far-red light, indicating that the steady-state level of LHCP mRNA is also under phytochrome control in pea, albeit different in detail from that observed for SSU mRNA. Phytochrome control of the steady-state LHCP mRNA level has also been reported for barley (Apel 1979) and for *Lemna gibba* (Tobin 1981*a*).

Does phytochrome regulate the levels of chloroplast proteins?

Figure 5 shows the levels of LHCP, LSU and SSU in pea plants exposed to various light régimes. From these results, it is clear that the level of SSU, like that of its mRNA, is under phytochrome control, with classical red–far-red reversibility. Note that the level of SSU generated in response to a single 15 min pulse of red light is lower than that produced in response to continuous white light. Thus the level of SSU in peas appears to be determined primarily by the steady-state level of the corresponding mRNA.

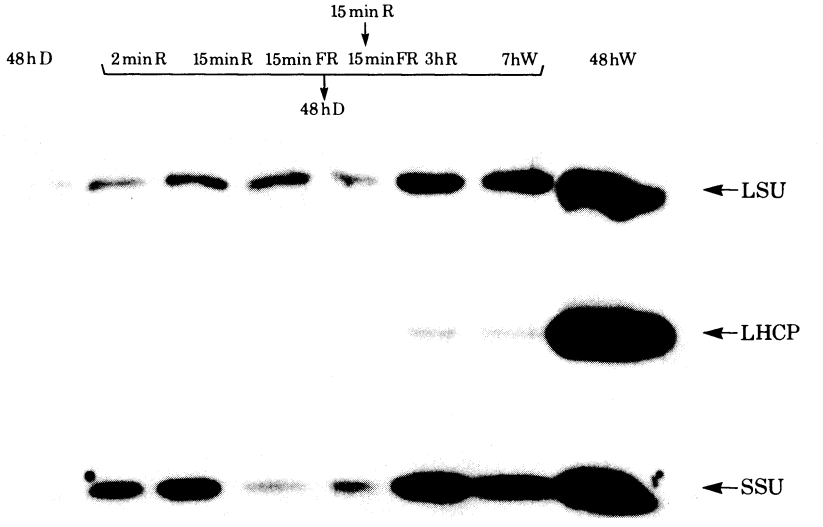

Figure 5. Investigation of the involvement of phytochrome in the accumulation of LSU, SSU and LHCP during de-etiolation of pea seedlings. See description of table 3 for a description of plant growth and illumination. See table 2 and figure 2 for details of the radioimmune assay.

The photocontrols on the LSU and LHCP are different from those on the SSU. LSU is induced approximately equally by red and far-red light. This result is in accord with that for the LSU mRNA and indicates that although the level of the LSU is not under simple phytochrome control, it does appear to be determined primarily by the steady-state level of its mRNA, at least in the situation studied here.

The LHCP is also not under simple phytochrome control. Although LHCP mRNA is partly dependent on phytochrome for its accumulation (table 3), it is clear from figures 2 and 5 that

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LHCP accumulates under continuous illumination but not in dark-grown plants or in plants exposed to light and then returned to darkness, even though in both situations the leaves contain readily detectable levels of LHCP mRNA. There are in fact several situations in which LHCP fails to accumulate in leaves containing the corresponding mRNA. In some cases the LHCP is known to be unstable, and in others instability has been inferred (Bennett 1983). Thus when etiolated pea leaves are de-etiolated for 16 h and then returned to darkness (Bennett 1981), or *Lemna gibba* is subjected in darkness to pulse-chase labelling with L-[^{35}S]methionine (Slovin & Tobin 1982) or bean plants are returned to darkness after a brief period of illumination (Argyroudi-Akoyunoglou *et al.* 1982), LHCP has been shown to break down.

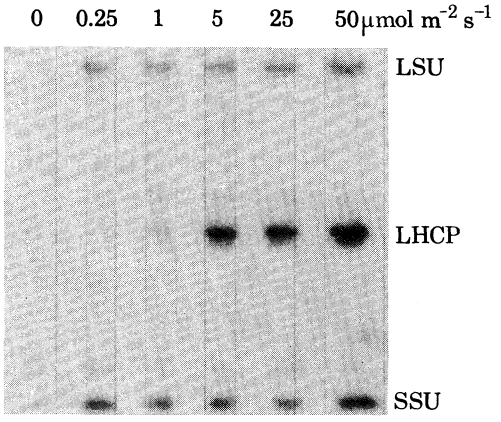

FIGURE 6. Effect of photon fluence rate on the accumulation of LSU, SSU and LHCP during de-etiolation of pea seedlings 8 days old. Plants were exposed to white light of the indicated fluence rates for 48 h before harvest. See table 2 and figure 2 for details of the radioimmune assay.

Bennett (1981) has suggested that LHCP is stabilized against breakdown by some event that occurs during chloroplast maturation. Although the synthesis of chlorophyll *a* and chlorophyll *b* is essential for the stabilization process, it is not sufficient. This point is established by the breakdown of pre-existing LHCP when pea (Bennett 1981), bean (Argyroudi-Akoyunoglou *et al.* 1982) or radish (Lichtenthaler *et al.* 1981) seedlings are placed in darkness. The stabilization of LHCP occurs under continuous illumination (and to a lesser extent in plants exposed to day–night cycles). What irradiance is required for LHCP stabilization?

Our pea plants are routinely grown under warm white fluorescent tubes. The photon fluence rate at the level of the plants is about 50 μmol m^{-2} s^{-1}, compared with about 2000 μmol m^{-2} s^{-1} for full sunlight. When we exposed etiolated pea plants 8 days old to white light of fluence rates in the range 0.25–50 μmol m^{-2} s^{-1} for 2 days and then assayed them for LHCP, LSU and SSU, the results shown in figure 6 were obtained. Dark-grown plants contained little if any of these polypeptides, but plants exposed to even the lowest fluence rate contained both LSU and SSU. Furthermore, the levels of the subunits of RuBC carboxylase–oxygenase were approximately constant over the entire range from 0.25 to 50 μmol m^{-2} s^{-1}. In contrast, only plants exposed to 5–50 μmol m^{-2} s^{-1} contained substantial levels of LHCP. At these photon fluence rates the chloroplasts were clearly able to mature to such a stage that the LHCP could be stabilized. Plants exposed to 1 μmol m^{-2} s^{-1} contained only a trace of LHCP, while plants exposed to 0.25 μmol m^{-2} s^{-1} contained no detectable LHCP. These results provide a new example of a situation where plants contain LHCP mRNA but fail to accumulate the protein.

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Concluding remarks

We have studied the photoregulation in pea seedlings of the accumulation of the three most abundant chloroplast polypeptides (namely LSU, SSU and LHCP). Only the SSU appears to be under simple phytochrome control and the data presented above indicate that the level of the polypeptide is determined largely by the level of the corresponding mRNA. The LSU also appears to be regulated largely at the level of the steady-state concentration of its mRNA but in this case the mRNA does not appear to be under simple phytochrome control.

Our present data do not provide any special insight into the mechanisms whereby the levels of the two RuBP carboxylase–oxygenase subunits are coordinated. Although tight coordination has been reported for the LSU and SSU during the cell cycle of *Chlamydomonas reinhardtii* (Iwanij *et al.* 1975), the coordination appears to be rather looser in soybean (Barraclough & Ellis 1979). Our protein assays indicate that in greening pea seedlings, the LSU and SSU accumulate approximately in parallel, but the photocontrols on the two mRNAs are not identical. Thus, for example, LSU mRNA is readily detectable in the dark whereas that for the SSU is not. Furthermore, both the SSU and its mRNA are more abundant after a pulse of red light than after a pulse of far-red light; in contrast, for both the LSU and its mRNA, there appears to be little difference in the inductive abilities of red and far-red light.

The LHCP mRNA is under phytochrome control in pea, as in *Lemna* (Tobin 1981*a*) and barley (Apel 1979). However, this mRNA is present in dark-grown plants to a considerable extent (as much as 18% of the mRNA found in continuously illuminated plants). This is in marked contrast with barley, where LHCP mRNA is undetectable in etiolated plants (Apel 1979), and with *Lemna*, where LHCP mRNA is very rapidly degraded when green fronds are placed in darkness (Tobin 1981*b*). Clearly, the photocontrols on a given mRNA may differ among plant species.

The absence of the LHCP from dark-grown peas, which nevertheless contain LHCP mRNA, could in principle be due to translational or post-translational controls. Giles *et al.* (1977) and Slovin & Tobin (1982) have provided evidence consistent with translational controls on LHCP mRNA. The 32 kDa translation product detected by Giles *et al.* (1977) is probably the precursor of the LHCP. However, Bennett (1981), Cuming & Bennett (1981) and other authors have argued in favour of post-translational control through the stabilization of LHCP by continuous chlorophyll synthesis. According to the latter view, the accumulation of LHCP is under dual photocontrol via both phytochrome and protochlorophyllide. Further work will be necessary to resolve this question.

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Discussion

J. W. Bradbeer (*Department of Plant Sciences, King's College London, U.K.*). It is important to recognize that the morphological complexity of the greening pea shoot causes some difficulty in understanding chloroplast development in this system. Leaf development in etiolated peas is arrested at an early stage and the etiolated pea shoot consists largely of stem tissue. On illumination, leaves develop from small leaf initials so that the long lag periods found for the increases in the mRNAs coding for chloroplast polypeptides are not unexpected.

J. Bennett. The immaturity of etiolated pea buds is of course well known and is one of the attractions of pea as a tissue for studying photoregulation of plant growth. The de-etiolation of peas involves a combination of leaf development and chloroplast development. In the first 40 h of de-etiolation, the total nucleic acid level of pea buds increases approximately threefold while the level of SSU mRNA increases about 50-fold. We take care to exclude the vast majority of the stem tissue when we harvest the buds, which at the stage of growth under study (8 days after germination) consist of the third and higher nodes only.

A. W. Galston (*Plant Breeding Institute, Cambridge, U.K.*). The authors state that both the small subunit (SSU) of RuBP carboxylase and its messenger RNA increase in quantity after a red light treatment. Do they have any further information about the comparative kinetics of the post-irradiation appearance of these two entities? Does the increased level of messenger precede the increased level of SSU, as we would expect?

J. Bennett. In etiolated pea plants the level of SSU mRNA measured by dot hybridization is only about 2% of the level found in plants de-etiolated for 48 h. The increase in SSU mRNA level is initially gradual but accelerates strongly after 12–24 h. The SSU itself is barely detectable by our radioimmune procedure in dark-grown plants or in plants exposed to up to 12 h of illumination. In plants exposed to 24 h of illumination, the SSU is readily detectable and increases a further tenfold, approximately, in the next 24 h.

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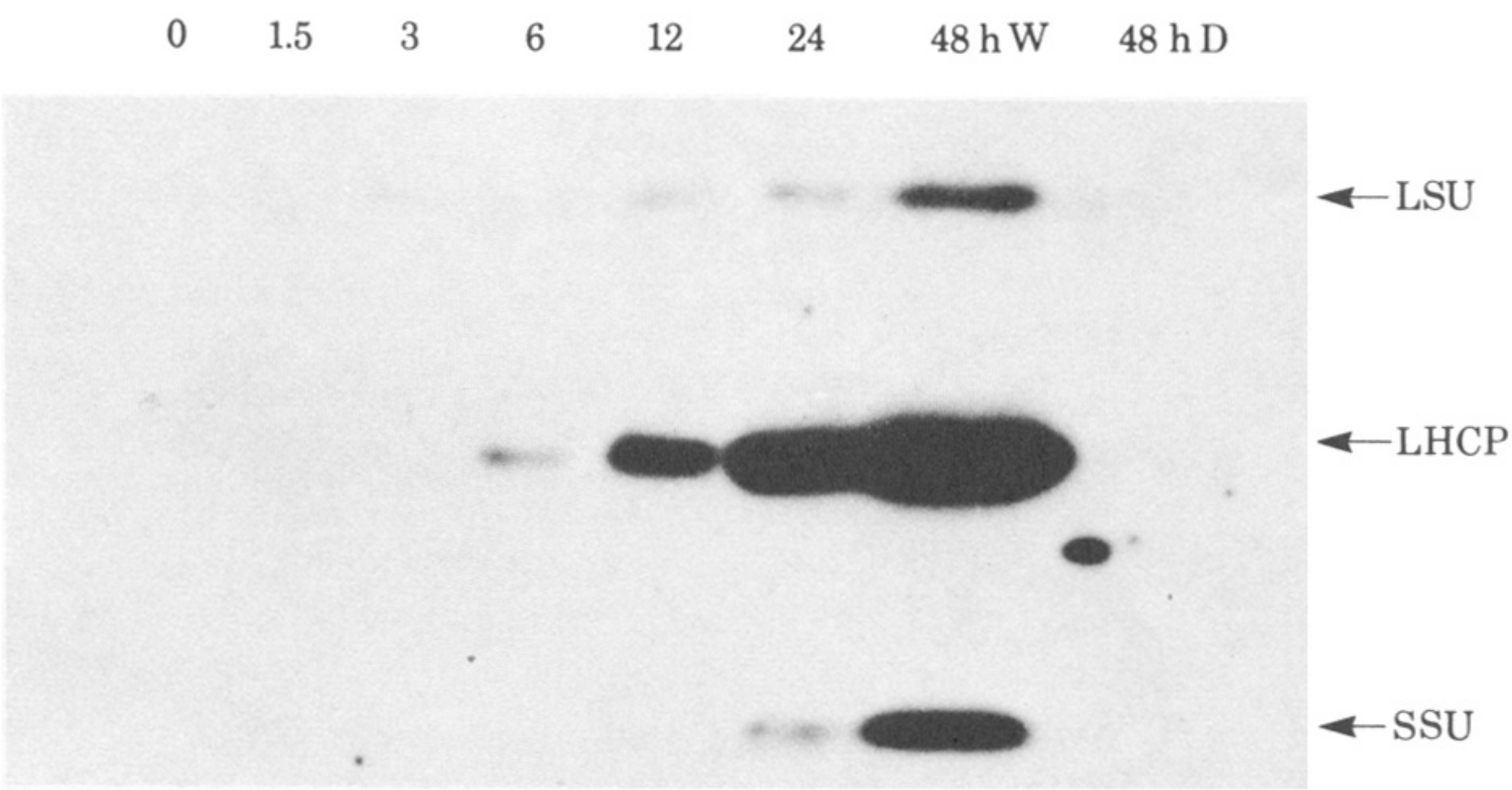

GURE 2. Accumulation of LSU, SSU and LHCP during de-etiolation of pea seedlings. Apical buds were harvested after 8 or 10 days of etiolation, or after 8 days of etiolation followed by 1.5–48 h of exposure to white light (W) (100 μmol m^{-2} s^{-1}). Samples corresponding to the sodium dodecyl sulphate extract of one-fiftieth of an apical bud were fractionated by sodium dodecyl sulphate polyacrylamide gel electrophoresis and analysed by the procedure outlined in table 2. The radioautogram shows the position and relative concentrations on the nitrocellulose sheet of the ternary complexes involving LSU (or SSU or LHCP), antibody and ^{125}I-labelled protein A.

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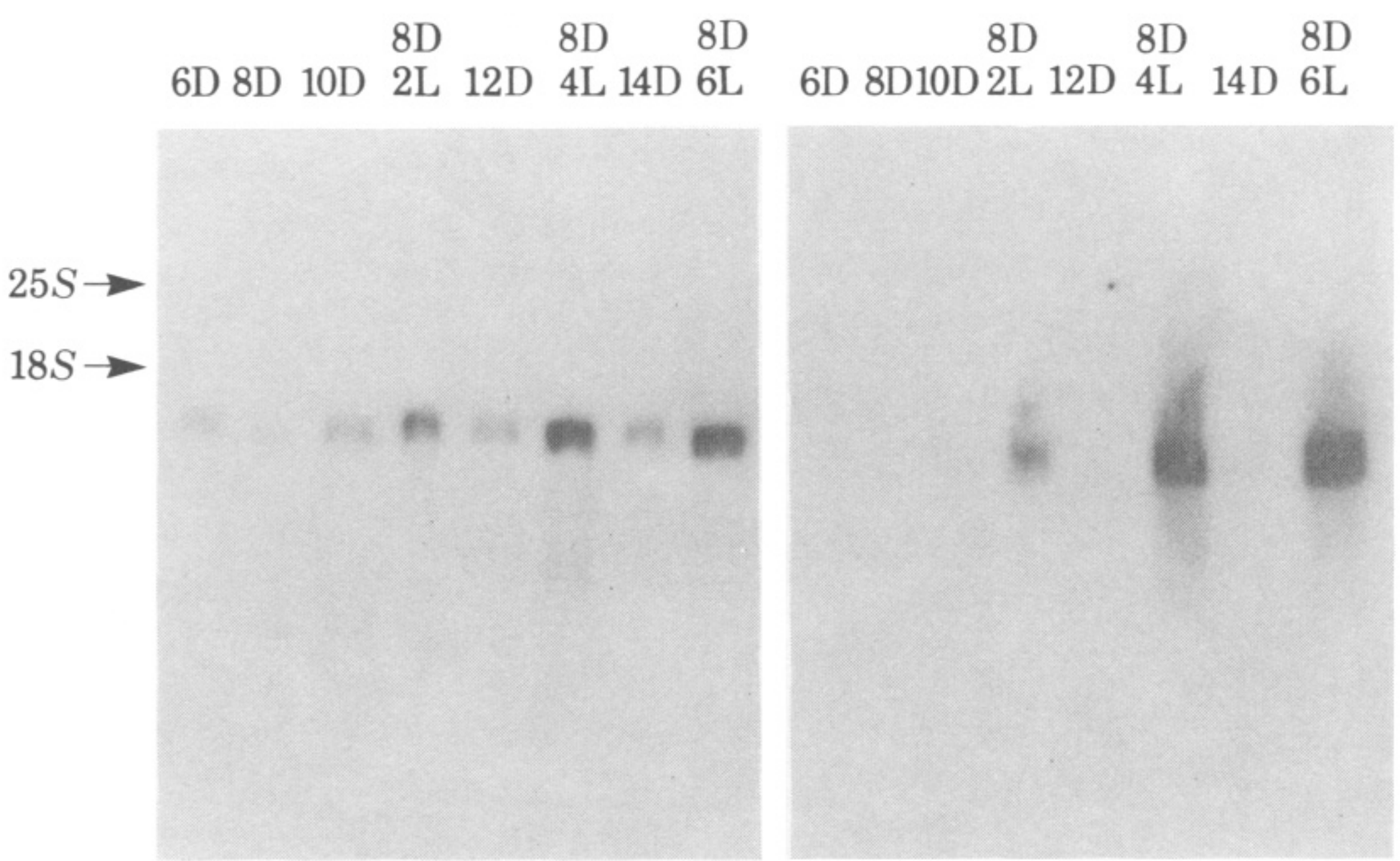

FIGURE 3. Light-dependent accumulation of SSU mRNA and light-stimulated accumulation of LHCP mRNA during de-etiolation of pea seedlings. Peas were grown in the dark (D) for the times indicated (in days), or transferred into continuous illumination with white light (L) after 8 days. Total RNA was extracted from the apical buds and 20 μg of each sample was fractionated in agarose-formamide gels. The RNA was blotted onto nitrocellulose sheets and hybridized with ^{32}P-labelled nick-translated SSU plasmid (pSSU 160) or LHCP plasmid (pFab 31) (radioactive count of each was 10^7 min^{-1}). See Gallagher & Ellis (1982) for a description of the origins of the clones. The nitrocellulose sheets were washed, dried and radioautographed.

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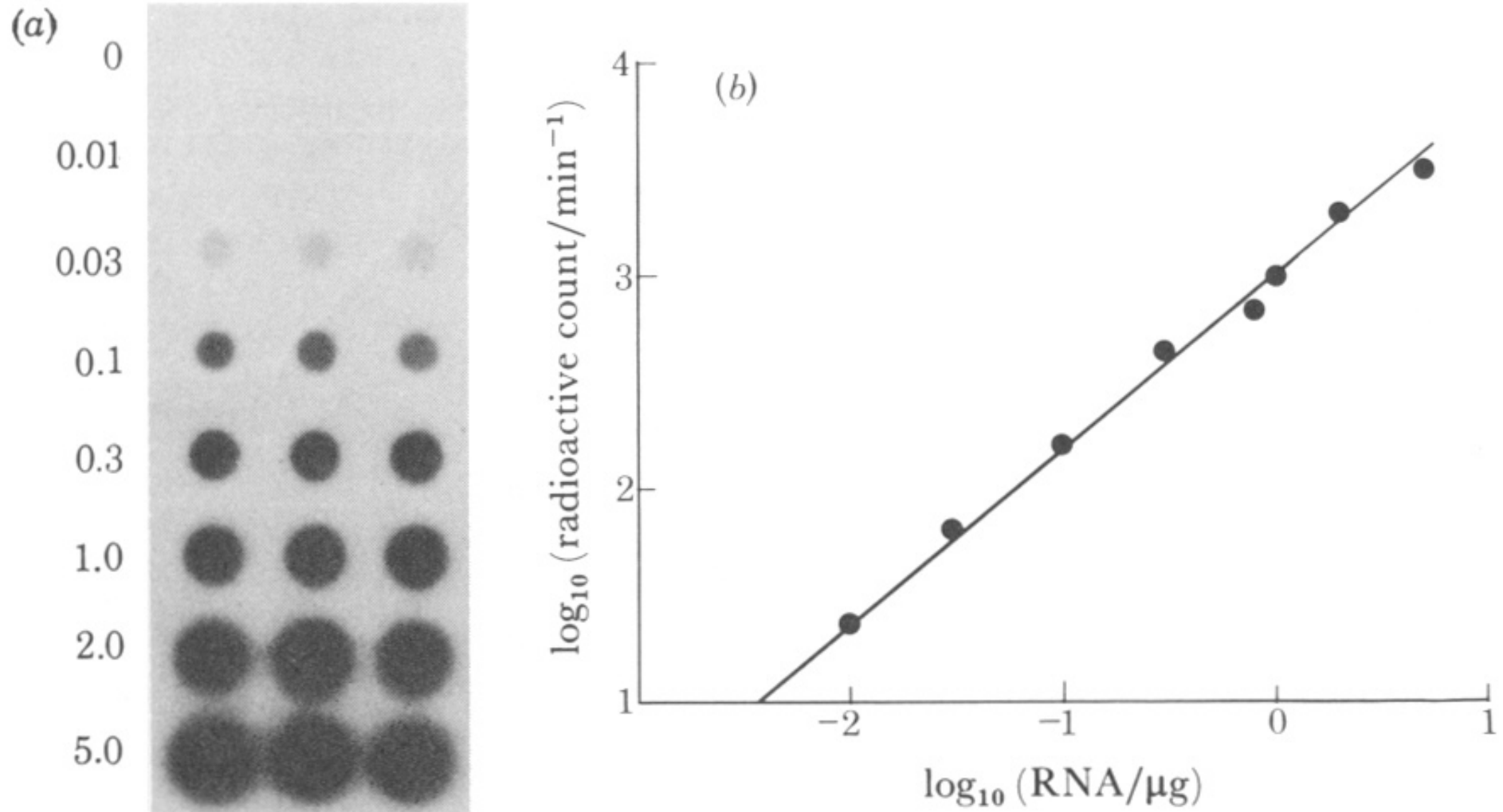
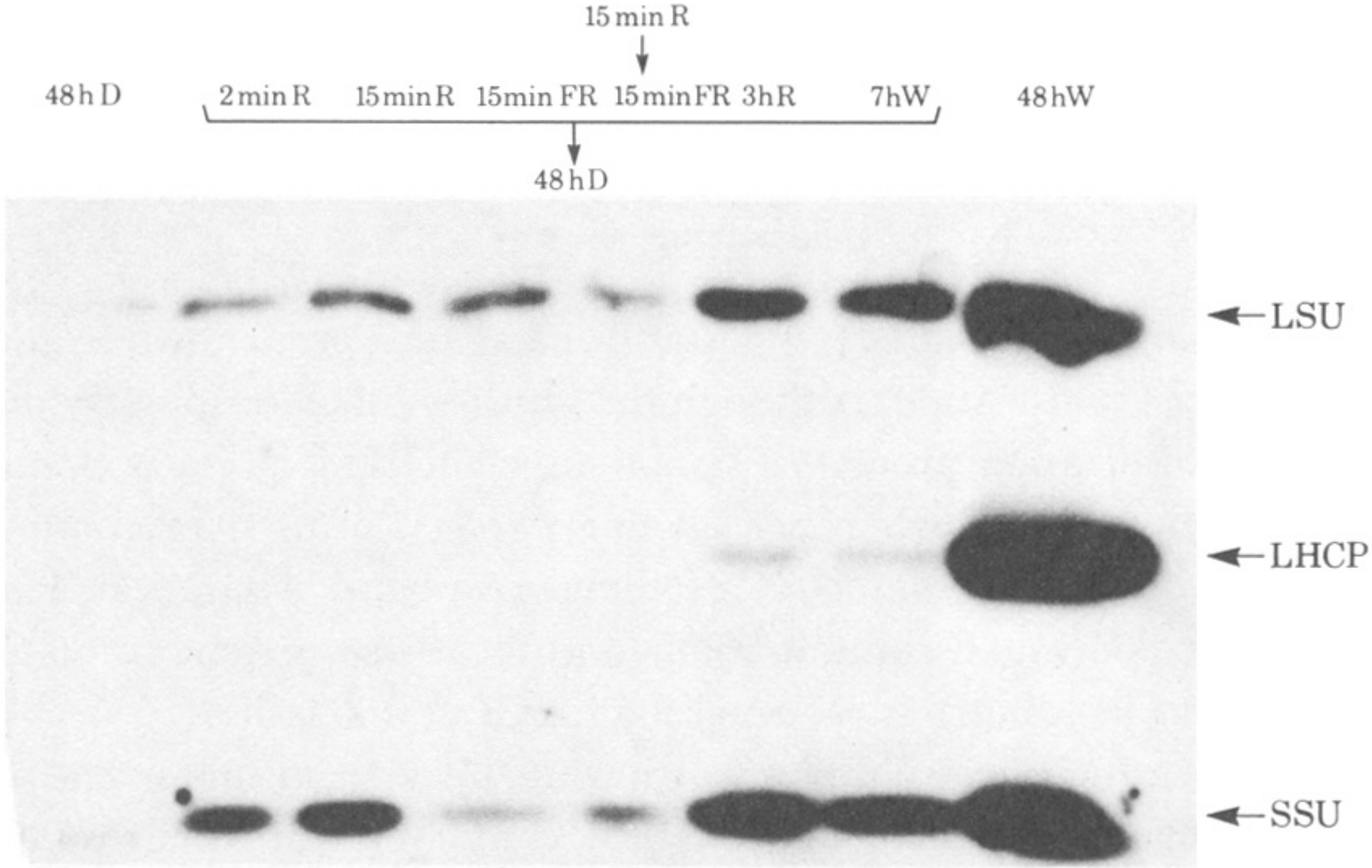


GURE 4. Assay of specific mRNAs by dot blotting. Total leaf RNA from plants exposed to 48 h of continuous white light was applied in the indicated quantities in 2.25 M NaCl, 0.225 M trisodium citrate, pH 7.0, to a nitrocellulose sheet held under a slight vacuum in a Perspex manifold. The LHCP mRNA content of each RNA dot was determined by hybridization of ^{32}P-labelled nick-translated LHCP probe (see description of table 3 for details). (*a*) Radioautogram of ^{32}P-labelled hybrids, showing triplication. Total RNA applied (micrograms) is shown on the left. (*b*) Double-logarithmic plot of ^{32}P label in hybrids as a function of total RNA content loaded per dot.



GURE 5. Investigation of the involvement of phytochrome in the accumulation of LSU, SSU and LHCP during de-etiolation of pea seedlings. See description of table 3 for a description of plant growth and illumination. See table 2 and figure 2 for details of the radioimmune assay.

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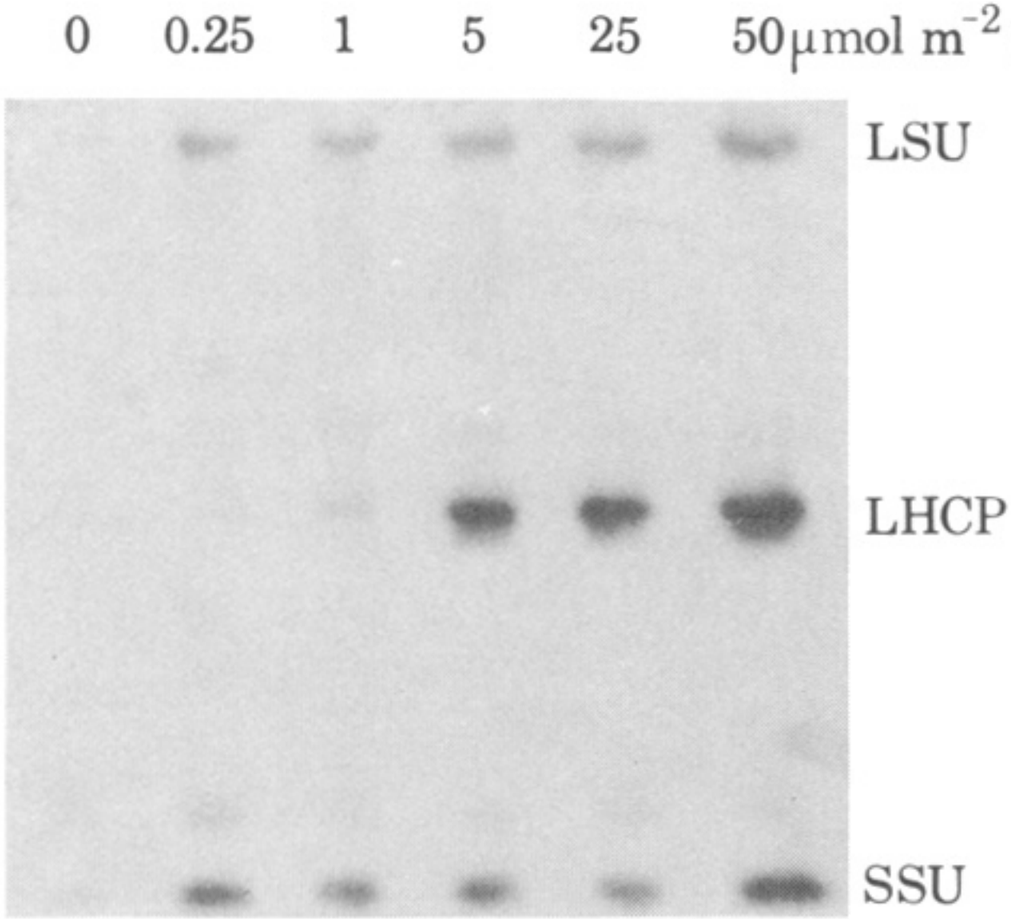

FIGURE 6. Effect of photon fluence rate on the accumulation of LSU, SSU and LHCP during de-etiolation of pea seedlings 8 days old. Plants were exposed to white light of the indicated fluence rates for 48 h before harvest. See table 2 and figure 2 for details of the radioimmune assay.