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The Complexity of Gene Expression in Higher Plants

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The complexity of gene expression in higher plants

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[Plate 1]

During light-induced chloroplast formation in higher plants the synthesis of several nuclear encoded plastid proteins is under the control of phytochrome. Light acting through the phytochrome system is able both to increase the transcription of certain nuclear genes and to decrease the transcription of others.

It has been generally assumed that regulation by phytochrome alone would be sufficient to account for the observed light-dependent changes in nuclear gene expression during chloroplast formation. However, it has recently become evident that the light-dependent control of nuclear gene expression may be far more complex than originally expected. There are at least two other factors that in addition to phytochrome may affect nuclear gene expression: (1) changes in chromatin organization from an inactive to a transcriptionally active state, and (2) a plastid-derived factor that seems to be involved in the transcriptional control of some nuclear genes encoding plastid-specific proteins.

Although the light-dependent control of transcription has been studied intensively for nuclear genes, much less is known about the light-dependent control of plastid gene expression. The P700 chlorophyll *a* protein of photosystem I is a major membrane protein whose massive accumulation is induced by light and whose genes have been located on the plastid DNA. In barley a high concentration of mRNA for the P700 chlorophyll *a* protein was detected within the total RNA as well as within the polysomal fraction of etioplasts and remained almost constant during greening. Based on these results it can be inferred that the accumulation of the P700 chlorophyll *a* protein during light-dependent chloroplast development in barley is not coupled to its transcript concentration but is controlled at a translational – or post-translational – level. The possible function of protochlorophyllide as photoreceptor in this light-dependent control of plastid gene expression is discussed.

Introduction

Angiosperms are unable to form chloroplasts in the absence of light. When seedlings are grown in the dark, the proplastids of the developing leaves differentiate into another plastid type, the etioplast. Upon illumination of dark-grown higher plants a rapid transformation of etioplasts into chloroplasts is induced. Protochlorophyllide is photoreduced to chlorophyllide, the prolamellar body disintegrates and an activation of the porphyrin pathway leads to the accumulation of chlorophyll. In parallel with chlorophyll accumulation a rapid formation of the thylakoid membranes occurs, leading to the assembly of the photosynthetic apparatus of the mature chloroplast (Boardman *et al.* 1978; Castelfranco & Beale 1983). These light-induced changes are based on an intimate interaction of the two genetic systems within the nucleus and the plastid (Bogorad 1975; Ellis 1981). Light exerts its control not only on processes occurring within the plastid but simultaneously triggers reactions in the cytoplasm that contribute to

chloroplast formation. It is therefore not surprising that not only protochlorophyllide within the plastid but also at least one additional photoreceptor, phytochrome, is involved in the light-dependent control of the complex interaction between plastids and the nucleus (Harpster & Apel 1985; Tobin & Silverthorne 1985). It has been demonstrated that light-dependent changes in the concentration of several nuclear-encoded plastid proteins during chloroplast formation are under the control of phytochrome. Phytochrome mediates both an increase in the transcription of certain genes and a decrease in the transcription of others (Gallagher & Ellis 1982; Silverthorne & Tobin 1984; Berry-Lowe *et al.* 1985; Mösinger *et al.* 1985).

It has been generally assumed that regulation by phytochrome alone would be sufficient to account for the observed light-dependent changes in nuclear gene expression during chloroplast formation. However, very recently it had become evident that the light-dependent control of nuclear gene expression may be far more complex than originally expected (Mayfield & Taylor 1984; Batschauer *et al.* 1986). There are at least two other factors that in addition to phytochrome may affect nuclear gene expression.

Changes in chromatin organization

The phytochrome-dependent regulation of nuclear gene transcription in leaves has been well documented. However, phytochrome is also detectable in other plant tissues such as the mesocotyl, roots and endosperm, as are the genes whose expression in the leaf is regulated by phytochrome. High levels of transcripts of these genes, however, were found only in leaves and mesocotyls, but not in such tissues as roots or endosperm (figure 1). Another transcript, which codes for one of the hordein storage proteins, was detectable only in the endosperm but in none of the other tissues tested (Steinmüller *et al.* 1986). Although phytochrome regulates the transcription of certain genes in leaves, other factors, responsible for the observed tissue-specific differences in transcript levels, must also be involved in the control of gene expression.

Based primarily on studies of animal cells, it has been proposed that controlled accessibility to regions of chromatin and specific DNA sequences may be one of the primary mechanisms by which gene expression in eukaryotic cells is modulated (Weisbrod 1982; Reeves 1984). Because the DNA in the nucleus is compacted too tightly to be available to the transcriptional apparatus, it is generally believed that the chromatin structure of genes that are transcribed has to be different from that of the bulk of DNA (Weisbrod 1982). Evidence that chromatin from actively transcribed genes exhibits a structure different from that of non-transcribed regions stems primarily from the use of relatively non-specific endonucleases as probes (Weisbrod 1982; Reeves 1984). In general, transcriptionally active sequences are found in an altered conformation that renders them preferentially sensitive to nucleases, particularly to DNase I. In addition, DNase I can recognize 'hypersensitive sites' in chromatin that may be important in gene control (Wu 1980; Elgin 1981). Considerable experimental evidence suggests that in animal cells at least two fundamentally different classes of mechanism are involved in the conversion of chromatin from an inactive to a transcriptionally active state: one that makes a selected subset of cellular genes available for transcription and a second that induces and regulates the actual expression of the potentially active genes (Weisbrod 1982; Reeves 1984).

In contrast to the abundant information on changes in the chromatin organization of animal genes, only a little is known about the possible role of chromatin structure in the regulation

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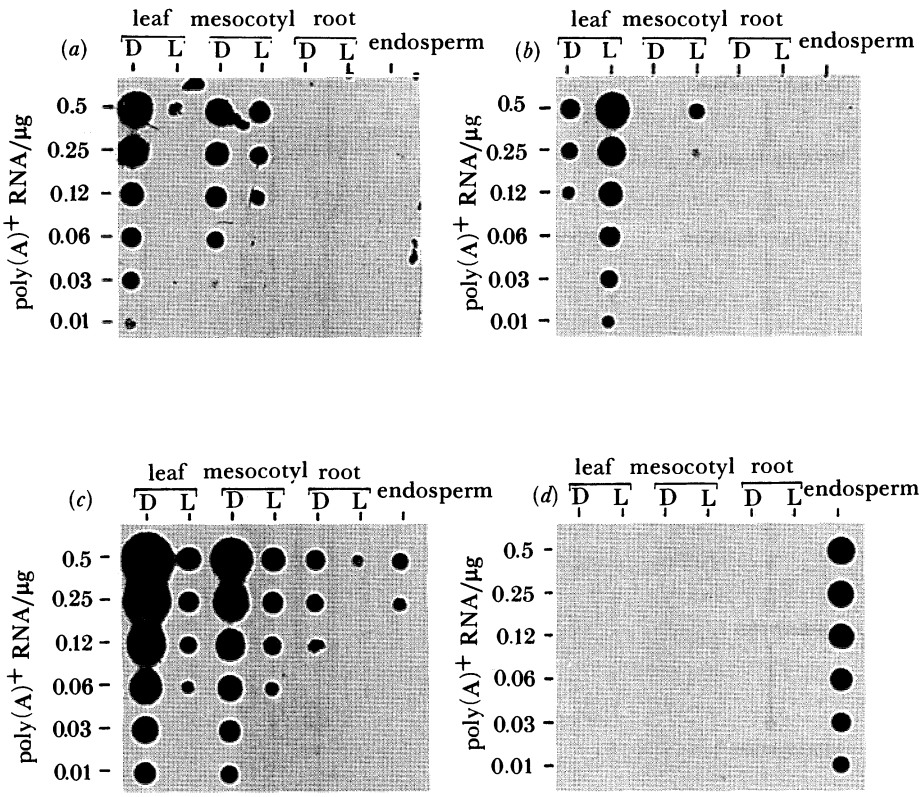

FIGURE 1. Tissue-specific distribution of transcripts in barley. Poly(A)-containing RNA was isolated from leaves, mesocotyls and roots of plants that had been grown in darkness for 5 days (D) or later illuminated with white light for 12 h (L). Endosperm was isolated 2–3 weeks after pollination. Dilution series of the poly(A)-containing RNA were dotted onto nitrocellulose and hybridized with ^{32}P-labelled DNA probes encoding (*a*) the NADPH-protochlorophyllide oxidoreductase (pHv DF1), (*b*) the light-harvesting chlorophyll *a*/*b* protein (pHv LF2), (*c*) a 15 kDa polypeptide of unknown function (pHv DA6) and (*d*) the B1-hordein (pc hor 2–4).

of gene expression in plants (Spiker 1985). In recent work on chromatin structure in barley, we have described a simple and efficient method for isolating plant chromatin that is suitable for studying DNase I sensitive domains and hypersensitive sites (Steinmüller & Apel 1986). We have used this approach to study the effects of tissue-specific exogenous factors on the chromatin structure of several genes during the development of barley seedlings. Etiolated leaves of barley were frozen in liquid nitrogen and ground with a mortar and pestle. The resulting powder was suspended in a buffer containing 40% glycerol. After two washing steps the chromatin pellet was resuspended in the isolation buffer without glycerol and was ready for DNase I treatment. After DNase I treatment for varying lengths of time, the DNA was purified from the different chromatin samples and digested to completion by added restriction endonucleases. The restriction fragments were separated electrophoretically and transferred onto nitrocellulose. The blots were hybridized with different ^{32}P-labelled DNA probes (figure 2). One of these DNAs encodes part of a B1-hordein, a protein whose synthesis is confined to the endosperm of barley seeds. The corresponding genes are not transcribed in leaf tissue and thus serve as a control for an inactive gene. The second DNA probe contains a DNA insert encoding a 15 kDa polypeptide of unknown function whose mRNA is one of the most prevalent

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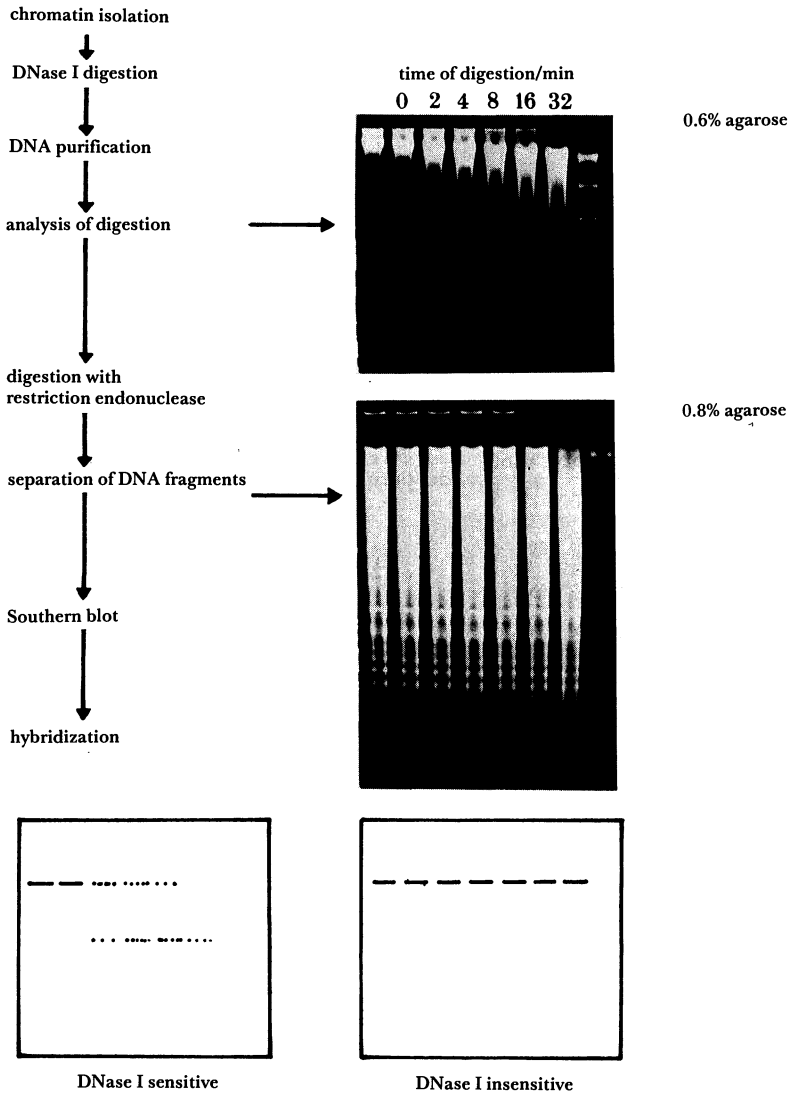

FIGURE 2. Schematic description of the DNase I assay for the determination of chromatin organization.

transcripts within the poly(A)-containing RNA fraction of etiolated barley leaves but which declines rapidly upon illumination (figure 1).

The results of the two hybridization experiments are shown in figures 3 and 4. The hordein-specific probe hybridized to several genomic DNA fragments, indicating that this storage protein is encoded by a complex multigene family. The hybridization pattern remained constant throughout the course of DNase I treatment (figure 3).

In contrast to the hordein genes, members of the multigene family encoding the 15 kDa polypeptide were highly susceptible to DNase I (figure 4). After the addition of small concentrations of nucleases to the chromatin, the intensity of the genomic DNA fragments decline rapidly and, except for one major band of 6 kilobase pairs (kb), they were no longer detectable after an incubation time of 8 min. At the same time as the original DNA fragments disappeared, other fragments of lower molecular mass appeared that hybridized to the copy

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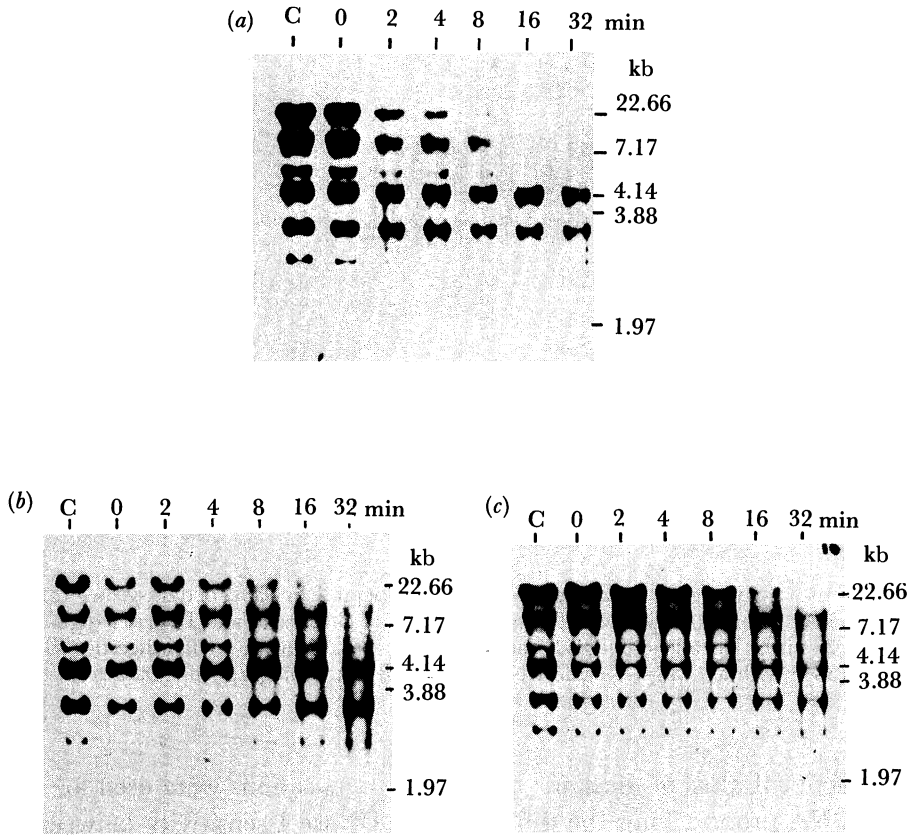

FIGURE 3. DNase I sensitivity of genes encoding the barley hordein gene family in endosperm (*a*) and leaves (*b*, *c*). Chromatin was isolated from leaves of plants that had been grown either in darkness for 5 days (*b*) or under a day/night cycle (8/16 h) for 5 days (*c*). Endosperm was isolated 2–3 weeks after pollination. The chromatin samples were digested with DNase I (0.2 μg ml^{-1}) for 0–32 min (lanes 2–7) at room temperature. The DNA was then purified from the chromatin by phenol/chloroform extraction and subsequently digested with *Eco*RI. The DNA fragments were separated on an 0.8 % agarose gel and transferred to nitrocellulose as shown in figure 2. The nitrocellulose blots were probed with a hordein-specific DNA probe and radioautographed. Control (lane 1): genomic barley DNA isolated by the procedure of Taylor & Powell (1982). Marker: λ gt WES·λB DNA digested with *Hin*dIII. (Data from Steinmüller *et al.* (1986).)

DNA (cDNA). Thus DNase I does not digest the DNA randomly but seems to recognize well-defined sites in the vicinity of the genes.

In agreement with studies on chromatin structure in animal tissue, alterations in the chromatin structure of plant genes are frequently tissue-specific. This was clearly demonstrated by the drastic differences in sensitivity to DNase I for the multigene families encoding the leaf-specific 15 kDa polypeptide and the storage protein hordein in leaves and endosperm of barley. In chromatin isolated from endosperm all members of the 15 kDa polypeptide multigene family were well protected against DNase I. Even after 32 min of incubation the relative concentration of all DNA fragments was almost the same as at the beginning of DNase I treatment (figure 4). A different pattern of DNase I sensitivity was observed for the hordein gene family. In contrast to leaf tissue, in which the different members of the hordein gene family were well protected against DNase I, in chromatin isolated from endosperm most of them were highly susceptible to DNase I (figure 3).

It is important to stress that for these studies of tissue-specific differences in chromatin

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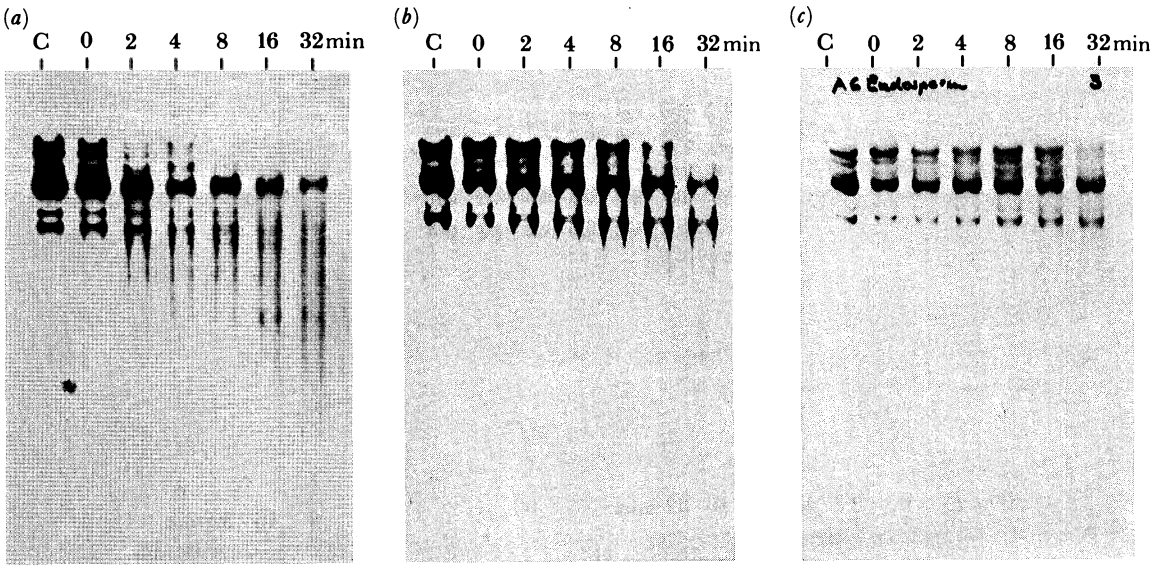

FIGURE 4. DNase I sensitivity of genes encoding the 15 kDa polypeptide in leaves of dark-grown (*a*) and illuminated (*b*) plants and endosperm (*c*). Growth of plants, isolation of chromatin and DNase I digestion were carried out as described in the legends to figures 2 and 3. *Eco*RI was used for the second digestion. The nitrocellulose blots were probed with a 15 kDa-specific DNA probe and autoradiographed. (Data from Steinmüller *et al.* (1986).)

organization identical blots of the same chromatin preparations were used for hybridization with the two DNA probes. Thus the differences in DNase I sensitivity between the hordein and the 15 kDa polypeptide multigene families are not due to an artificial variability among different chromatin preparations, but rather reflect internal differences in the organizational state of these two gene families in one particular tissue type.

Furthermore, as shown previously, these alterations appear not to be due to internal modifications of the DNA itself but rather reflect differences in the organization of these genes within the chromatin of the two different tissues (Steinmüller & Apel 1986). Our results concur with the observations of others, who have also found differences in chromatin conformation for a storage protein gene in different tissues of French beans (Murray & Kennard 1984).

Within one particular tissue type the chromatin configuration may also be affected by environmental factors such as light. In chromatin of etiolated leaves, the various members of the multigene family encoding the 15 kDa polypeptide were highly susceptible to DNase I. When chromatin from leaves of fully green plants was assayed under identical conditions, DNA fragments were well protected against DNase I treatment, indicating that the organization of the corresponding genes within the leaf chromatin had undergone drastic changes during light treatment (figure 4). However, it is important to stress that these alterations in chromatin organization were not detectable if etiolated plants were exposed to continuous white light for shorter periods, of up to 24 h. Even though the light-induced decline in the rate of transcription of the 15 kDa polypeptide multigene family had reached its minimum value after 12 h of light, the chromatin configuration of these genes was still similar to that in the etiolated leaves. It therefore seems possible that a general change in chromatin structure from a DNase I-sensitive to a DNase I-insensitive configuration – and vice versa – may require longer lasting processes. Although the light-induced changes in chromatin organization of the 15 kDa polypeptide genes may cause the transformation of an active gene into an inactive one, they appear not to be

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involved in the regulation of the actual expression of a potentially active gene. In agreement with these results on changes in plant chromatin, in animal cells the sensitivity to DNase I of actively transcribed genes seems to reflect primarily a potential for a gene to be transcribed rather than transcription itself (Reeves 1984).

The implication of a plastid-derived factor in the transcriptional control of nuclear genes

In addition to changes in chromatin organization, which seem to play an important role in determining the transcriptional commitment of a gene during tissue specification, another factor has been found to be involved in the light-dependent regulation of nuclear gene expression during chloroplast formation. Although the control of the light-dependent chloroplast development by the nucleus has been well documented, much less is known about the involvement of plastids in the photocontrol of developmental processes in extraplastidic compartments of the cell. The possible existence of such a control has been discussed several times (see, for example, Bradbeer *et al.* 1979). However, only recently has indirect evidence in favour of the control of nuclear processes by plastid-derived factors been obtained in work with pigment-deficient plants (Mayfield & Taylor 1984; Batschauer *et al.* 1986).

In carotenoid-deficient *albina* mutants of barley, and in barley plants treated with the herbicide Norflurazon, the light-dependent accumulation of the mRNA for the light-harvesting chlorophyll *a*/*b* protein (LHCP) is blocked (Batschauer *et al.* 1986). Thus the elimination of a functional chloroplast, either as a result of mutation or as a result of herbicide treatment, can lead to the specific suppression of the expression of a nuclear gene encoding a plastid-localized protein. These results confirm and extend earlier observations on maize (Mayfield & Taylor 1984). The inhibition of mRNA accumulation appears to be specific for the LHCP; the mRNAs encoding the small subunit of ribulose 1,5-bisphosphate carboxylase and the NADPH-protochlorophyllide oxidoreductase are relatively unaffected. The failure of the *albina* mutants and of Norflurazon-treated plants to accumulate the LHCP mRNA is not exclusively caused by an instability of the transcript but rather by the inability of the plants to enhance the rate of transcription of the LHCP genes during illumination. Several chlorophyll-deficient *xantha* mutants of barley, which are blocked after protoporphyrin IX or Mg-protoporphyrin, and the chlorophyll-*b*-less mutant *chlorina f*2 accumulate the LHCP mRNA to almost normal levels during illumination. Thus, if any of the reactions leading to chlorophyll formation is involved in the control of LHCP mRNA accumulation it should be one between the formation of protochlorophyllide and the esterification of chlorophyllide *a* (Batschauer *et al.* 1986).

While the nature of the regulatory factor(s) has not been identified our results suggest that, in addition to phytochrome (Pfr), plastid-dependent factors are required for a continuous light-dependent transcription of nuclear genes encoding the LHCP.

Translational control of the light-dependent accumulation of the P700 chlorophyll protein of photosystem I

So far we have concentrated only on the light-dependent control of nuclear genes. However, chloroplast formation also depends on the activity of plastid-specific genes. Whereas light-dependent control at the level of transcription has been studied intensively for nuclear genes, much less is known about the light-dependent control of plastid gene expression.

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A phytochrome-dependent change in the rate of transcription similar to that of nuclear genes has been proposed as a major control by which light may regulate the expression of plastid-specific genes (Rodermel & Bogorad 1985; Zhu *et al.* 1985). However, recent studies of several plastid-specific genes including those encoding the 32 kDa herbicide-binding polypeptide of photosystem II and the large subunit of ribulose 1,5-bisphosphate carboxylase have indicated that the expression of these genes during the light-dependent formation of chloroplasts may not be regulated exclusively at the level of transcription but also at one of the subsequent steps of protein biosynthesis (Fromm *et al.* 1985; Inamine *et al.* 1985).

The P700 reaction centre chlorophyll *a* protein of photosystem I is a major plastid membrane protein whose accumulation is induced by light (Vierling & Alberte 1983). The genes encoding polypeptides of the P700 chlorophyll *a* protein complex have been located on the plastid DNA of different higher plants (Westhoff *et al.* 1983; Fish *et al.* 1985).

In maize a light-induced increase in the rate of transcription of these genes seems to be involved in the control of the assembly of the chlorophyll–protein complex (Rodermel & Bogorad 1985; Zhu *et al.* 1985). In other plant species, however, the apoprotein of the P700 chlorophyll *a* protein complex was readily detectable in dark-grown seedlings and its relative amount did not change drastically upon exposing the plants to light and during greening (Nechushtai & Nelson 1985). These results are in contrast to earlier reports on a light-induced appearance of the P700 chlorophyll *a* protein and would suggest that a light-dependent transcriptional control of the corresponding genes may not be required for the synthesis of the chlorophyll *a* protein of the photosystem I reaction centre. To resolve these discrepancies and to understand the light-dependent control of plastid gene expression during chloroplast formation we have studied the appearance of the P700 chlorophyll *a* protein in barley.

Leaf material of plants that had been exposed for various lengths of time to continuous white light was ground to a fine powder under liquid nitrogen before the protein was solubilized with a heated sodium dodecyl sulphate (SDS)-containing extraction buffer. The extracted proteins were separated electrophoretically and blotted onto nitrocellulose, and the P700 chlorophyll *a* protein was immunostained by using a monospecific antiserum raised against the purified P700 chlorophyll *a* protein of barley. Under these conditions a rapid and drastic increase of the P700 chlorophyll *a* protein of photosystem I was detected during the light-induced transformation of etioplasts into chloroplasts (figure 5).

In view of the discrepancies between the published results of several groups mentioned above we felt it necessary to test the reliability of our data by a second experimental approach. The method of immunogold labelling of ultrathin sections of embedded leaf material allows the detection and localization of specific antigenic sites within the intact cell. In control experiments a non-specific adsorption of the immunogold particles could be excluded: by the method of immunogold labelling we could verify that the occurrence of the light-harvesting chlorophyll *a/b* protein is restricted to the plastid compartment. This result, together with those published previously on the localization of other plastid-specific proteins (Dehesh *et al.* 1986), indicates that the original topography of cellular components has not been visibly altered during the preparation of tissue sections. We also found a drastic increase in the amounts of the P700 chlorophyll *a* protein during illumination of dark-grown barley seedlings by this second approach – a similar result to that obtained by the method of immunoblotting (figure 5).

There are several different possibilities as to how light could have induced the accumulation of the P700 chlorophyll *a* protein within the plastids. One mechanism that has been implicated

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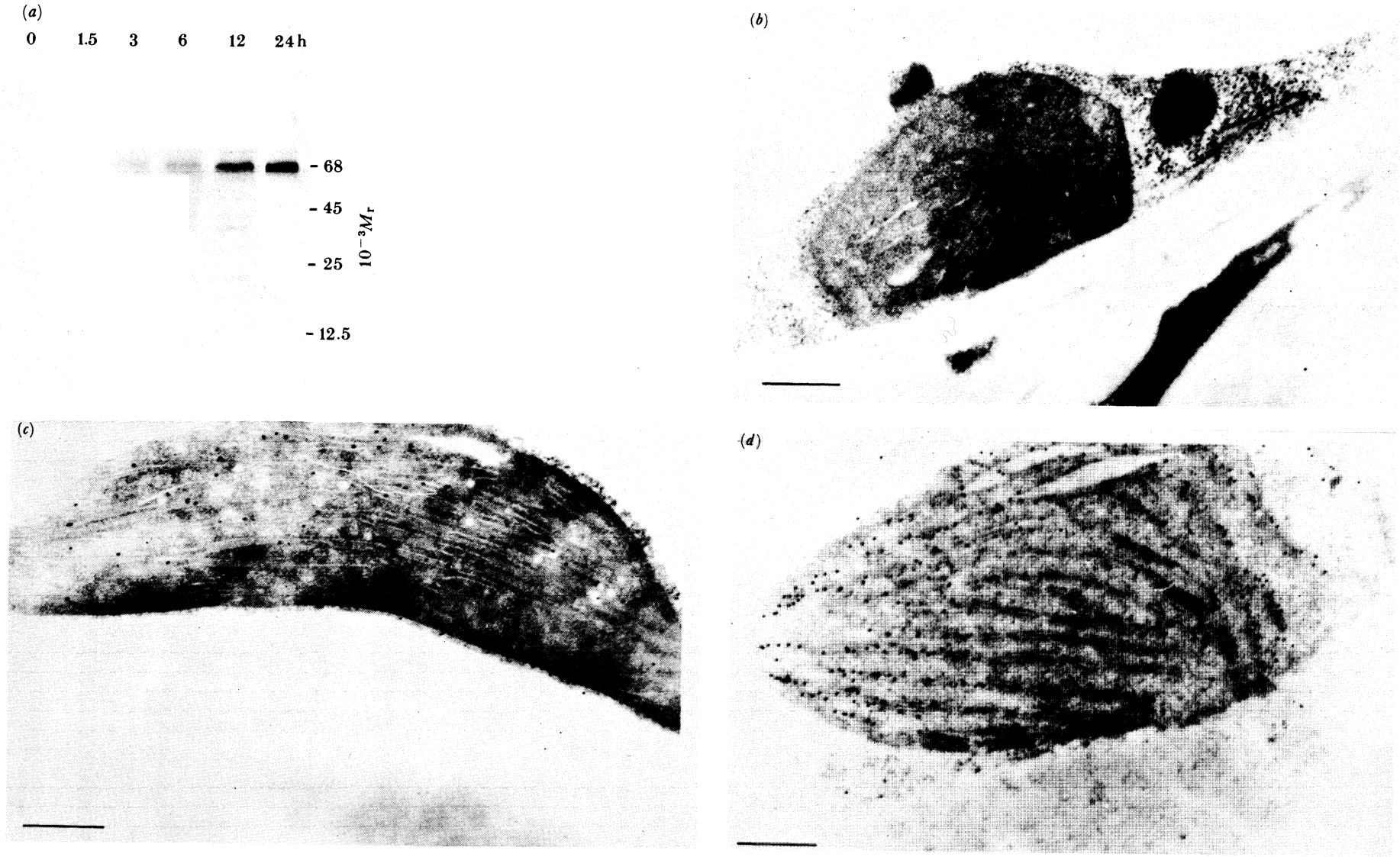

FIGURE 5. The light-induced accumulation of the P700 chlorophyll *a* apoprotein in dark-grown and illuminated barley seedlings. (*a*) Immunoblotting of the chlorophyll-binding protein: equal amounts of total leaf protein were subjected to SDS polyacrylamide gel electrophoresis and subsequently electroblotted on to nitrocellulose. The nitrocellulose blot was reacted with an antiserum against the P700 chlorophyll *a* apoprotein and immunostained. The six tracks represent different times of illumination. (*b–d*) Immunogold labelling of the P700 chlorophyll *a* protein (*b*, *c*) and the light-harvesting chlorophyll *a*/*b* protein (*d*) in leaves of dark-grown (*b*) and 12 h illuminated barley seedlings (*c*, *d*). Sections of Lowicryl-embedded tissue slices were reacted with the antisera followed by 10 nm protein-A–gold. Bars represent 0.5 μm. (Data from Kreuz *et al.* (1986) and Dehesh *et al.* (1986).)

(*Facing p.* 488)

previously in the light-dependent control of protein accumulation is a light-induced increase in the rate of transcription of the corresponding gene(s) (Rodermel & Bogorad 1985; Zhu *et al.* 1985).

We have determined the relative level of transcripts encoding the P700 chlorophyll *a* protein of photosystem I reaction centre. Equal amounts of total RNA isolated from leaves of dark-grown and 12 h illuminated barley plants were separated electrophoretically and blotted onto nitrocellulose. The blot was hybridized with the spinach gene probe for the P700 chlorophyll *a* apoprotein. Only one mRNA species of approximately 4.7 kb was detectable, which hybridized to the radioactively labelled DNA probe (figure 6).

The amount of this transcript relative to the total cellular RNA, which is mainly ribosomal RNA, did not change drastically upon illumination of dark-grown seedlings. This observation was further substantiated by quantitative dot-blot hybridization assays. The data summarized in figure 6 show that the steady-state level of hybridizable mRNA complementary to the probe for the P700 chlorophyll *a* apoprotein remained fairly constant throughout an extended illumination period except for a slight transient increase after 3 h of illumination. In contrast, the mRNA sequence level for the 32 kDa herbicide-binding protein increased drastically in response to light. Also in this case there is a readily detectable transcript pool already present in dark-grown plants.

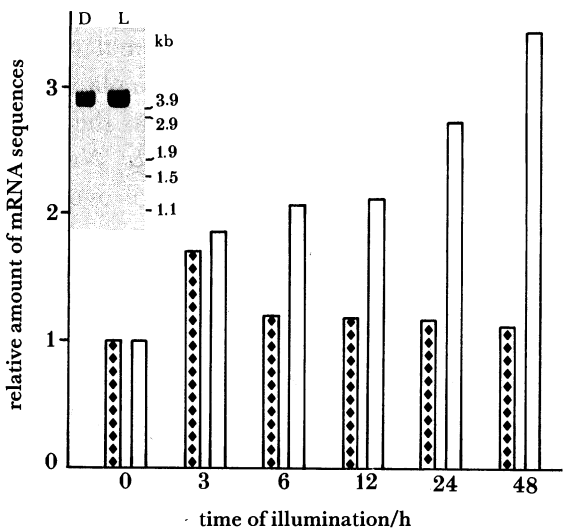

FIGURE 6. Light-induced changes in the relative amounts of transcripts encoding the P700 chlorophyll *a* apoprotein and the 32 kDa herbicide-binding protein of barley. Seedlings were grown either in total darkness or under continuous white light for various lengths of time. Total leaf RNA was prepared from the different plant samples and the relative amounts of mRNA sequences for the P700 chlorophyll *a* apoprotein and the 32 kDa herbicide-binding protein were determined by dot-blot hybridization. The dark levels of transcripts were set at unity. Open columns, mRNA sequence levels for the 32000 protein; hatched columns, mRNA sequence levels for the P700 chlorophyll *a* apoprotein. Inset: Northern blot analysis of the transcript of the P700 chlorophyll *a* apoprotein gene in dark-grown (D) and illuminated (L) barley seedlings. Numerals indicate the sizes (in kilobases) of the cytoplasmic and plastid rRNAs of barley and of *E. coli* 23 *S* and 16 *S* rRNA. (Data from Kreuz *et al.* (1986).)

Based on the results presented above it can be inferred that the accumulation of the P700 chlorophyll *a* protein during light-dependent chloroplast formation in barley is not coupled to its transcript level.

Further attempts were made to address the question of whether or not translational control

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may be involved in the light-dependent accumulation of the P700 chlorophyll *a* protein. The levels of hybridizable mRNA in polysomes from etioplasts and chloroplasts isolated during the early phase of greening, when a maximum rate of protein accumulation occurred, were estimated by the quantitative dot-blot hybridization procedure. A high concentration of mRNA for the P700 chlorophyll *a* protein was detected within the polysomal fraction of etioplasts; the concentration remained almost constant during greening. The uptake of a given mRNA into the polysomes might be regarded as indicative of the active translation of this mRNA. In such a case the synthesis of the P700 chlorophyll *a* protein of photosystem I would occur in dark-grown plants but since there is no chlorophyll available the protein would be rapidly destroyed. Such an interpretation would be in agreement with the observed close coordination of protein accumulation with the production of chlorophyll during the light-induced chloroplast formation (Vierling & Alberte 1983). As has been previously proposed, for the synthesis of the light-harvesting chlorophyll *a*/*b* protein (Apel & Kloppstech 1980), association of chlorophyll with the protein may be required for stable insertion of the protein into the developing thylakoid membranes. Besides such a post-translational control, however, the existence of other regulatory mechanisms is not ruled out.

We have measured only the relative but not the absolute concentrations of transcripts within the polysomal fraction because of technical limitations that do not permit us to determine the absolute amounts of polysomes present in leaves of dark-grown and illuminated barley leaves. Furthermore, we do not know whether or not the polysomal mRNAs are indeed actively translated in dark-grown plants as efficiently as in light-grown plants. Evidence is accumulating that stromal and thylakoid fractions of chloroplasts contain the same mRNA species while synthesizing different proteins (Leu *et al.* 1984; Minami & Watanabe 1984). It has been suggested that the thylakoid membrane itself exerts control over the translation of specific mRNAs (Leu *et al.* 1984). However, it is also conceivable that other factors, absent in the dark, are required for the translation of a polysomal mRNA. The only known photoreceptor within the plastid compartment of higher plants that might be responsible for a direct light-dependent control of plastid gene expression at a translational or post-translational level is protochlorophyllide. The function of protochlorophyllide is different from that of phytochrome in that it does not act as a typical sensor pigment. Instead its light-dependent reduction is the prerequisite for the formation of chlorophyll, which is a crucial constituent for the assembly of photosynthetic units within the thylakoid membrane. Even though other light-dependent reactions in the surrounding cytoplasm might affect more indirectly the expression of plastid-specific genes, we feel that a more penetrating investigation of the light-dependent chlorophyll formation and the enzymes involved in it may eventually help to identify the mechanism by which the plastid compartment seems to control the light-dependent expression of its own genes.

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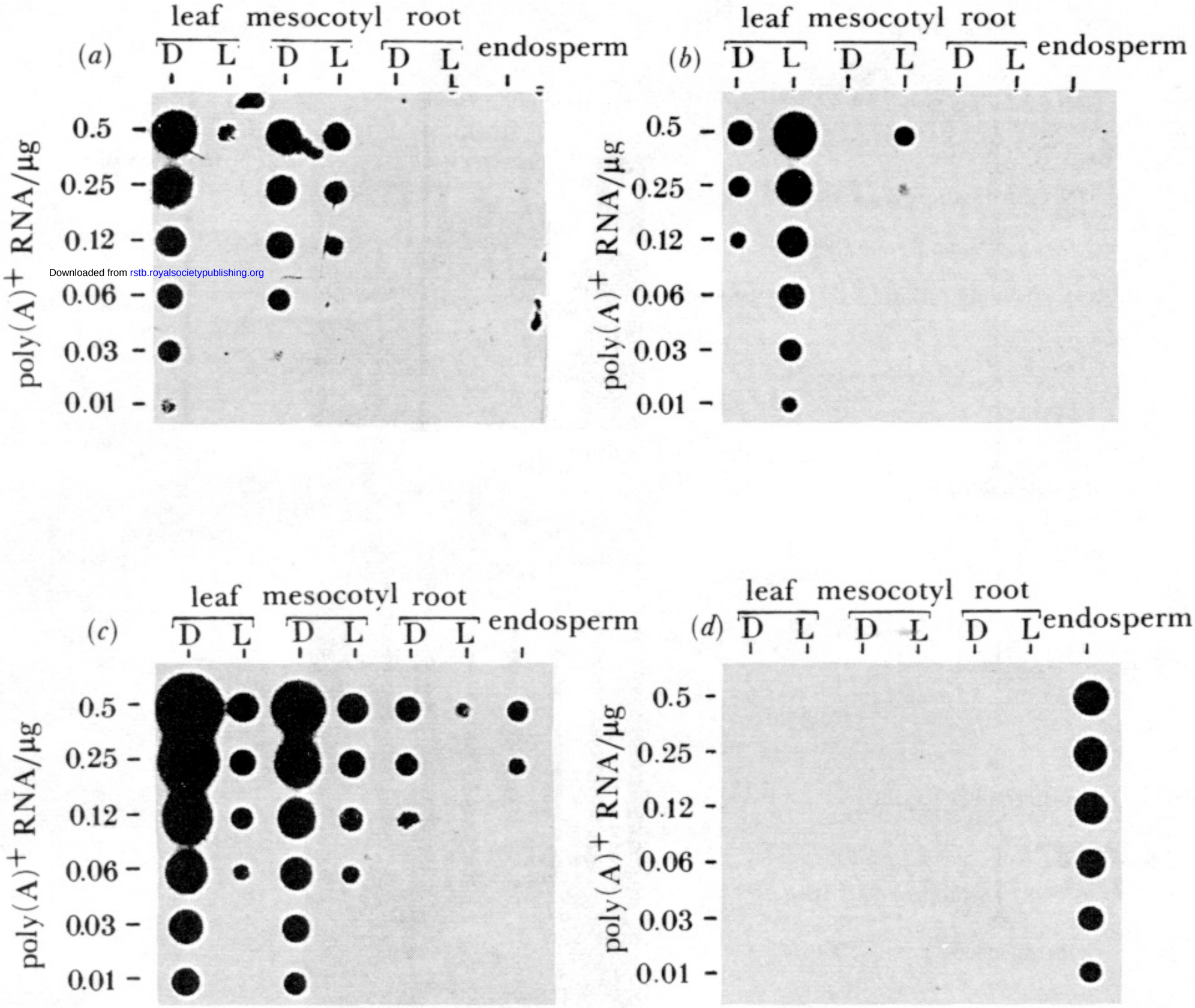


FIGURE 1. Tissue-specific distribution of transcripts in barley. Poly(A)-containing RNA was isolated from leaves, mesocotyls and roots of plants that had been grown in darkness for 5 days (D) or later illuminated with white light for 12 h (L). Endosperm was isolated 2–3 weeks after pollination. Dilution series of the poly(A)-containing RNA were dotted onto nitrocellulose and hybridized with ^{32}P-labelled DNA probes encoding (*a*) the NADPH-protochlorophyllide oxidoreductase (pHv DF1), (*b*) the light-harvesting chlorophyll *a*/*b* protein (pHv LF2), (*c*) a 15 kDa polypeptide of unknown function (pHv DA6) and (*d*) the B1-hordein (pc hor 2–4).

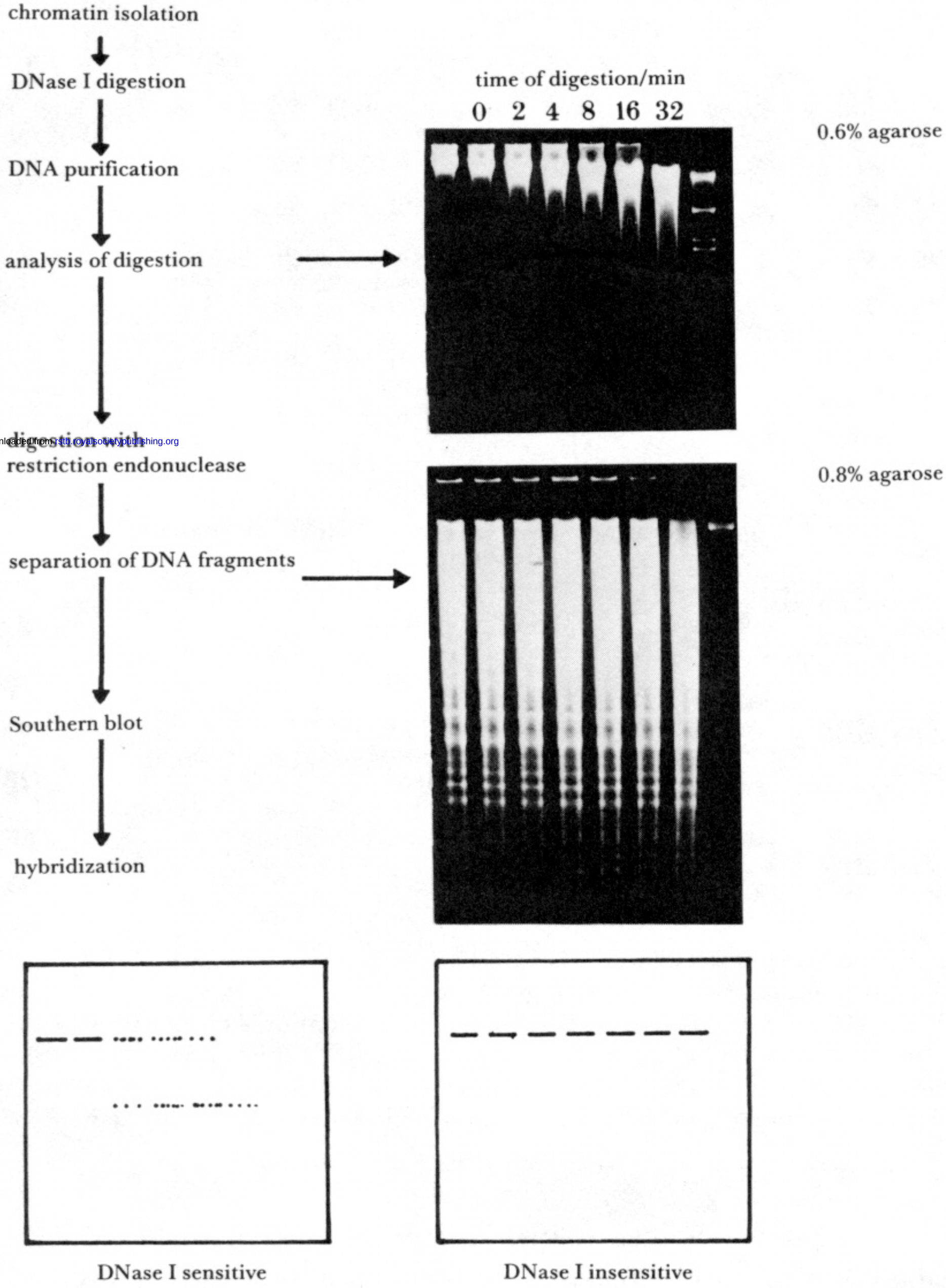
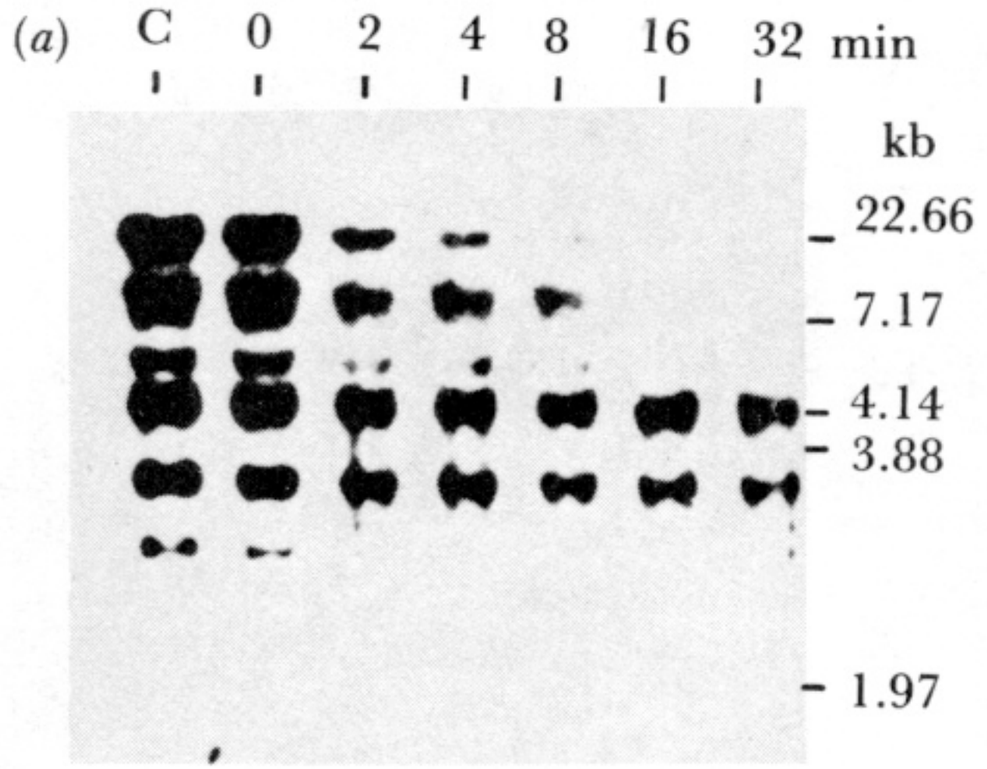
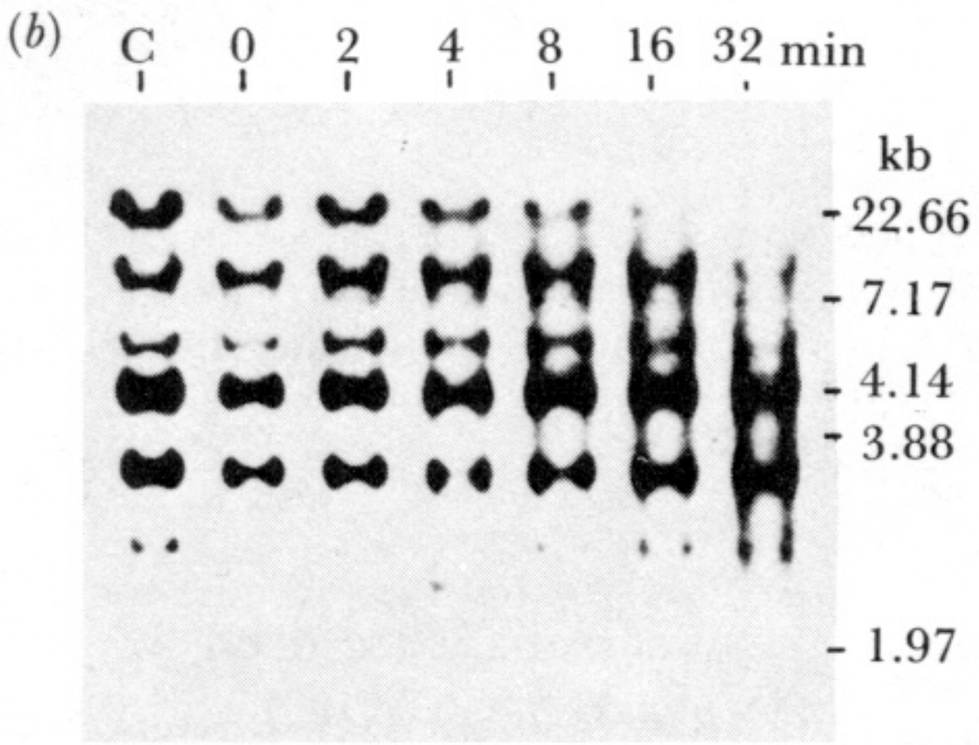


FIGURE 2. Schematic description of the DNase I assay for the determination of chromatin organization.





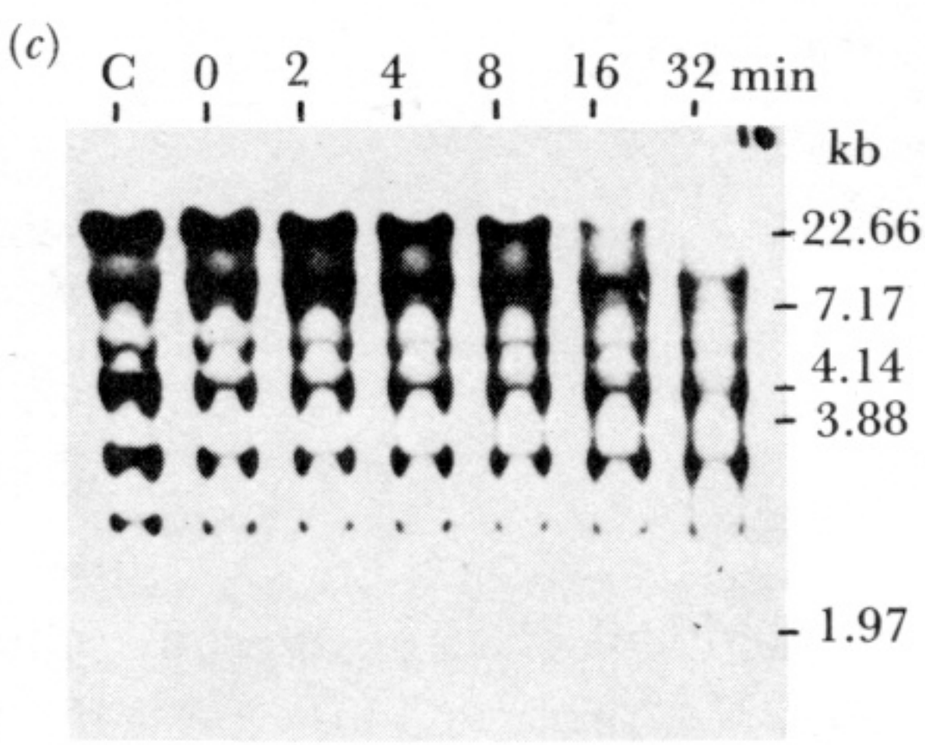


FIGURE 3. DNase I sensitivity of genes encoding the barley hordein gene family in endosperm (*a*) and leaves (*b*, *c*). Chromatin was isolated from leaves of plants that had been grown either in darkness for 5 days (*b*) or under a day/night cycle (8/16 h) for 5 days (*c*). Endosperm was isolated 2–3 weeks after pollination. The chromatin samples were digested with DNase I (0.2 μg ml^{-1}) for 0–32 min (lanes 2–7) at room temperature. The DNA was then purified from the chromatin by phenol/chloroform extraction and subsequently digested with *Eco*RI. The DNA fragments were separated on an 0.8 % agarose gel and transferred to nitrocellulose as shown in figure 2. The nitrocellulose blots were probed with a hordein-specific DNA probe and radioautographed. Control (lane 1): genomic barley DNA isolated by the procedure of Taylor & Powell (1982). Marker: λ gt WES·λB DNA digested with *Hin*dIII. (Data from Steinmüller *et al.* (1986).)

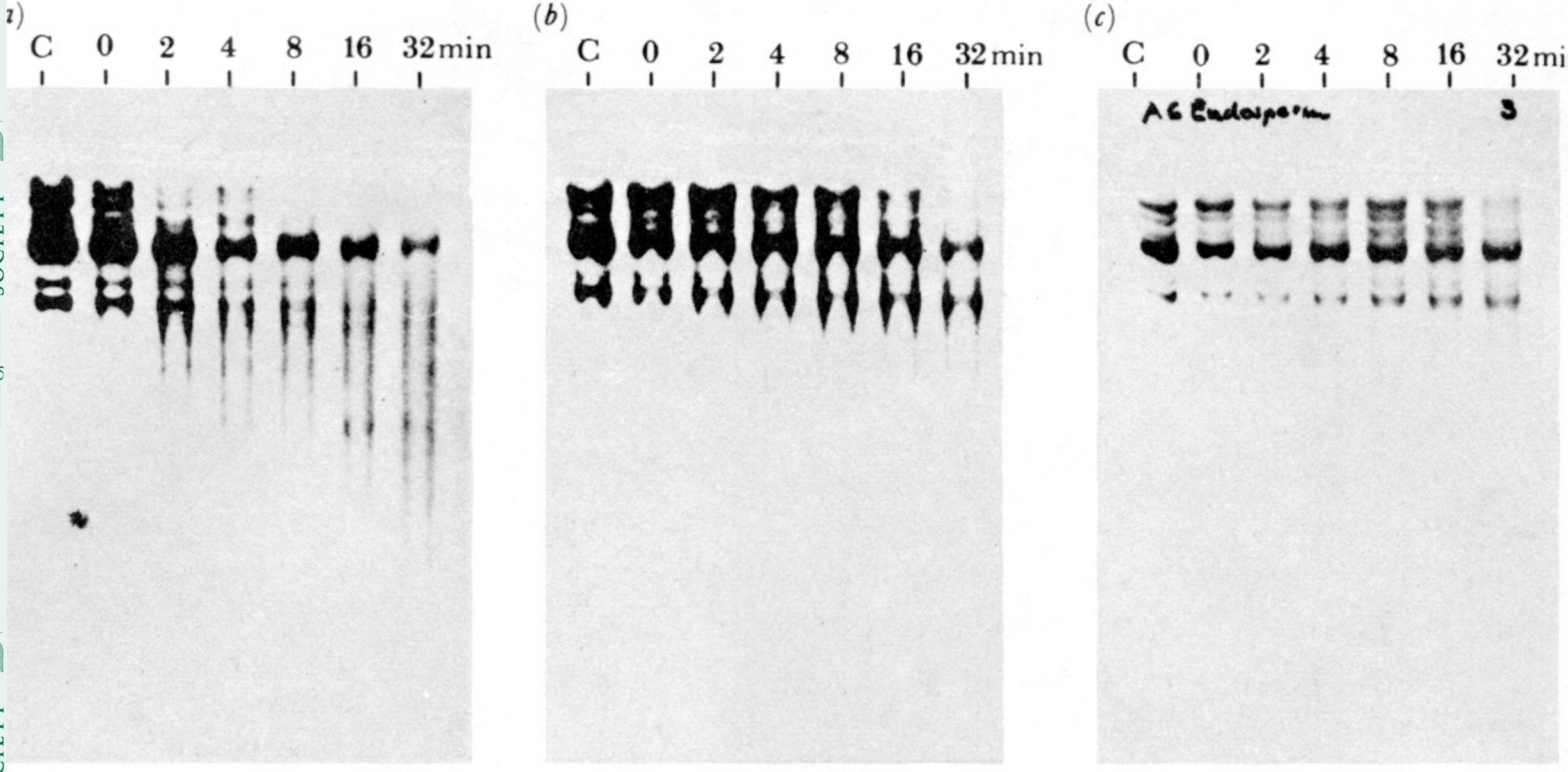


FIGURE 4. DNase I sensitivity of genes encoding the 15 kDa polypeptide in leaves of dark-grown (*a*) and illuminated (*b*) plants and endosperm (*c*). Growth of plants, isolation of chromatin and DNase I digestion were carried out as described in the legends to figures 2 and 3. *Eco*RI was used for the second digestion. The nitrocellulose blots were probed with a 15 kDa-specific DNA probe and autoradiographed. (Data from Steinmüller *et al.* (1986).)

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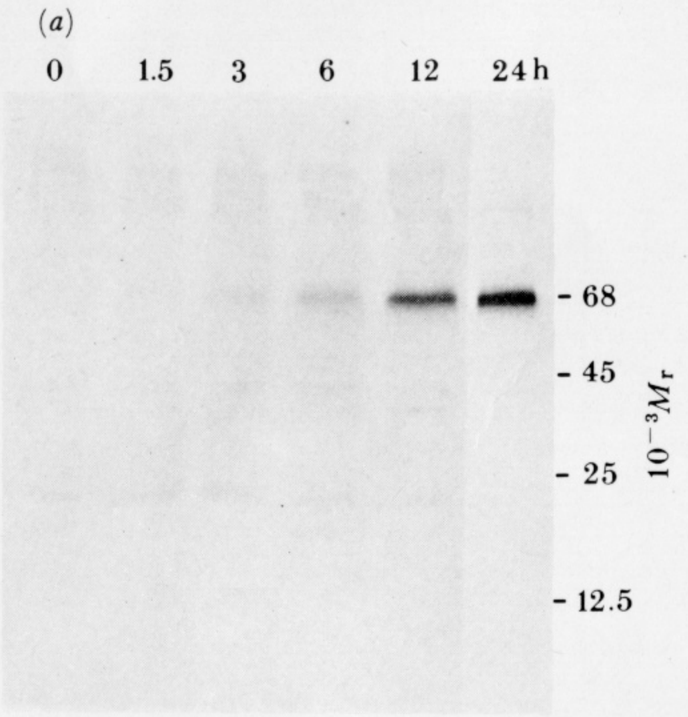

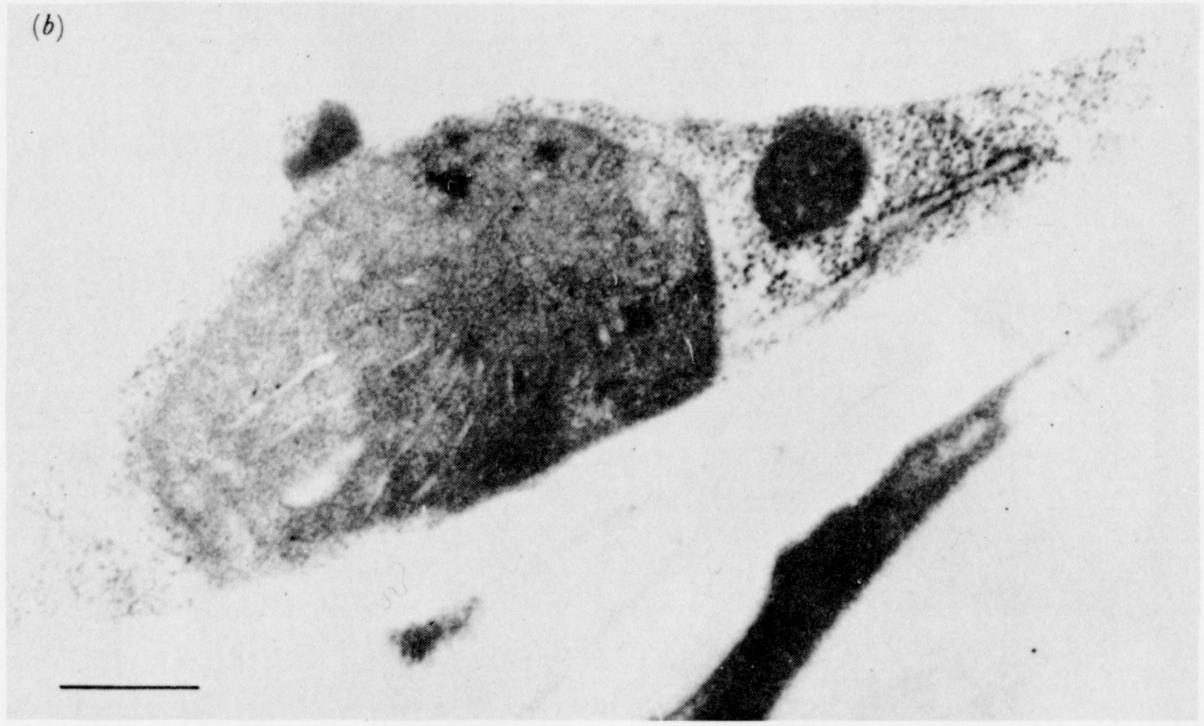

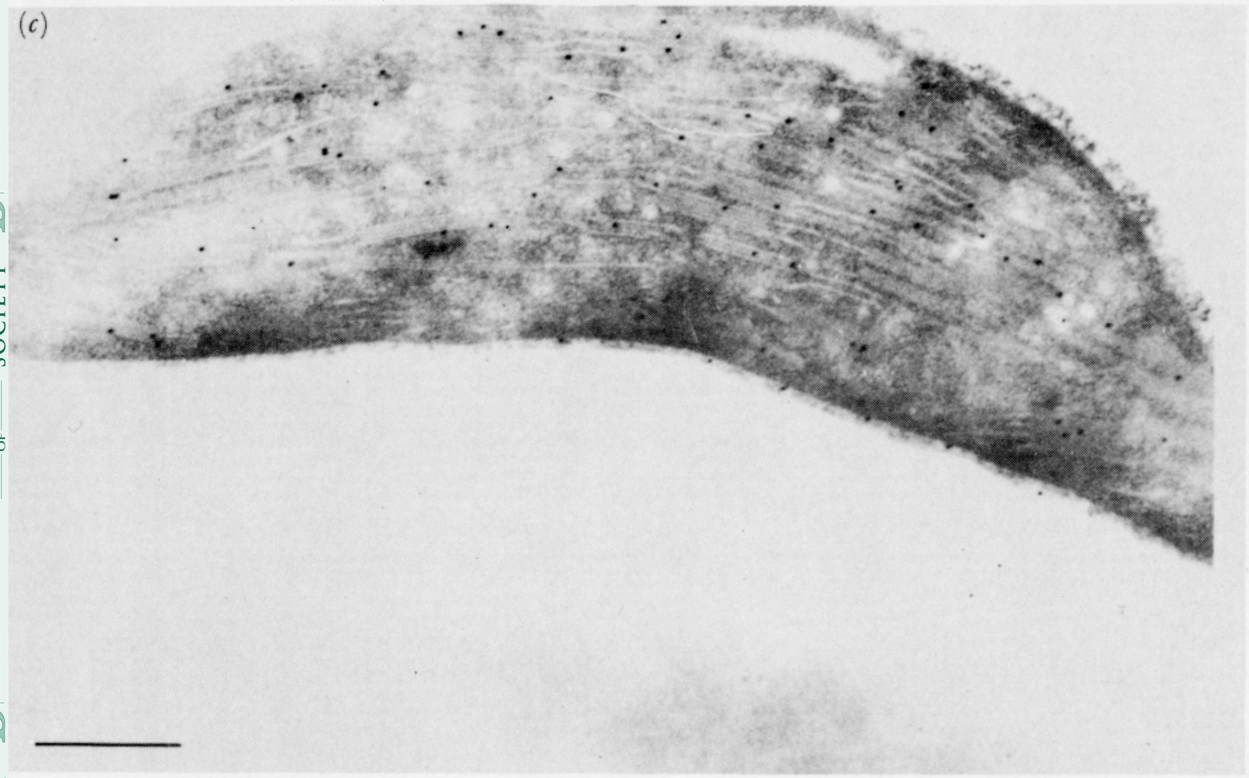

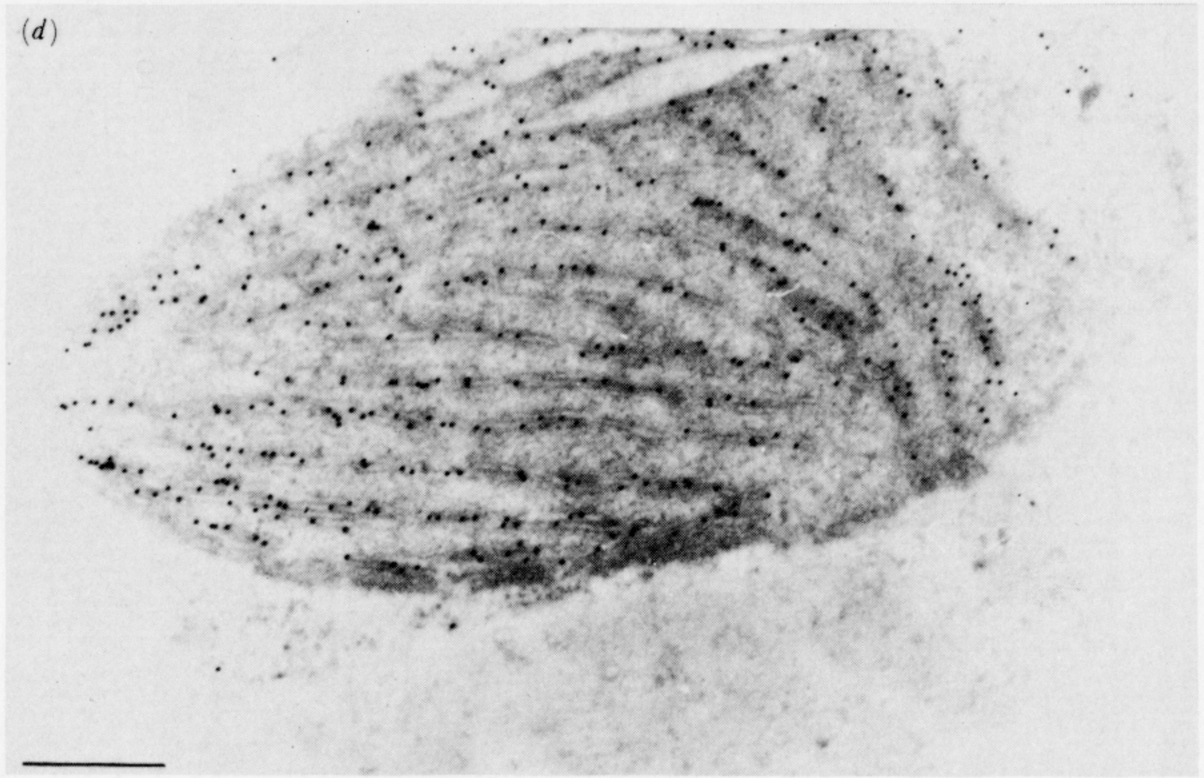

FIGURE 5. The light-induced accumulation of the P700 chlorophyll *a* apoprotein in dark-grown and illuminated barley seedlings. (*a*) Immunoblotting of the chlorophyll-binding protein: equal amounts of total leaf protein were subjected to SDS polyacrylamide gel electrophoresis and subsequently electroblotted on to nitrocellulose. The nitrocellulose blot was reacted with an antiserum against the P700 chlorophyll *a* apoprotein and immunostained. The six tracks represent different times of illumination. (*b–d*) Immunogold labelling of the P700 chlorophyll *a* protein (*b*, *c*) and the light-harvesting chlorophyll *a*/*b* protein (*d*) in leaves of dark-grown (*b*) and 12 h illuminated barley seedlings (*c*, *d*). Sections of Lowicryl-embedded tissue slices were reacted with the antisera followed by 10 nm protein-A–gold. Bars represent 0.5 μm. (Data from Kreuz *et al.* (1986) and Dehesh *et al.* (1986).)

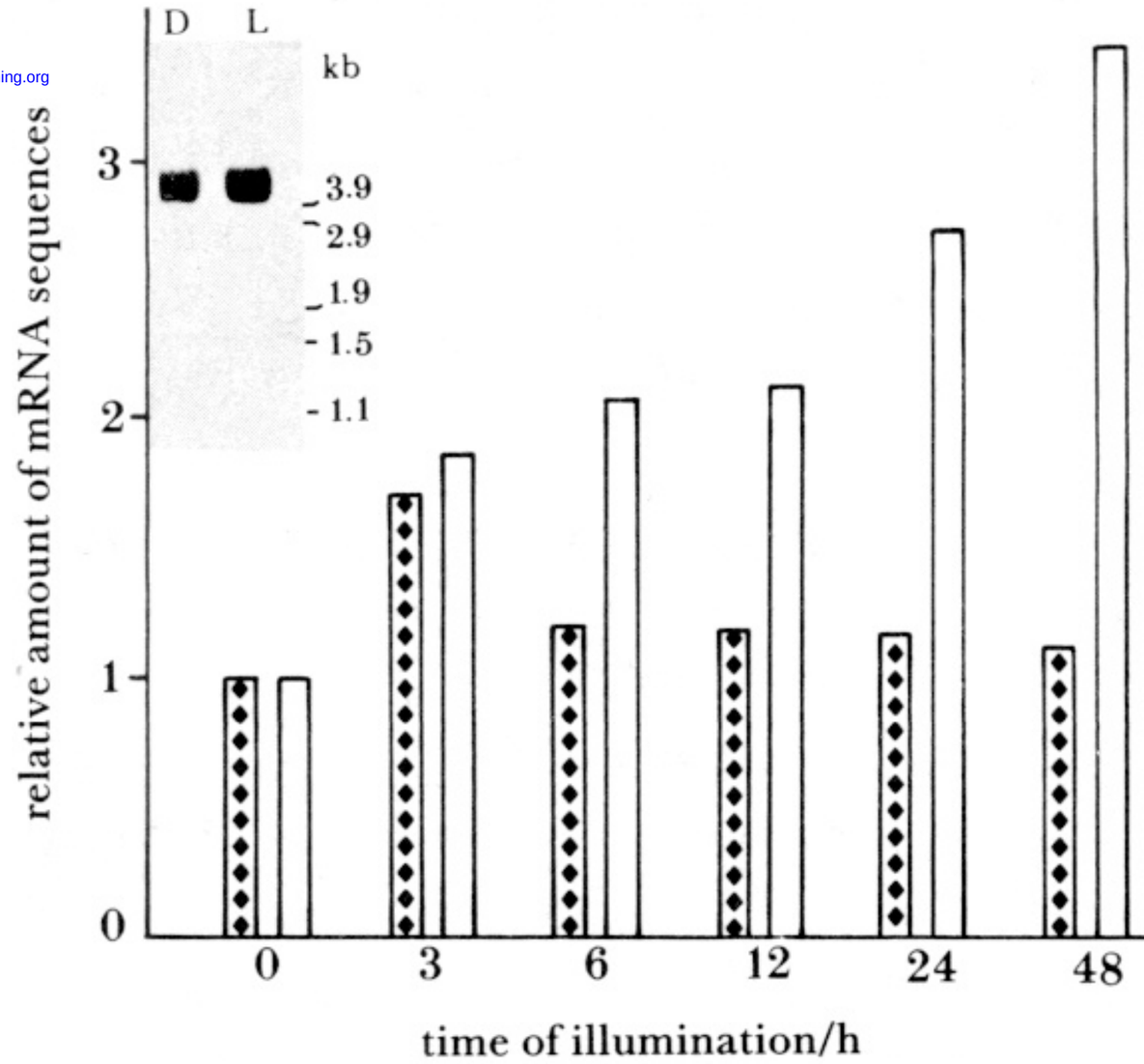

FIGURE 6. Light-induced changes in the relative amounts of transcripts encoding the P700 chlorophyll *a* apoprotein and the 32 kDa herbicide-binding protein of barley. Seedlings were grown either in total darkness or under continuous white light for various lengths of time. Total leaf RNA was prepared from the different plant samples and the relative amounts of mRNA sequences for the P700 chlorophyll *a* apoprotein and the 32 kDa herbicide-binding protein were determined by dot-blot hybridization. The dark levels of transcripts were set at unity. Open columns, mRNA sequence levels for the 32000 protein; hatched columns, mRNA sequence levels for the P700 chlorophyll *a* apoprotein. Inset: Northern blot analysis of the transcript of the P700 chlorophyll *a* apoprotein gene in dark-grown (D) and illuminated (L) barley seedlings. Numerals indicate the sizes (in kilobases) of the cytoplasmic and plastid rRNAs of barley and of *E. coli* 23 *S* and 16 *S* rRNA. (Data from Kreuz *et al.* (1986).)