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Light Suppresses 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase Gene Expression in *Arabidopsis thaliana*[1]

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3-Hydroxy-3-methylglutaryl (HMG) coenzyme A reductase mRNA accumulates preferentially in dark-grown Arabidopsis plants. As one step toward understanding the role that light plays in the regulation of the mevalonate pathway in plants, we characterized the suppression of *HMG1* gene expression in response to illumination wavelength, duration, and fluence rate. The accumulation of *HMG1* mRNA by dark treatment is suppressed by continuous exposure to white light and is dependent on the amount of light perceived during the period of illumination. By using promoter/reporter gene fusions we also demonstrate that this reaction is mediated by *cis*-acting elements that reside in the Arabidopsis *HMG1* promoter and, therefore, is likely to be controlled at the transcriptional level. *HMG1* expression is differentially responsive to continuous blue and red light but not to far-red light. In contrast, changes in *HMG1* mRNA levels were not observed in response to brief light pulses of any spectrum, suggesting that continuous illumination is required for sustained and maximal suppression of HMG coenzyme A reductase expression. Taken together, these data indicate that light-mediated control of the *HMG1* gene is mediated by a regulatory circuit that monitors aspects of both spectral quality and fluence and involves either multiple photoreceptors or a single photoreceptor that is differentially sensitive to both blue and red light.

Light affects the characteristics of plant growth and development at virtually every stage of the plant life cycle and influences both morphogenic features and intracellular metabolism. In many cases, the physiological responses triggered by photoperception are mediated by changes in gene expression and are accomplished by the coordinate activation or suppression of specific batteries of light-regulated genes (Tobin and Silverthorne, 1985; Gilmartin et al., 1990).

Light has been shown to activate the expression of gene families that encode proteins involved in photosynthesis, nitrogen metabolism, and flavonoid biosynthesis. For many genes, such as those encoding the small subunit of ribulose bisphosphate carboxylase, the Chl *a*/*b*-binding proteins, and chalcone synthase, among others, the role of light in this induction is well understood and is mediated by phytochrome and/or a blue-light receptor at the level of transcriptional initiation (Coruzzi et al., 1984; Silverthorne and Tobin, 1984; Berry-Lowe and Meagher, 1985; Simpson et al., 1985; Fluhr et al., 1986; Kaulen et al., 1986; Feinbaum and Ausubel, 1988; Quail, 1991). Light has also been shown to regulate nitrate reductase gene expression and appears to be involved both directly, by acting in etiolated plants through phytochrome and perhaps a blue-light receptor (Rajasekhar et al., 1988; Melzer et al., 1989), and indirectly, by affecting the rate of photosynthesis in green plants (Cheng et al., 1992). Promoter analysis of these genes has revealed some of the *cis*-acting elements and *trans*-acting regulatory factors that may function in this light-activated transcriptional control (Li et al., 1993). In addition, recent genetic studies have provided important insights into the signal transduction pathways and their component factors that mediate developmental control in response to changes in the light environment (Chory, 1992, 1993; Deng, 1994; Quail, 1994).

In contrast to our understanding of the control mechanisms by which gene expression is induced by light, relatively little is known about light-mediated repression of gene expression. In fact, only a few identified genes, including phytochrome (Colbert et al., 1983; Lissemore and Quail, 1988; Kay et al., 1989), *NPR1–3* (Okubara and Tobin, 1991), Pchlide reductase (Mosinger et al., 1985), and Asn synthetase (Tsai and Coruzzi, 1991), have been demonstrated to be regulated in this fashion. The negative regulation of these genes by low-fluence light exhibits the reversible red/far-red spectral response that is characteristic of many phytochrome-mediated events. Thus, the same primary event, red-light perception by phytochrome, is capable of triggering both induction and suppression of gene expression.

Recently, the genes that encode HMG CoA reductase have been isolated from *Arabidopsis thaliana* (Caelles et al., 1989; Learned and Fink, 1989; Enjuto et al., 1994), and a preliminary report indicated that the levels of both *HMG1* and *HMG2* mRNA are elevated in dark-grown plants (Enjuto et al., 1994). Here, we explicitly demonstrate that *HMG1* gene expression is suppressed by light and describe some of the novel characteristics of this regulatory response. HMG CoA reductase (EC 1.1.1.34) catalyzes the NADPH-dependent reduction of HMG CoA to MVA in the first committed step of the isoprenoid biosynthetic pathway. In higher plants, MVA serves as the precursor for a broad array of isoprenoid compounds that play a variety of

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Abbreviations: *CAB*, genes encoding Chl *a*/*b*-bindng proteins; CaMV, cauliflower mosaic virus; HMG, 3-hydroxy-3-methylglutaryl; *HMG1*, the gene encoding HMG CoA reductase; MVA, mevalonic acid; rDNA, ribosomal DNA; *rRNA*, genes encoding 18S and 28S rRNAs.

roles in the metabolism, growth, and development of the plant. Multiple branches in the pathway give rise to numerous products, including sterols, dolichol, growth regulators (such as cytokinin, GA, and ABA), photosynthetic pigments, phytotoxins, phytoalexins, and a variety of specialized terpenoids. However, even though the regulated biosynthesis of these metabolites is thought to be essential to the normal processes of plant growth and development, the mechanisms that control isoprenoid biosynthesis are currently poorly understood, and the key control points in the pathway have not yet been identified (Gray, 1987).

As a first step toward understanding HMG CoA reductase regulation in Arabidopsis, we examined the effects of light on *HMG1* mRNA accumulation and studied the expression of the *HMG1* gene in response to illumination wavelength, duration, and fluence rate. This study provides evidence for the light-mediated suppression of *HMG1* transcription and is an initial framework for investigating the role of environmental cues in the regulation of the mevalonate pathway.

MATERIALS AND METHODS

Materials

[α-^{32}P]dATP (3000 Ci/mmol) was from Amersham or New England Nuclear. All enzymes were obtained from commercial sources and used according to suppliers' specifications.

Construction of the Chimeric *HMG1:uidA* Gene

The 2550-nucleotide *Hin*dIII/*Stu*I fragment of genomic *HMG1* DNA previously isolated from Arabidopsis (Learned and Fink, 1989) was transferred from pBluescript S/K to the *Hin*dIII and *Sma*I sites of pBI101.3 (Jefferson et al., 1987), creating a translational fusion between the *HMG1* upstream regulatory sequences and the *uidA* gene from *Escherichia coli*. The resulting construct includes approximately 2500 bp of the upstream flanking sequences from the *HMG1* gene, as well as the untranslated leader region and 19 nucleotides from the *HMG1* coding sequence (−2459 to +90, relative to the site of transcriptional initiation). Translation of RNA derived from this chimeric gene is initiated at the AUG initiation codon used in native *HMG1* mRNA. The resulting plasmid was designated pML36, and the details of the promoter fusion are shown in "Results."

Plant Transformation and Regeneration

The binary vector plasmid containing the chimeric *HMG1:uidA* gene (pML36) was introduced by direct transfection into LBA4404, a disarmed strain of *Agrobacterium tumefaciens* (Hoekma et al., 1983). Transformation of Arabidopsis roots (No-0 ecotype) and regeneration of kanamycin-resistant calli into fertile plants was carried out using the procedure of Valvekens et al. (1988). T_1 seed obtained from self-fertilization of the primary transformants were grown aseptically on germination media supplemented with 25 μg/mL kanamycin under continuous light and scored for resistance to the antibiotic. Kanamycin-resistant plants were transferred to soil and the T_2 seeds resulting from self-fertilization were harvested. A collection of 15 independent transformants was analyzed for the light-dependent patterns of *HMG1:uidA* gene expression, and based on the characteristics of β-glucuronidase expression, six of these transgenic plants were identified for more extensive analysis.

Plant Growth Conditions

Plant Material

The Columbia ecotype of *Arabidopsis thaliana* (L.) Henyh was used exclusively in this work, except as indicated. General methods for the growth and handling of Arabidopsis were as described by Somerville and Ogren (1982). Seeds were sterilized by treatment with 25% bleach for 15 min, followed by extensive rinsing with sterile water. Seeds were treated for 1 to 2 d in the dark at 4°C and then sown on germination medium nutrient agar (Valvekens et al., 1988) containing 0.5 × Murashige-Skoog salts (GIBCO-BRL) supplemented with 1% Suc. Sterile cellophane discs were routinely placed on the agar surface to prevent root penetration and to facilitate harvesting of the plants for RNA isolation. Plants were grown at 20°C under Sylvania cool-white fluorescent lights at an intensity of approximately 80 μmol m^{-2} s^{-1}. Light was excluded from plants by wrapping the Petri plates in several layers of aluminum foil and/or transfer to a growth chamber maintained in complete darkness.

Light Sources

For experiments involving continuous white light, 40-W Sylvania cool-white fluorescent bulbs provided the source of illumination. White light irradiance was 80 μmol m^{-2} s^{-1} unless indicated otherwise. The treatment of plants with specific wavelengths of light was accomplished by filtering white light generated from a 40-W Sylvania cool-white bulb (F40C) through red (2450) or blue (2424) plexiglass filters. The irradiance produced by these light sources measured at the plant level was 34 or 18 μmol m^{-2} s^{-1}, respectively. Far-red light was provided by two Sylvania F48T12/232 fluorescent tubes filtered through far-red FRS700 plexiglass (dye No. 58015; Rohm and Haas, Philadelphia, PA) and placed approximately 10 cm from the plants. Under these conditions, this light source delivered 34 μmol m^{-2} s^{-1} at the plant level. For experiments involving high-intensity light pulses, a Sylvania quartz halogen CZA CZB projector lamp was used as the light source. The fluence rate measured at plant level for unfiltered white light was 2.3×10^3 μmol m^{-2} s^{-1}, whereas the irradiance for light filtered through blue plexiglass or red plexiglass was 0.3×10^3 or 0.8×10^3 μmol m^{-2} s^{-1}, respectively. Narrow-waveband light was generated using a 250-W quartz-halogen lamp filtered through a Ditric Optics (Hudson, MA) 10-nm bandpass interference filter (660 nm) for the red-light experiments or through 0.125-inch black plexiglass (Rohm and Haas FRS 700, dye No. 58015) for the far-red light experiments. Under these conditions, the irra-

diance at the sample was approximately 30 μmol m^{-2} s^{-1}. In all cases, fluence rates were measured with an LI-1800 spectroradiometer (Li-Cor, Lincoln, NE).

Isolation of RNA and Blot Hybridization Analysis

Total RNA was prepared from whole-plant tissue by grinding in liquid nitrogen and extraction of the nucleic acids as described by Nagy et al. (1988). Five micrograms of each RNA sample were covalently modified by treatment with glyoxal (McMaster and Carmichael, 1977) and fractionated by electrophoresis through a 1% agarose gel containing 10 mM $NaPO_4$, pH 6.5. RNA was blotted by capillary transfer to a sheet of Nytran$^+$ membrane (Schleicher & Schuell) overnight in 10× SSC, according to the recommendations of the manufacturer. Following transfer, the RNA was covalently cross-linked to the membrane by UV irradiation. Filter hybridization to ^{32}P-labeled probes was performed in 5× SSC, 10mM KPO_4, pH 7.4, 50% formamide, 100 μg/mL denatured herring sperm DNA, 0.1% (w/v) SDS, 10% dextran sulfate for 14–24 h at 42°C (Ausubel et al., 1987). The membrane was then washed twice with 2× SSC, 0.1% SDS at room temperature and twice with 0.1× SSC, 0.1% SDS at 65°C for 15 min. The blots were exposed to x-ray film (Kodak XAR-5) for 5 to 36 h. Relative amounts of mRNAs were quantified on a BAS1000 Phosphorimager (Fuji, Tokyo, Japan) following exposure of the imaging screen to the RNA blot.

Radiolabeled probes were prepared by random priming of gel-purified DNA fragments (Feinberg and Vogelstein, 1983). The probe for *HMG1* mRNA was a 2.3-kb full-length Arabidopsis *HMG1* cDNA (Learned and Fink, 1989). This fragment is gene specific and shows no cross-hybridization with the Arabidopsis *HMG2* gene under the conditions described (data not shown). mRNA derived from the family of Chl *a*/*b*-binding proteins was detected using the 1.4-kb *Hin*dIII fragment from the *CAB140* gene of Arabidopsis (Leutwiler et al., 1986). The 1.0- and 1.6-kb fragments containing 18S and 25S coding sequences from the radish rDNA repeats (Delseny et al., 1983) were used as probes for rRNA.

RESULTS

HMG CoA Reductase Gene Expression Is Regulated by Light in Seedlings and Mature Plants

The number and diversity of essential MVA-derived compounds in higher plants dictates that adequate quantities of this precursor be available in all cells that carry out isoprenoid biosynthesis. Therefore, the enzymatic production of MVA can be considered a general housekeeping function that requires basal expression of the gene(s) encoding HMG CoA reductase. Regulated expression of HMG CoA reductase could occur in response to specific metabolic requirements or environmental signals, allowing the levels of HMG CoA reductase and the corresponding rates of MVA synthesis to be adjusted according to physiological conditions. Previous studies indicated that the levels of HMG CoA reductase enzyme activity were higher in etiolated seedlings than in light-grown green seedlings (Brooker and Russell, 1975), suggesting the possibility that MVA synthesis might be regulated in response to changing light conditions. With the development of specific probes for the *HMG1* gene, we initiated a series of experiments to examine the molecular basis for light-mediated suppression of HMG CoA reductase in Arabidopsis.

To determine whether light affects the expression of the *HMG1* gene, blot hybridization analysis was used to compare mRNA levels from Arabidopsis plants grown under continuous light with those from plants that had been subjected to a dark treatment. In this experiment, RNA was isolated from seedlings that were grown for 6 d under continuous light, 6 d in complete darkness, or for 5 d under continuous light followed by dark adaptation for 24 h (Fig. 1, lanes 1, 2, and 3, respectively). RNA was also prepared and analyzed from mature Arabidopsis plants that had been grown for 3 weeks under continuous light and from plants that were dark adapted for 24 h (Fig. 1, lanes 4 and 5, respectively). At both developmental stages, the steady-state levels of *HMG1* mRNA were approximately 5-fold higher in the plants exposed to darkness for 24 h compared to plants that had been maintained in the light. Furthermore, both etiolated and dark-adapted seedlings exhibited the same high levels of *HMG1* mRNA. These data demonstrate that the *HMG1* gene is expressed in both light-grown and dark-grown Arabidopsis plants but that the steady-state levels of *HMG1* mRNA accumulate to high levels in plants subjected to dark treatment.

Kinetics of Light-Mediated Changes in *HMG1* mRNA Levels in Arabidopsis Plants

As a first step toward characterizing this regulatory response to white light, we examined the time course of light effects on *HMG1* expression. In these experiments, the steady-state levels of *HMG1* mRNA in Arabidopsis plants were monitored during the 24-h period following a change in illumination. Figure 2A shows the kinetics of *HMG1* mRNA accumulation after plants were transferred from continuous white light into complete darkness. The increase in *HMG1* mRNA was detectable after 2 to 4 h of dark treatment and reached a maximum level after approximately 12 to 24 h (Fig. 2A, lanes 4–6). The high steady-state level of *HMG1* mRNA acquired during dark adaptation was maintained for an indefinite period in the absence of light.

The reciprocal response was studied by examining *HMG1* gene expression in plants that were dark adapted for 48 h and subsequently returned to continuous white light. Following light deprivation, *HMG1* mRNA is detected at the high levels characteristic of dark-adapted plants (Fig. 2B, lane 1). However, within several hours of exposure to white light, *HMG1* mRNA levels begin to diminish and ultimately return to their low basal concentration after approximately 24 h of light treatment (Fig. 2B, lane 6). The data provide further evidence that *HMG1* gene expression is regulated in response to light conditions. Furthermore, the reciprocal nature of this response indicates that the accumulation of *HMG1* mRNA in Arabidop-

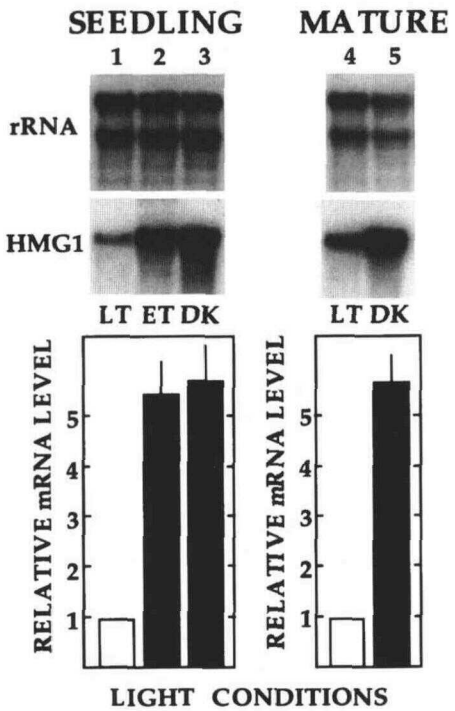

Figure 1. Light regulation of HMG CoA reductase gene expression in Arabidopsis. Total RNA was isolated from 6-d-old Arabidopsis seedlings or from 21-d-old mature plants grown under different light conditions and fractionated by agarose gel electrophoresis. Blot hybridization analysis was carried out using ^{32}P-labeled DNA probes derived from the Arabidopsis *HMG1* cDNA (Learned and Fink, 1989). As an internal control, rRNA was detected using radish rDNA as a probe (Delseny et al., 1983). The sources of RNA are as follows: lane 1, 6-d-old seedlings grown under continuous light (LT); lane 2, 6-d-old seedlings grown under continuous dark (ET); lane 3, 6-d-old seedlings grown for 5 d under continuous light followed by dark treatment for 24 h (DK); lane 4, mature plants grown under continuous light (LT); and lane 5, 21-d-old plants grown under continuous light for 20 d followed by 24 h of dark adaptation (DK). Bottom, The intensities of radioactive signals visualized by autoradiography were quantified using a BAS1000 Phosphorimager. Relative levels of *HMG1* mRNA are shown, normalized to the values determined in light-grown plants, and represent the means of at least three independent trials. The vertical bars indicate SE.

sis is induced by dark treatment and suppressed by exposure to white light.

HMG CoA Reductase Gene Expression Is Responsive to Light Irradiance

Next, we wanted to test the possibility that light-regulated expression of the *HMG1* gene is dependent on the amount of light delivered to the plant, and to address this question, we measured the steady-state levels of HMG CoA reductase mRNA as a function of fluence rate. Five-day-old etiolated seedlings were removed from the dark and transferred to a growth incubator that was illuminated by a single bank of fluorescent lamps located at the top of the chamber. In this way, a gradient of light intensity was established and samples placed on different levels within the chamber were exposed to different fluence rates depending on their distance from the light source. RNA was isolated from these plants after 24 h of illumination, and blot hybridization analysis was performed. The results of this experiment are shown in Figure 3 and summarized in the bottom panel. In this experiment, the accumulation of *HMG1* mRNA was inversely related to fluence rate, with the highest levels corresponding to plants maintained in complete darkness (Fig. 3, lane 1), and the lowest levels associated with plants illuminated under normal light conditions (Fig. 3, lane 6). Intermediate levels of *HMG1* mRNA were detected when plants were exposed to reduced light irradiance for the 24-h photoperiod (Fig. 3, lanes 2–5). It is interesting that, although maximal suppression of *HMG1* expression required illumination by light sources of at least moderate intensity (80 μmol m^{-2} s^{-1}), complete darkness was not necessary for the accumulation of high levels of *HMG1* mRNA. Therefore, the negative effect of white light on *HMG1* gene expression appears to depend on fluence rate, with the levels of mRNA being determined by the amount of light perceived during the period of continuous illumination.

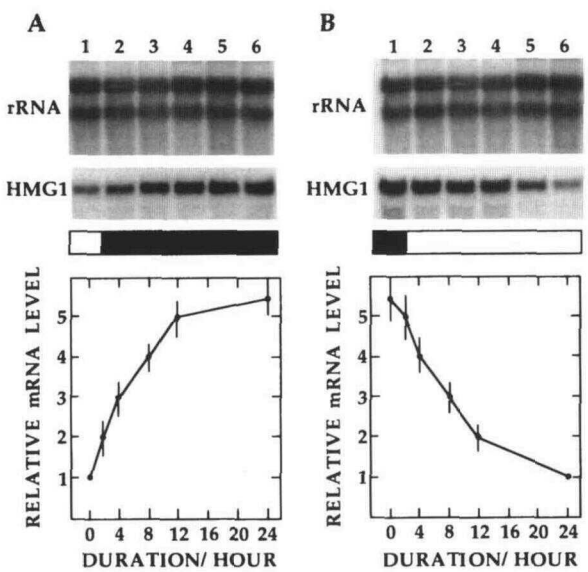

Figure 2. Alterations in the steady-state levels of *HMG1* mRNA in response to changes in illumination. A, Total RNA was isolated from mature Arabidopsis plants grown under continuous light (lane 1) and transferred to the dark for 2, 4, 8, 12, or 24 h (lanes 2–6, respectively). B, Total RNA was isolated from mature Arabidopsis plants grown under continuous illumination, transferred to the dark for 48 h (lane 1), and returned to the light for 2, 4, 8, 12, or 24 h (lanes 2–6, respectively). Following transfer to a nylon membrane, RNA was detected using uniformly labeled DNA probes from either the Arabidopsis *HMG1* cDNA or radish rDNA. The intensities of radioactive signals visualized by autoradiography were quantified using a BAS1000 Phosphorimager. Relative levels of *HMG1* mRNA are shown, normalized to the values determined in light-grown plants, and represent the means of three independent trials. The vertical bars indicate SE.

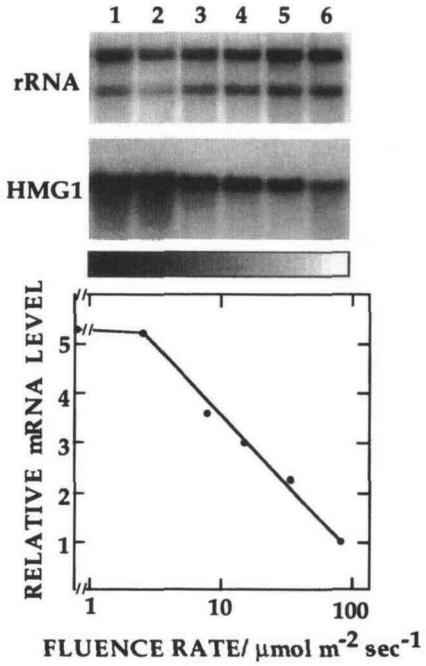

Figure 3. Irradiance-dependent *HMG1* expression in Arabidopsis. Arabidopsis plants were grown in complete darkness for 5 d on sterile nutrient medium and transferred to an illuminated growth chamber for 24 h. This incubator had a single light source, and fluence rate was varied by distributing plants at different positions within chamber. Total RNA was prepared from these plants and fractionated by agarose gel electrophoresis, and blot hybridization was used to compare the levels of *HMG1* mRNA in the different samples. rRNA levels were also measured as a control. RNA was isolated from plants grown under the following light conditions: continuous darkness (lane 1), or continuous light exhibiting fluence rates of 2 μmol m^{-2} s^{-1} (lane 2), 8 μmol m^{-2} s^{-1} (lane 3), 15 μmol m^{-2} s^{-1} (lane 4), 30 μmol m^{-2} s^{-1} (lane 5), and 80 μmol m^{-2} s^{-1} (lane 6). Bottom, The intensities of radioactive signals visualized by autoradiography were quantified using a BAS1000 Phosphorimager. Relative levels of *HMG1* mRNA are shown, normalized to the values determined in light-grown plants, and represent the means of two trials.

Effects of Continuous White, Blue, or Red Light on *HMG1* mRNA Levels in Etiolated Arabidopsis Seedlings

Plants have a variety of photoreceptors that are capable of absorbing and interpreting light by monitoring features such as spectral quality, intensity, duration, and direction. The effects of filtered light on *HMG1* gene expression were investigated in an attempt to identify the photoreceptor system(s) that mediate the light response. Five-day-old etiolated seedlings were removed from complete darkness and treated with continuous white light (80 μmol m^{-2} s^{-1}), blue light (18 μmol m^{-2} s^{-1}), red light (34 μmol m^{-2} s^{-1}), or far-red light (34 μmol m^{-2} s^{-1}) for 24 h, at which time plants were harvested and RNA samples were prepared.

As shown in Figure 4, the amount of *HMG1* mRNA was reduced by treatment with either blue light (Fig. 4, lane 3) or red light (Fig. 4, lane 4) when compared to the induced levels of *HMG1* mRNA detected in dark-grown plants (Fig. 4, lane 1). Under these conditions, *HMG1* expression was suppressed more effectively by blue light than red light, although exposure to either light source established a limited response in which *HMG1* mRNA levels failed to attain the basal level observed following treatment with white light (Fig. 4, lane 2). In contrast, illumination with far-red light had virtually no effect on the accumulation of *HMG1* mRNA, and high levels of expression were observed even after prolonged exposure to far-red light (Fig. 4, lane 5).

One explanation for these observations is that the effects of light on *HMG1* expression are mediated by multiple photoreceptors and that changes in the *HMG1* mRNA levels are regulated independently in response to light from the red and blue portions of the spectrum. Two experiments were designed to address this possibility. First, 5-d-old etiolated seedlings were illuminated with continuous red light for 24 h and then treated with blue light for an additional 24 h. As shown in Figure 4, *HMG1* mRNA levels decreased dramatically upon exposure to continuous blue light following a red-light treatment (lane 6). In contrast, when the order of exposure was reversed and seedlings were adapted first to blue light and then to red light, *HMG1* mRNA accumulated to the levels characteristic of etiolated plants treated with red light alone (Fig. 4, lane 7). Finally, when illumination was provided simultaneously by both the blue- and red-light sources, nearly full suppression of *HMG1* expression was observed (Fig. 4, lane 8). These data provide further evidence that *HMG1* gene expression responds to light from both the red and blue portions of the electromagnetic spectrum and, furthermore, that neither red light nor blue light alone is sufficient at the fluence rates tested for full, white-light-mediated suppression in Arabidopsis seedlings.

Second, to further characterize the role of spectral quality in the light-regulated expression of HMG CoA reductase and to distinguish among the responses triggered by white, blue, and red light, we examined the kinetics of *HMG1*

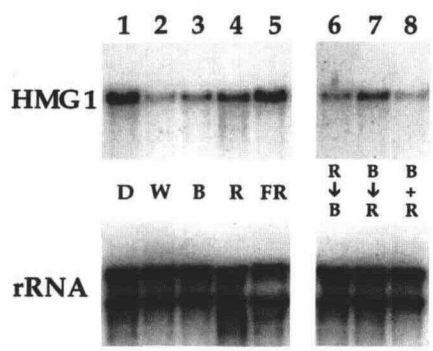

Figure 4. Suppression of *HMG1* gene expression by blue and red light in etiolated Arabidopsis seedlings. Five-day-old dark-grown Arabidopsis seedlings were maintained in complete darkness (D, lane 1) or transferred to continuous white light (W, lane 2), blue light (B, lane 3), red light (R, lane 4), or far-red light (FR, lane 5) for 24 h. Additional sets of etiolated seedlings were treated either with red light for 24 h followed by blue light for an additional 24 h (R → B, lane 6), with blue light followed by red light (B → R, lane 7), or with a combination of both blue- and red-light sources for 24 h (B + R, lane 8). Total RNA was fractionated by agarose gel electrophoresis and subjected to analysis by blot hybridization analysis using either labeled *HMG1* cDNA or rDNA as a probe.

suppression. In this experiment, etiolated seedlings (6 d) were exposed to continuous white, blue, red, or far-red light and harvested at 8-h intervals for the first 48 h and after 72 h of illumination. Total RNA was isolated from these samples and subjected to blot hybridization analysis. As shown in Figure 5, high steady-state levels of *HMG1* mRNA were detected in samples isolated from plants maintained in complete darkness throughout the duration of the experiment (Fig. 5: DARK, lanes 1–8). In contrast, when seedlings were exposed to continuous white light, the steady-state levels of *HMG1* mRNA progressively decreased during the first 16 to 24 h (Fig. 5: WHITE, lanes 1–4), at which time the minimum basal expression level was established and maintained for at least 72 h (Fig. 5: WHITE, lanes 5–8). Similarly, illumination with blue light also reduced the amount of *HMG1* mRNA (Fig. 5: BLUE, lanes 1–8). Moreover, the response kinetics were similar for plants exposed to either white or blue light, characterized by an initial phase during the first 24 h of illumination when *HMG1* mRNA levels were being reduced relatively rapidly. Less dramatic changes in *HMG1* expression were observed during the subsequent 48 h of illumination. In contrast, the time course for red-light-mediated suppression was comparatively slow and appears to occur more gradually over the entire 72-h photoperiod (Fig. 5: RED, lanes 1–8). At the early times, red light was less effective in triggering this photoresponse than either white or blue light, whereas at the later times, less marked differences between different light sources were observed. Finally, *HMG1* gene expression was unaffected by exposure to continuous far-red light for up to 72 h of illumination (Fig. 5: FAR-RED, lanes 1–8). Treatment with continuous light from any of these sources had no affect on the steady-state levels of rRNA in Arabidopsis seedlings (data not shown).

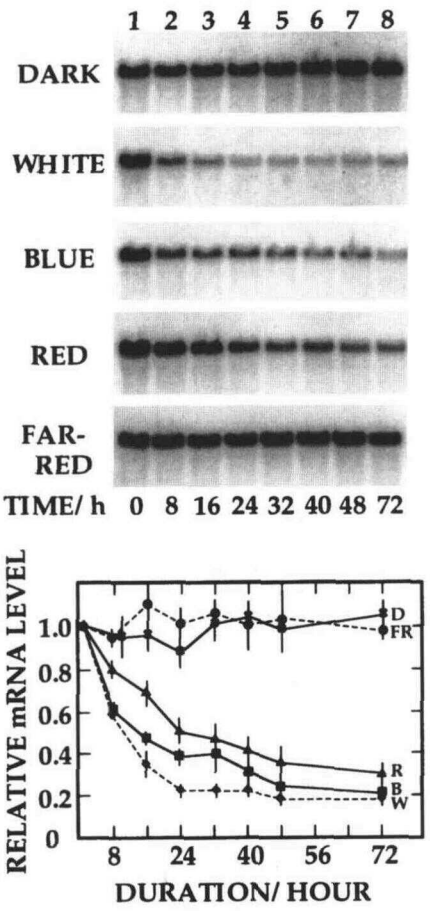

Figure 5. Kinetics of HMG1 suppression in response to continuous blue, red, and far-red light. Five-day-old dark-grown Arabidopsis seedlings were maintained in complete darkness (DARK) or exposed to continuous white light (WHITE), blue light (BLUE), red light (RED), or far-red light (FAR-RED). Plants were harvested after 8 h (lane 2), 16 h (lane 3), 24 h (lane 4), 32 h (lane 5), 40 h (lane 6), 48 h (lane 7), and 72 h (lane 8) of illumination. Total RNA was fractionated by agarose gel electrophoresis and subjected to analysis by blot hybridization using either labeled *HMG1* cDNA or rDNA as a probe. Bottom, The graph shown summarizes the results of this experiment. The intensities of radioactive signals visualized by autoradiography were quantified using a BAS1000 Phosphorimager, and the relative levels of *HMG1* mRNA were calculated by normalizing each experimental value to the level detected in dark-grown seedlings. Each data set is represented by the mean of three independent trials. Vertical bars indicate the SE. D, Complete darkness; FR, far-red light; R, red light; B, blue light; W, white light.

Effects of Light Pulses on *HMG1* Expression in Etiolated Seedlings

The above results establish that the suppression of HMG CoA reductase gene expression is regulated at the level of mRNA accumulation by exposure to continuous light. Because many photoregulated responses to light, including the modulation of gene expression, can be triggered by brief periods of illumination and do not require continuous exposure to light, we tested the effect of high-intensity light pulses on the steady-state levels of *HMG1* mRNA. Etiolated Arabidopsis seedlings (6 d) were treated for 5 min with white (2.3×10^3 μmol m^{-2} s^{-1}), blue (0.3×10^3 μmol m^{-2} s^{-1}), or red (0.8×10^3 μmol m^{-2} s^{-1}) light. Following illumination, the plants were returned to complete darkness for periods of 6 or 12 h, at which time seedlings were harvested. Blot hybridization analysis of these RNA samples is shown in Figure 6. These experiments showed that brief exposure to light from any of these sources failed to elicit detectable changes in the levels of *HMG1* mRNA isolated either 6 or 12 h after illumination (Fig. 6, lanes 1–14). Thus, these high-intensity light pulses were ineffective at suppressing *HMG1* expression, even though the effects of both filtered and unfiltered light were clearly observed when seedlings were exposed to relatively long periods of continuous illumination.

To ensure that these pulsed light treatments were effective in eliciting an appropriate response from a photoregulated target gene, we monitored changes in the levels of mRNA encoded by the *CAB* gene family. As expected, short pulses of either white (Fig. 6, lanes 3 and 4), blue (Fig. 6, lanes 5 and 6), or red (Fig. 6, lanes 7 and 8) light were sufficient to induce dramatic increases in steady-state levels of *CAB* mRNA monitored either 6 or 12 h after illumination. In addition, expression from the *CAB* genes was affected when etiolated seedlings were treated with a pulse of narrow-waveband light. *CAB* mRNA accumulated to

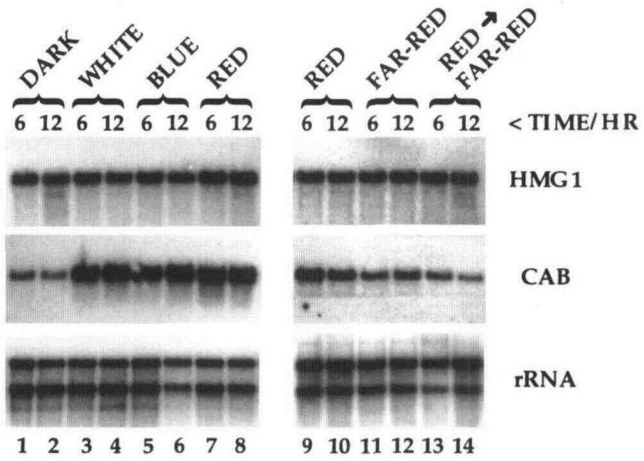

Figure 6. Effects of light pulses on the accumulation of *HMG1* mRNA in etiolated seedlings. Etiolated Arabidopsis seedlings (6 d) received a 5-min pulse of illumination and were subsequently returned to complete darkness for either 6 h (lanes 1, 3, 5, 7, 9, 11, and 13) or 12 h (lanes 2, 4, 6, 8, 10, 12, and 14). Total RNA was isolated from these samples and subjected to blot hybridization analysis using *HMG1*, *CAB140*, or rDNA for probes as indicated. Light treatments were as follows: darkness (lanes 1 and 2), white (unfiltered) light (lanes 3 and 4), blue light (lanes 5 and 6), and red light (lanes 7 and 8). Plants were also treated with narrow-waveband light as follows: red light (lanes 9 and 10), far-red light (lanes 11 and 12), and red light followed immediately by far-red light (lanes 13 and 14).

high levels in response to a single pulse of red light (Fig. 6, lanes 9 and 10), whereas far-red light alone was sufficient to induce much lower levels of *CAB* expression (Fig. 6, lanes 11 and 12). Furthermore, the induction of *CAB* expression by red light could be partially reversed by subsequent treatment with far-red light (Fig. 6, lanes 13 and 14), although under these conditions steady-state levels of *CAB* mRNA did not return to the basal level observed in etiolated seedlings. Taken together, these results suggest that continuous exposure to light is required for sustained and maximal suppression of HMG CoA reductase expression.

Light-Suppressed Expression of Arabidopsis *HMG1* Is Regulated at the Level of Transcription

Finally, we wanted to determine whether the effects of light on *HMG1* gene expression are mediated at the transcriptional level and, in particular, through *HMG1* promoter sequences. Our approach to this question was to exploit our ability to construct chimeric genes in vitro and to stably introduce these genes into Arabidopsis plants for analysis. Using *Agrobacterium*-mediated transformation (Valvekens et al., 1988), we introduced two different promoter/reporter gene fusions into Arabidopsis root explants (No-0 ecotype). First, a translational fusion was constructed in which expression of the *uidA*-coding region from *E. coli* was placed under the control of putative regulatory sequences (−2459 to +90 relative to transcription initiation) from the Arabidopsis *HMG1* gene (Fig. 7, middle). Conversely, to study the role that the HMG CoA reductase transcription unit plays in photoregulated expression, the Arabidopsis *HMG1* cDNA was placed under the transcriptional control of the highly active CaMV 35S promoter. The resulting construct includes the 5′ and 3′ untranslated regions of the *HMG1* mRNA, as well as the entire HMG CoA reductase-coding sequence, with translational initiation being directed by the AUG initiation codon used in the native *HMG1* mRNA (Fig. 7, bottom; Re et al., 1995). In this way, we can uncouple contributions to *HMG1* photoregulation from promoter-dependent transcriptional events and promoter-independent posttranscriptional processes. Light-regulated expression of these transgenes was analyzed in a collection of independently transformed plants, and the results from representative individual transformants are shown in Figure 7.

To determine whether light affects the expression of these genes, blot hybridization analysis was used to compare mRNA levels in plants grown under different illumination conditions. In this experiment, RNA was isolated from seedlings that were grown for 6 d under continuous light, 6 d under complete darkness, or 5 d under continuous light followed by dark adaptation for 24 h (Fig. 7, lanes 1, 2, and 3, respectively). RNA was also prepared and analyzed from mature Arabidopsis plants that had been grown for 3 weeks under continuous light, as well as from plants that were dark adapted for 24 h (Fig. 7, lanes 4 and 5).

First, we wanted to confirm that the suppression of *HMG1* mRNA accumulation we observed in wild-type Arabidopsis (Columbia ecotype) was also displayed in parental Nossen (No-0) strains of Arabidopsis. As shown in Figure 7, the steady-state levels of *HMG1* mRNA in both seedlings and mature plants were substantially higher in plants deprived of light compared to plants that had been continuously maintained in the light. Thus, light regulation of the *HMG1* gene is not confined to the Columbia ecotype in Arabidopsis. Furthermore, the identical pattern of *HMG1* mRNA accumulation was observed in transgenic plants carrying the *HMG1:uidA* gene fusion (R.M. Learned, unpublished data).

Next, we isolated RNA from No-T36 plant lines, containing the *HMG1:uidA* transgene, grown under different light conditions, and monitored changes in the levels of *uidA* mRNA in response to changing illumination. Blot hybridization analysis revealed that when *uidA* expression was controlled by sequences in the *HMG1* promoter *uidA* mRNA accumulated to a much higher level in both seedlings and mature plants that were subjected to dark treatment (Fig. 7, lanes 2, 3, and 5). Thus, both the native *HMG1* gene and the *HMG1:uidA* transgene exhibited the same pattern of photoregulated expression (Fig. 7). These results suggest that the sequences between −2459 and +90 in the *HMG1* gene are sufficient to confer light-regulated expression onto the *uidA* reporter gene.

In contrast, when the CaMV 35S promoter was used to drive the expression of the *HMG1* cDNA, high levels of *HMG1* mRNA were detected in both light- and dark-grown transgenic plants (Fig. 7). In these samples, *HMG1* mRNA was enriched approximately 40-fold relative to the parental No-0 strain and represents contributions from both the *CaMV 35S:HMG1* transgene and the native *HMG1* gene (Re

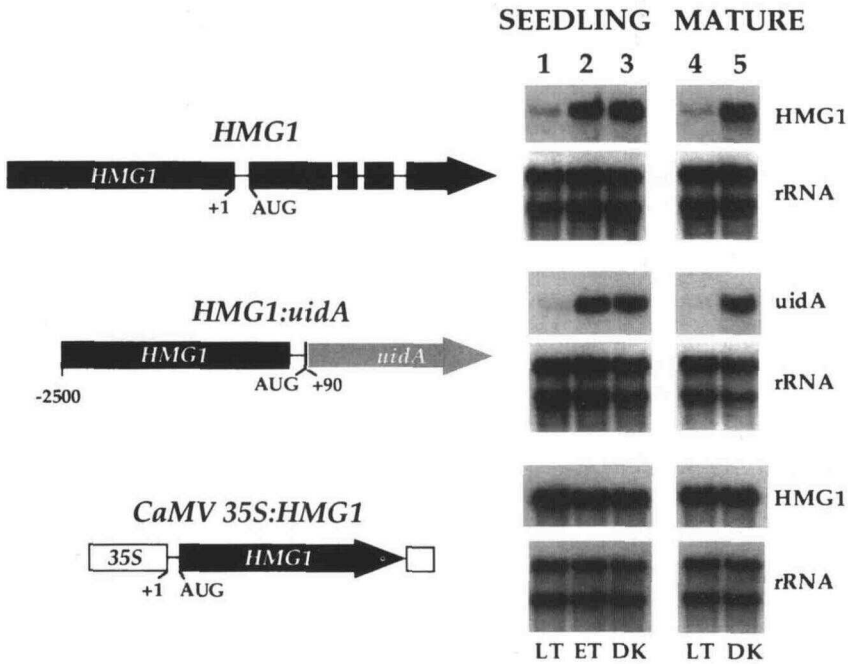

Figure 7. Light responsiveness of promoter/reporter fusion genes in transgenic Arabidopsis. RNA was isolated from Arabidopsis plants grown under different light conditions and subjected to blot hybridization analysis using ^{32}P-labeled DNA probes derived from *E. coli uidA*, Arabidopsis *HMG1*, or radish rDNA as indicated. The sources of RNA are as follows: lane 1, 6-d-old seedlings grown under continuous light; lane 2, 6-d-old seedlings grown under continuous dark; lane 3, 6-d-old seedlings grown for 5 d under continuous light followed by dark treatment for 24 h; lane 4, 21-d-old mature plants grown under continuous light; and lane 5, 21-d-old plants grown under continuous light for 20 d followed by 24 h of dark adaptation. Blot hybridization analysis was carried out on the following plant lines: top, wild-type Arabidopsis plants (No-0 ecotype); center, Arabidopsis transformed with the *HMG1:uidA* chimeric gene (No-T36.09); and bottom, Arabidopsis transformed with the *CaMV 35S:HMG1* fusion gene (No-T43.06). Maps of the relevant genes are shown in each panel. Black boxes indicate *HMG1* sequences, with narrow lines showing noncoding sequences within the transcribed region of the gene. The stippled box indicates the coding sequence for the *E. coli uidA* gene. The clear boxes represent sequences in the CaMV 35S transcription unit. Numbers below the maps refer to nucleotide positions relative to the transcriptional initiation site in the Arabidopsis *HMG1* gene, and "AUG" indicates the position of the *HMG1* initiation codon in each gene.

et al., 1995). As shown in Figure 7, it appears that the transcribed sequences represented in the *HMG1* cDNA cannot mediate light control in the absence of sequences present in the upstream-flanking region of *HMG1*. Based on these results, we propose that the suppression of *HMG1* mRNA accumulation by continuous illumination is controlled, at least in part, at the transcriptional level and is mediated by *cis*-acting elements that reside in the Arabidopsis *HMG1* promoter.

DISCUSSION

Here we present our initial study of HMG CoA reductase photoregulation in plants and, in particular, examine the effects of light on Arabidopsis *HMG1* mRNA accumulation. In this report, we have shown that HMG CoA reductase mRNA can be detected in Arabidopsis plants grown under a variety of light conditions but that *HMG1* is expressed preferentially in etiolated and dark-adapted plants, with high levels of *HMG1* mRNA accumulating in plants deprived of light. Time-course experiments indicate that accumulation of HMG CoA reductase mRNA in response to changing light conditions requires approximately 12 to 16 h to attain the new steady state. In contrast, short light pulses have no apparent effect on *HMG1* mRNA levels. We also established a dose-response curve for white-light-mediated regulation and showed that *HMG1* gene expression is sensitive to the amount of light delivered to the plant. Thus, short pulses, even from high-intensity light sources, may be insufficient to trigger the suppression of *HMG1* expression through the action of a regulatory system that appears to monitor aspects of both light intensity and duration. Prolonged exposure to high fluence rates of white light is required for sustained and maximal suppression of HMG CoA reductase expression, and basal levels of *HMG1* mRNA are maintained only under conditions of continuous illumination.

As a first step toward elucidating the molecular basis of photoregulated *HMG1* expression in Arabidopsis, we examined the potential role of noncoding sequences in the *HMG1* gene as targets for gene regulation. In this report, we demonstrate that light-responsive expression of HMG CoA reductase is mediated by *cis*-acting sequences in the *HMG1* promoter and suggest that the regulated accumula-

tion of *HMG1* mRNA is a consequence of coordinating promoter activity with features of the light environment. A systematic dissection of of the *HMG1* regulatory region using both in vivo and in vitro approaches is currently in progress and is expected to provide important insights into the mechanisms that mediate the light-repressed expression of HMG CoA reductase in Arabidopsis.

In a complementary series of experiments, we began to characterize features of the primary light signal to which *HMG1* promoter activity responds. Light profoundly influences plant growth and development in two fundamental ways. First, the light environment is monitored for informational cues by an assembly of photoreceptors that interpret these signals and initiate a series of light-dependent morphological and developmental responses (Chory, 1992, 1993; Deng, 1994; Quail, 1994). The effects of filtered light on *HMG1* gene expression were investigated in a preliminary attempt to identify the photoreceptor system that mediates the light response. Exposure to either continuous red or blue light established a limited response but failed to achieve the minimal basal level of *HMG1* expression observed following treatment with white light. Instead, complete suppression was observed following treatment with continuous white light or with a combination of red and blue light. At the fluence rates tested, *HMG1* mRNA levels were reduced more effectively by treatment with blue light than with red light; a primary role for blue light in *HMG1* photoregulation was revealed both by the rapid suppression of *HMG1* mRNA accumulation in response to blue light and by the lower fluence rates required to generate the response. The distinct patterns of suppression that were observed in these experiments are consistent with a regulatory response that is mediated either by multiple photoreceptors that monitor different regions of the spectrum or by a single photoreceptor that is differentially sensitive to blue and red light.

However, none of the characteristics of *HMG1* photoregulation described in this report are, in themselves, diagnostic of responses mediated by the well-studied classes of regulatory photoreceptors in higher plants. If light perception mediated by a photoreceptor, such as phytochrome or blue-light receptor, is involved in the control of HMG CoA reductase gene expression, several novel features distinguish *HMG1* photoregulation from previously described receptor-mediated responses. First, although examples of irradiance-dependent gene regulation have been described previously (Prioul and Reyss, 1988; Bertoni and Becker, 1993; Gao and Kaufman, 1994), the expression of these genes is uniformly activated by light, whereas *HMG1* expression is down-regulated following illumination. Second, the examples of phytochrome-mediated suppression that have been described exhibit the red/far-red reversibility that is characteristic of a low fluence response (Colbert et al., 1983; Mosinger et al., 1985; Lissemore and Quail, 1988; Kay et al., 1989; Tsai and Coruzzi, 1990; Okubara and Tobin, 1991). Because suppression of *HMG1* expression is not observed using brief light pulses, we have been unable to confirm the role of phytochrome in this light response. In addition, although we have demonstrated a role for red light in down-regulating *HMG1* mRNA accumulation, far-red light was found to have no effect on the levels of *HMG1* mRNA in etiolated Arabidopsis seedlings. Consequently, it is not clear whether far-red light is a neutral condition with respect to *HMG1* expression or whether it plays an active role in receptor-mediated induction of *HMG1* mRNA synthesis. Finally, in this report we establish that blue light participates in the suppression of *HMG1* gene expression; however, the decline in mRNA accumulation that we observe with *HMG1* appears to be a much less well-documented response to blue light than the induction of gene expression. Although these results do not directly implicate the operative regulatory photoreceptor, the data do provide compelling evidence that *HMG1* gene expression responds to light from multiple portions of the spectrum. Moreover, these studies represent only the first step in the characterization of the light response by the *HMG1* gene in Arabidopsis and establish the foundation for more detailed photobiological experiments. In particular, analysis of Arabidopsis mutants defective in photoperception, including plants that carry mutations in the *PHYA, PHYB, HY1, HY4, HY6,* and *BLU* genes (Koornneef et al., 1980; Liscum and Hangarter, 1991; Nagatani et al., 1993) will provide important insight into the contributions of specific photoreceptors in the light-regulated synthesis of HMG CoA reductase mRNA.

Alternatively, *HMG1* gene expression may not be responding directly to light as the trigger for photomorphogenesis but, rather, in its other role as energy source. Plants synthesize a number of pigments, including the Chls, that absorb light and channel that energy into photosynthetic carbon fixation and carbohydrate synthesis, resulting in profound changes in plant cell metabolism. It is interesting to note that in our studies relatively long periods of illumination are necessary to modulate *HMG1* expression levels and, moreover, that *HMG1* mRNA accumulation was suppressed only by light sources providing illumination capable of promoting photosynthesis. Consequently, it seems possible that *HMG1* regulation may not represent a primary physiological response to light but rather is a direct response to these light-dependent metabolic changes.

One prediction of such a model for indirect light regulation is that the metabolic signal occupies a more proximal position in the signal transduction pathway than light perception and, therefore, could modulate levels of *HMG1* expression independently of light. In fact, this type of metabolic regulation appears to operate during repression of photosynthetic gene expression by sugars and acetate (Sheen, 1990), as well as the induction of nitrate reductase gene transcription by sugars (Cheng et al., 1992). As expected, metabolic control of these genes overrides other modes of regulation, including light. It is interesting that continuous white light stimulates nitrate reductase mRNA accumulation in an irradiance-dependent fashion (Melzer et al., 1989) in a response that appears similar to the light-mediated suppression we observe with HMG CoA reductase. However, although our experiments are consistent with a light-dependent metabolic basis for HMG CoA re-

ductase regulation, because the plants were grown routinely on nutrient medium supplemented with Suc, it is unlikely that simple sugars are sufficient for this control. As a means of identifying the molecular signal, we are currently trying to uncouple light perception from *HMG1* regulation using metabolic intermediates or inhibitors of specific metabolic pathways, including photosynthesis. In this way, we can begin to elucidate the signal transduction pathway that is responsible for the light-mediated suppression of HMG CoA reductase gene expression.

Whatever the ultimate identity of the physiological trigger, the biological imperative for light-mediated control of HMG CoA reductase gene expression must reside in either MVA synthesis or utilization. Whereas in animal cells most of the MVA is committed to cholesterol biosynthesis, the multivalent regulation of HMG CoA reductase provides a sensitive feedback control mechanism that responds to the levels of serum cholesterol and coordinates isoprenoid metabolism to ensure the availability of both sterol and nonsterol products (Goldstein and Brown, 1990). In contrast, bulk carbon flow through the plant isoprenoid pathway is directed toward the synthesis of several classes of MVA derivatives, including sterols and photosynthetic pigments. In addition, the terminal reactions in the branch pathways leading to the synthesis of these isoprenoids are compartmentalized in multiple organelles within the plant cell and take place under different light conditions. Thus, although a metabolic basis for *HMG1* regulation is an attractive model, the relationship between MVA utilization and illumination offers no facile explanation for the suppression of HMG CoA reductase expression by light, and feedback inhibition of HMG CoA reductase seems an unlikely mechanism for controlling the coordinated synthesis of the different classes of plant isoprenoids. Alternatively, light-mediated control of *HMG1* may not be a feedback mechanism responding to the accumulation of isoprenoid end products but rather may coordinate the commitment of metabolic precursors into the mevalonate biosynthetic pathway in response to changing environmental and physiological conditions. In particular, acetyl-CoA is a central metabolite in plant cells, connecting photosynthetic carbon assimilation in the chloroplast with the biosynthesis of amino acids, nucleotides, fatty acids, and isoprenoids.

The experiments described here provide a conceptual framework and experimental foundation for our continuing study of HMG CoA reductase gene regulation and its role in controlling plant isoprenoid biosynthesis. To this end, we are exploiting both molecular and genetic approaches to identify components of the signal transduction pathway, as well as the response elements in the *HMG1* promoter. These studies will allow us to distinguish between regulation by light repression or by dark activation and to begin to elucidate the molecular basis for photoregulated HMG CoA reductase gene expression. Furthermore, although we have concentrated on control at the level of mRNA synthesis, we are also evaluating the contribution of posttranscriptional control mechanisms to the regulated expression of HMG CoA reductase in *A. thaliana* (Re et al., 1995).

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