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Red/far-red and blue light-responsive regions of maize *rbcS*-m3 are active in bundle sheath and mesophyll cells, respectively

(C4 photosynthesis/transient *in situ* expression/cell type-specific photoregulation/reporter genes)

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ABSTRACT **Leaves of the C4 plant maize have two major types of photosynthetic cells: a ring of five large bundle sheath cells (BSC) surrounds each vascular bundle and smaller mesophyll cells (MC) lie between the cylinders of bundle sheath cells. The enzyme ribulose bisphosphate carboxylase/oxygenase is encoded by nuclear *rbcS* and chloroplast *rbcL* genes. It is not present in MC but is abundant in adjacent BSC of green leaves. As reported previously, the separate regions of *rbcS*-m3, which are required for stimulating transcription of the gene in BSC and for suppressing expression of reporter genes in MC, were identified by an *in situ* expression assay; expression was not suppressed in MC until after leaves of dark-grown seedlings had been illuminated for 24 h. Now we have found that transient expression of *rbcS*-m3 reporter genes is stimulated in BSC via a red/far-red reversible phytochrome photoperception and signal transduction system but that blue light is required for suppressing *rbcS*-m3 reporter gene expression in MC. Blue light is also required for the suppression system to develop in MC. Thus, the maize gene *rbcS*-m3 contains certain sequences that are responsive to a phytochrome photoperception and signal transduction system and other regions that respond to a UVA/blue light photoperception and signal transduction system. Various models of "coaction" of plant photoreceptors have been advanced; these observations show the basis for one type of coaction.**

In photosynthetic CO_2 fixation ribulose-bisphosphate carboxylase/oxygenase [Rubisco; 3-phospho-D-glycerate carboxylyase (dimerizing), EC 4.1.1.39] catalyzes the synthesis of two molecules of 3-phosphoglycerate from one of CO_2 and one of ribulose 1,5-bisphosphate. But the Rubisco-catalyzed reaction does not discriminate completely between CO_2 and O_2. This and its relatively low affinity for CO_2 are surprising features for an enzyme that operates in the low CO_2 partial pressures and relatively high O_2 tensions of the earth's atmosphere today. However, these characteristics are not surprising for an enzyme that originated and evolved in an atmosphere rich in CO_2 and poor in O_2. C4 photosynthesis has evolved independently among flowering plants a number of times (1, 2) to circumvent these "deficiencies" in Rubisco.

Leaves of the C4 plant maize contain two types of photosynthetic cells: bundle sheath cells (BSC) are present as a single ring of cells around each vascular bundle and mesophyll cells (MC) surround the BSC and occupy the remaining space in the leaf. CO_2 is fixed first in MC by phospho*enol*pyruvate carboxylase to produce oxaloacetate, which is reduced to malate. The malate is transported to BSC where it is oxidatively decarboxylated. The CO_2 "pumped" into BSC in malate is fixed by Rubisco. MC lack Rubisco; BSC contain Rubisco. The eight large polypeptide subunits of angiosperm Rubisco are encoded by the chloroplast *rbcL* gene, and the eight small subunits are encoded by a family of nuclear *rbcS* genes. Both MC and BSC in dark-grown maize seedlings contain transcripts of *rbcS* and *rbcL*. Upon illumination, the pools of *rbcS* and *rbcL* transcripts increase in size in BSC but remain constant and then decrease in MC (3). There are three photoregulated steps in these processes (4): First, expression of *rbcS*-m3 is stimulated in BSC during the first 24 h of illumination of dark-grown seedlings [the *rbcS*-m3 sequence region extending from −211 (taking the transcription start site as +1) to +64 is sufficient (ref. 4; J.-F. Viret and L.B., unpublished data)]. Second, expression of a *rbcS*-m3 reporter gene is suppressed in MC of dark-grown seedlings but only if they have already been illuminated for 24 h (regions extending from −907 to −444 and from +720 to +957 are required together with the −93 to +64 sequence). The third light-requiring step is the development, during the first 24 h of illumination, of the capacity to downregulate *rbcS* expression in MC later.

The best known plant photoperception signal transduction systems are those based on phytochrome photoreceptors (5, 6). Five genes encoding phytochrome apoproteins have been identified in *Arabidopsis thaliana* and closely related sequences occur in other plants (reviewed in ref. 6); phytochromes A and B have different roles in controlling the elongation of etiolated hypocotyls (7, 8) and in controlling germination, seed development, and flowering in *A. thaliana* (9). It seems likely that the other phytochromes will also have distinct functions but it is also conceivable that a single phytochrome photoreceptor feeds into more than one signal transduction chain. Understanding these pathways is a major goal of contemporary phytochrome research. Other photomorphogenic systems are driven through UVA/blue light- and UVB-absorbing photoreceptors (10). In several species the increase in expression of *rbcS* upon illumination of dark-grown seedlings has been shown to be mediated by a phytochrome system (11–16). But blue light (B) is required to induce *rbcS* expression in tobacco cells in suspension culture (17) and to restore the levels of transcripts of two pea *rbcS* genes (either *in situ* or in transgenic petunia plants) that are reduced after the plants have been in darkness for 4 days; phytochrome is also involved for the latter (ref. 18; also see ref. 19). Transcription of *rbcS* in light-grown *Phaseolus vulgaris* increases in response to B (20). In none of these cases is it known whether signals cross over between postphotoperception signal transduction chains.

In the present experiments, we have found that expression of *rbcS*-m3 is enhanced in BSC through a phytochrome photoperception and signal transduction system, whereas the two steps required for suppressing expression in MC are effected through one or more B-absorbing photoreceptors. A phytochrome-based system may also be involved in the two latter processes directly or indirectly.

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Abbreviations: R, red light; B, blue light; FR, far-red light; GUS, β-glucuronidase; BSC, bundle sheath cell(s); MC, mesophyll cell(s).

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MATERIALS AND METHODS

Plant Material. Second leaves of 10-day-old dark-grown maize (*Zea mays* L.; FR9 cms × FR 37; Illinois Foundation Seeds, Champaign) seedlings (grown at 28°C) were harvested and manipulated under a dim green safelight. For bombardment, four 3.5-cm-long segments from within the upper halves of these leaves were flattened side by side on 1.2% agar Murashige and Skoog medium (ref. 21; GIBCO) in 50-mm Petri dishes with the lower epidermis facing upward (22).

Light Treatment. Greening for 24 h was under 54 microeinsteins (μE)·m^{-2}·s^{-1} from warm white fluorescent lamps at 24°C. Far-red light (FR; emission maximum, 725 nm; energy fluence rate, 8 μE·m^{-2}·s^{-1}) was provided by an Airequipt model 125 500-W projector with a Baird-Atomic interference filter; a 3-cm water filter was also used to avoid heat transfer to the filter and sample. Red light (R) was provided by GTE-Sylvania red fluorescent lamps (F4OR) filtered through two layers of Roscolux (Rosco, Portchester, NY) Fire no. 19 plastic and one layer of no. 2423 Plexiglas (23). The R source had a peak transmittance at 660 nm and an energy fluence rate of 9 μE·m^{-2}·s^{-1} at the level of the plants. B (15 μE·m^{-2}·s^{-1}; maximum at 440 nm) was provided by light from GTE F40B fluorescent lamps filtered through two layers of Roscolux no. 69 (24). In experiments in which plants or leaf segments were illuminated with R and B simultaneously, R was provided as described above and B (8 μE·m^{-2}·s^{-1}) was obtained by passing light from an Airequipt model 125 500-W projector through a 4-mm-thick Corning Glass CS 5-58 filter (25); a 3-cm water filter was also used to avoid heating the filter and sample.

Construction of Chimeric Genes. The plasmid pMT*nos* (22) was the basic construct used to create all other chimeric constructs tested in the present study. Methods for cloning were adapted from Sambrook *et al.* (26). pMT*nos* contains 2.1 kbp of *rbcS*-m3 (27) from upstream of the transcription start site plus 434 bp of transcribed sequence (including the *rbcS*-m3 intron) fused to the *Escherichia coli uidA* gene coding sequence [β-glucuronidase (GUS) reporter], followed by 260 bp of the nopaline synthase (*nos*) terminator of *Agrobacterium tumefaciens* (28). The plasmid pMT*nos244* was obtained by the deletion, with endpoint at 244 bp, of the 3′ end of pMT*nos*3′ (4); it was created by PCR. The PCR-amplified fragment was digested and cloned in its position in *Sma* I/*Spe* I sites after changing 3 nucleotides in the primer to create a *Spe* I site (ACTAGT). pMT*nos*3′, p3′MT*nos*, pMT9073′, pMT4443′, and pMT2113′ have been described (4).

Transient *in Situ* Expression Assay. The expression of GUS reporter genes in MC and BSC was determined by an *in situ* transient expression assay (22, 28–32). A suspension of tungsten microprojectiles (1.1 μm) on which 1.5 μg of reporter gene DNA had been precipitated was shot into segments of etiolated maize leaves with a PDS-1000 Biolistic apparatus (Bio-Rad) under a green safelight. After a period under illumination or in darkness, the leaf segments were incubated with the GUS substrate 5-bromo-4-chloro-3-indolyl glucuronide (Biosynthag, Skokie, IL) as described (31, 39). The numbers of GUS-expressing MC and BSC per shot were determined by optical sectioning (4). Each blue spot was counted as a single expression event irrespective of the number of contiguous blue cells showing GUS activity. In no case did a single spot include both MC and BSC. Each GUS construct was tested at least three times using the *in situ* assay and each experiment was performed at least twice.

RESULTS

Expression of *rbcS*-m3 in BSC Is Stimulated by R: FR Reverses the Effect of R. A reporter gene containing *rbcS*-m3 sequences extending from −211 to +434 (taking +1 as the nucleotide at which transcription is initiated), the GUS protein coding sequence of the *E. coli* gene *uidA*, and a *nos* terminator region (pMT211*nos*; Fig. 1) is expressed about twice as strongly in MC as in BSC of leaf segments placed in darkness for 24 h after the reporter gene is introduced. Exposure to light from warm white fluorescent lamps for the same 24-h period has little effect on the level of expression in MC but generally results in about a doubling of expression in BSC (Fig. 2*A*; data taken from ref. 4). Similar results were obtained (Fig. 2*B*) when leaf segments were illuminated not with continuous white light but with R for 1 min at 0, 3, 6, 9, and 12 h after pMT211*nos* reporter gene DNA was introduced. The segments were maintained in darkness for an additional 12 h before the GUS assay was initiated. Exposure to 5 min of FR after each exposure to R essentially eliminated the effect of illumination

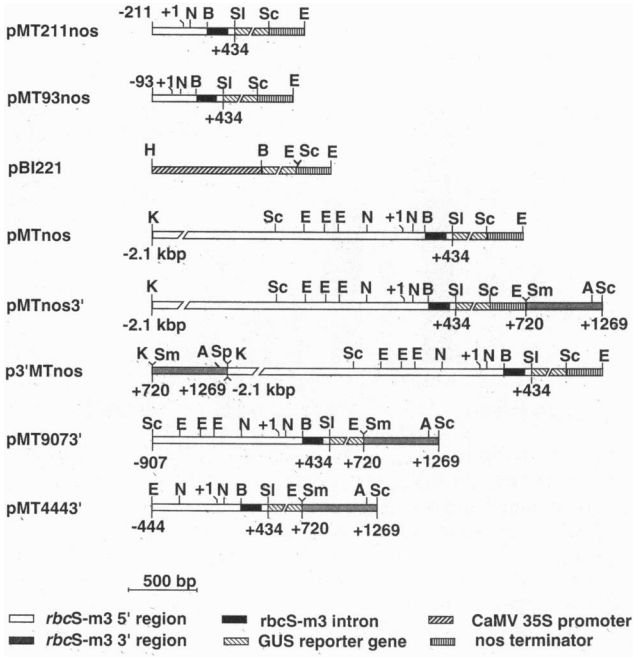

FIG. 1. Diagrams showing chimeric GUS reporter genes. B, *Bam*HI; E, *Eco*RI; K, *Kpn* I; N, *Nco* I; Sc, *Sac* I; Sl, *Sal* I; Sm, *Sma* I; Sp, *Spe* I. Segments of *rbcS*-m3 are delineated in base pairs from the gene's own transcription start site (+1). The polyadenylylation site of the normal *rbcS*-m3 transcript is indicated (A). CaMV, cauliflower mosaic virus.

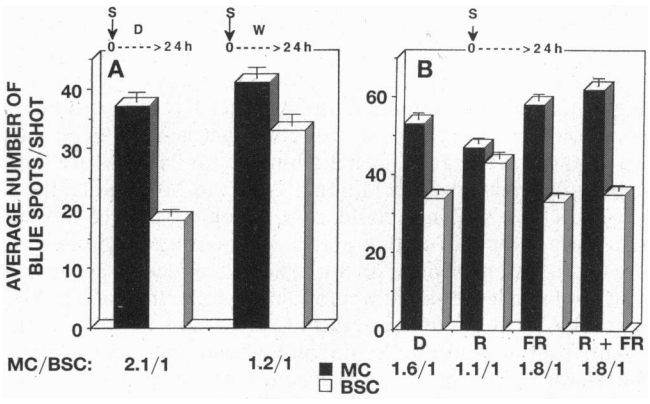

FIG. 2. Effects of darkness and white light, R, or FR on *in situ* transient expression of pMT211*nos* in leaves of etiolated maize seedlings. DNA was introduced on microprojectiles (S) into segments of leaves from 10-day-old dark-grown maize seedlings. (*A*) Segments were then maintained in white light (W) or in darkness (D) for 24 h. Average number (±SEM) of blue spots per shot is shown (data are from ref. 4). (*B*) Segments were then returned to darkness for 24 h or exposed to R, FR, or R followed by FR as described in the text.

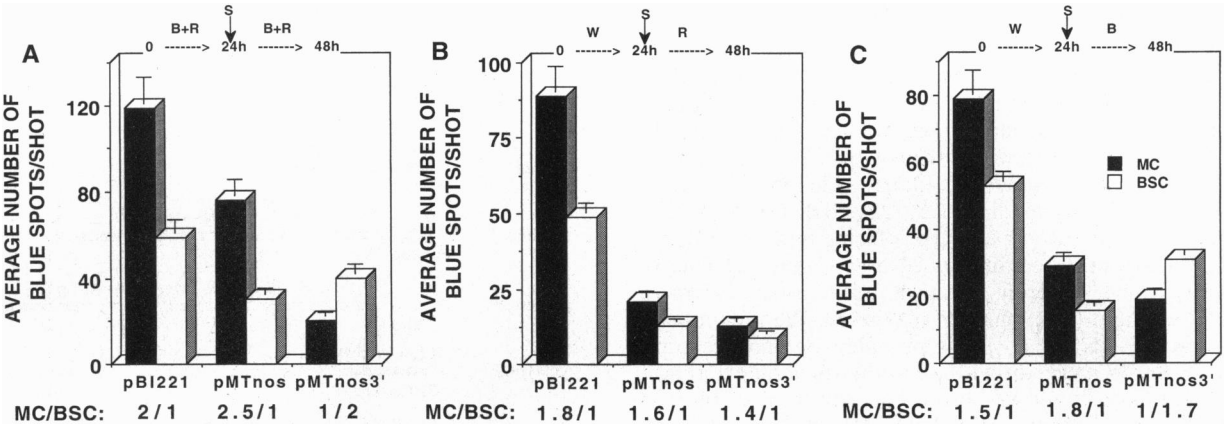

FIG. 3. *In situ* transient expression assays to determine whether R or B is effective for suppressing expression of the reporter gene pMT*nos*3′ in MC. (*A*) DNA of the pMT*nos* control and of pMT*nos*3′ was introduced into segments of leaves of 10-day-old dark-grown seedlings that had been maintained in B plus R for 24 h. Segments then remained in B plus R for an additional 24 h before the GUS assay was begun. Average number (±SEM) of blue spots per shot is shown. Ratios of expression of the constructs in MC/BSC are of the same order as when white light is used (4). Reporter pMT*nos* is expressed preferentially in MC while pMT*nos*3′ is expressed preferentially in BSC. (*B* and *C*) DNA of the reporter genes pMT*nos* and pMT*nos*3′ were introduced into segments of leaves of dark-grown maize seedlings illuminated with white light (W) for 24 h. When they were exposed to R during the subsequent 24 h, the ratio of expression in MC and BSC was about the same for the two reporter genes. If they were exposed to B for the second 24 h, pMT*nos*3′ was expressed preferentially in BSC, whereas pMT*nos* was expressed preferentially in MC.

with R. The same response pattern was exhibited by a reporter gene in which the *rbcS*-m3 sequence extends only to −93 rather than −211 (data not shown). The ratio of expression in MC vs. BSC is similar for pMT*nos*3′ (data not shown).

Thus, *rbcS*-m3 reporter gene expression is stimulated by a phytochrome-mediated R/FR reversible signal perception transduction system, which is apparently ineffective (or only marginally effective) in MC.

Suppression of *rbcS*-m3 Expression in MC Is Mediated Via a B-Absorbing Photoreceptor. After dark-grown maize seedlings have been exposed to white light for 24 h, expression of the reporter gene pMT*nos*3′ (Fig. 1), containing *rbcS*-m3 sequences −907 to +434 and +720 to +957, is suppressed in MC during the second 24 h of illumination but a reporter gene lacking the +720 to +957 sequence (pMT*nos*) is not suppressed (4). Exposure of dark-grown seedlings to R and B simultaneously throughout the first and second 24 h of illumination results in the same types of MC/BSC expression ratios for the two reporter genes as does exposure to white light (Fig. 3*A*). Thus, the B and R sources together contain all wavelengths of light required for the repression response in MC.

The control construct pBI221 (cauliflower mosaic virus 35S promoter linked to GUS linked to the 3′-terminal region of *nos*) is expressed about equally in MC and BSC in etiolated leaf segments during illumination for 24 h after DNA is introduced (4). However, when this control construct is introduced into leaves that have already been illuminated for 24 h and are then exposed to light for an additional 24 h, the MC/BSC ratio is 1.5–2:1 (Fig. 3). This could result from a change in the structure of the leaf so that under the shooting conditions we use the tungsten particles do not penetrate as deeply as they do into etiolated leaves and therefore more come to reside in MC than BSC. Alternatively, reduced expression in BSC could result from altered physiology of MC and BSC after illumination for 24 h. Viret *et al.* (4) concluded that the first of these two possibilities is the more likely. This suggests that it might be appropriate to introduce a correction into the data, which would lower the MC/BSC expression ratios for pMT*nos*3′. However, no such correction has been introduced in presenting the data here.

To determine whether exposure to R or B alone is sufficient to suppress expression of pMT*nos*3′ in MC in the 24–48 h after illumination was initiated, dark-grown seedlings were exposed to white light for 24 h, and DNA was shot into leaf segments that were illuminated with R or B during hours 24–48. The reporter genes pMT*nos* and pMT*nos*3′ are expressed to about the same relative extents in the two cell types (MC/BSC = 1.6:1 and 1.4:1, respectively) in leaf segments illuminated with R alone during the second 24 h (Fig. 3*B*). On the other hand, exposure to B during the second 24 h (Fig. 3*C*) results in expression of pMT*nos*3′ being suppressed in MC (MC/BSC, 1:1.7) but the pMT*nos* control, which lacks gene sequences necessary for suppression, is expressed with a ratio of 1.8:1. These results are comparable to those elicited by exposure to white light (pMT*nos* MC/BSC = 2.1:1; pMT*nos*3′ MC/BSC = 1:2.1; data not shown) or B + R (pMT*nos* MC/BSC = 2.5:1; pMT*nos*3′ MC/BSC = 1:2) during the same period. Thus, activation of a UVA/B photoreceptor is required for the suppression response.

A B Photoreceptor Is Involved in Development of Sensitivity to B for Suppression in MC. The capacity to photodownregulate *rbcS*-m3 reporter gene expression in MC develops during the first 24 h that dark-grown seedlings are illuminated (4). Exposure to B during the second 24 h of illumination can be used to determine whether illumination with R or B during the first 24 h facilitates development of the suppression system. As shown in Fig. 4*A*, leaf segments taken from dark-grown seedlings illuminated with R for 24 h and then, after reporter gene DNAs are introduced, illuminated for 24 h with B, express both pMT*nos* (MC/BSC = 2.2:1) and pMT*nos*3′ (MC/BSC = 1.6:1) more strongly in MC than BSC. However, leaf segments illuminated with B during hours 0–24 as well as during hours 24–48 (i.e., after reporter gene DNA had been introduced; Fig. 4*B*) expressed the reporter pMT*nos*3′ preferentially in BSC (MC/BSC = 1:2.5) but the control construct pMT*nos*, which is comparable to pMT*nos*3′ but lacks the *rbcS*-m3 3′ sequence, was expressed preferentially in MC (MC/BSC = 1.6:1). Thus, B was sufficient and R was insufficient for the MC to develop the capacity to suppress pMT*nos*3′ expression.

Earlier experiments in which white light was used throughout the 0- to 24-h and 24- to 48-h periods (4) showed that both the 3′ *rbcS*-m3 +720 to +957 and the 5′ −907 to −445 sequences are required for suppression of reporter gene expression during the 24–48 h of illumination. This is also the case under B; compare pMT*nos* with pMT*nos*3′ in Fig. 4*B* regarding the necessity for the +720 to +1269 region, but the sequence +720 to +957 is equally effective (data not shown) and the reporter

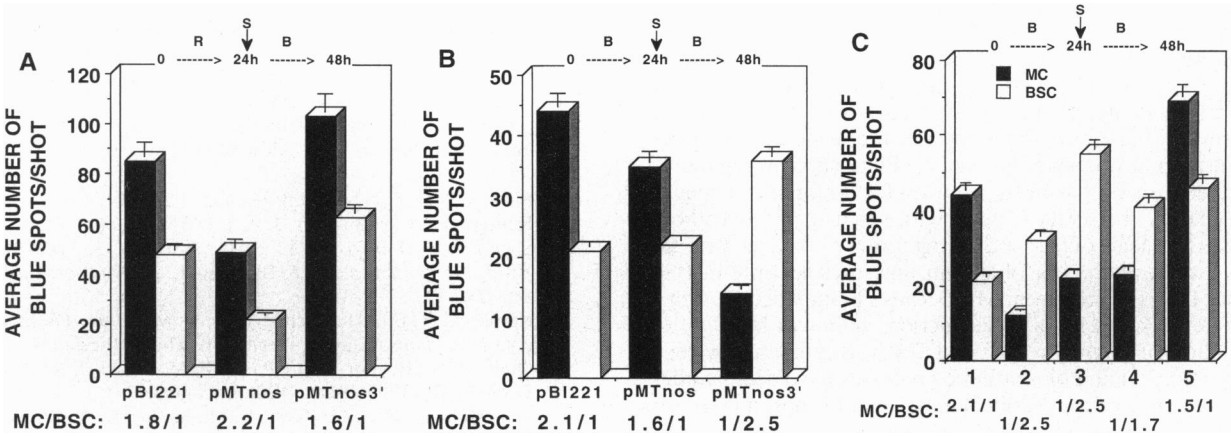

FIG. 4. *In situ* transient expression assays to determine whether R or B is effective for development of the system for suppressing *rbcS*-m3 reporter gene expression in MC. (*A* and *B*) Ten-day-old dark-grown maize seedlings were exposed to B or R for 24 h. Leaf segments were then prepared and reporter gene DNA was introduced. Segments were exposed to B for the next 24 h to measure the effectiveness of R or B for development of the suppression system. The construct pMT*nos*3′ was expressed preferentially in BSC of leaves irradiated with B during the first 24 h but not in those exposed to R during the same time. The control gene pMT*nos* exhibited preferential expression in MC under both circumstances. (*C*) Ten-day-old dark-grown seedlings were illuminated with white light for 24 h and then leaf segments were bombarded with microprojectiles carrying various chimeric reporter genes. After illumination with B for an additional 24 h, the GUS assay was started. The average (±SEM) of blue spots in MC and BSC per shot is shown for each gene. Results are discussed in the text. Chimeric DNAs (see Fig. 1): 1, pBI221; 2, pMT*nos*3′; 3, p3′MT*nos*; 4, pMT9073′; 5, pMT4443′.

gene pMT9073′ is suppressed in MC but pMT4443′ is not (Fig. 4*C*), showing the necessity for the region −907 to −445. Thus, in B as in white light, the sequence from −907 to −444—as well as that from +720 to +957—is required for suppression of *rbcS*-m3 reporter gene expression in MC. Also as in white light, the 3′ sequence is as effective in suppressing expression of the reporter gene in MC (Fig. 4*C*) when it is relocated to upstream of −2100 (construct p3′MT*nos*; Fig. 1) as when it is at its normal position.

Phytochrome Systems Also Affect Suppression of *rbcS*-m3 Expression in MC. The experiments described above demonstrate that B is required and, conversely, that exposure to R alone is not sufficient to bring about the stronger relative suppression of expression of the *rbcS*-m3 reporter in MC than

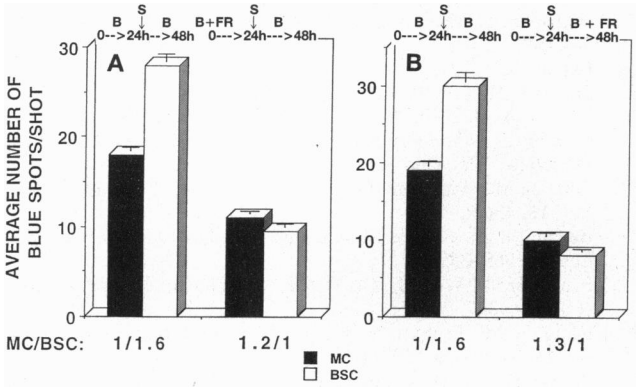

FIG. 5. Investigations of phytochrome involvement in suppression of pMT*nos*3′ expression in MC by examining the effects of FR pulses on *in situ* transient expression of pMT*nos*3′ in plants maintained under continuous B. (*A*) Ten-day-old dark-grown maize seedlings were illuminated continuously with B or with B plus intermittent FR (see text for conditions) in the course of the first 24 h. Then pMT*nos*3′ reporter gene DNA was introduced into leaf segments, which were placed under B for the next 24 h. At the end of that time, the GUS assay was initiated. Distribution of spots in MC and BSC is shown. (*B*) Dark-grown seedlings were illuminated with B for 24 h. Reporter gene DNA was then introduced into segments of leaves of these plants. The segments were exposed to continuous B or B plus intermittent illumination with FR over the next 24 h. Average number (±SEM) of blue spots in MC and BSC is shown for each chimeric gene.

in BSC. However, the B source contains wavelengths that are also absorbed by phytochrome. The expression of *rbcS*-m3 reporter genes—e.g., pMT*nos*3′—was not suppressed in MC by periodic illumination of leaf segments with white light during hours 24–48 after continuous illumination during hours 0–24 (data not shown). Nor did illumination with dim B (6 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 30 min every 2 h against a background of continuous R (e.g., see ref. 23) result in preferentially suppressing pMT*nos*3′ expression in MC. However, by illuminating with FR periodically, we were able to examine the effect of driving the phytochrome into the R-absorbing form in leaf tissues under continuous B in order to investigate whether a phytochrome system is involved in development of the B-requiring system for *rbcS*-m3 suppression and in the B-requiring repression process itself.

Exposure to 5 min of FR 0, 3, 6, 9, and 12 h after the beginning of continuous illumination with B during hours 0–24 results in expression of pMT*nos*3′, in leaf segments maintained in B for hours 24–48, at a ratio of ≈1.2:1 in MC/BSC rather than 1:1.5–1:2.5; the latter values are normally found after leaf segments from dark-grown seedlings are exposed to B during hours 24–48 (Fig. 5*A*). These results could be taken to show that FR blocks development of the MC-localized expression repressing apparatus, but expression in BSC is also reduced.

To determine whether a phytochrome system, as well as a B photoreceptor and signal transduction chain, is involved in controlling *rbcS*-m3 expression in MC during hours 24–48 of illumination, leaf segments that had been illuminated with B for 24 h and into which pMT*nos*3′ DNA was then introduced either were illuminated with B continuously during hours 24–48 or were exposed to continuous B plus FR for 5-min intervals 24, 27, 30, 33, and 36 h after the beginning of illumination. Intermittent illumination with FR resulted in reduced expression in both BSC and MC and a MC/BSC expression ratio of 1.3:1 in contrast to a ratio of 1:1.6 for the same construct in leaf segments illuminated by B alone.

DISCUSSION

Viret *et al.* (4) identified the separate regions of *rbcS*-m3 that are involved in regulating stimulation of expression of reporter genes in BSC and suppressing expression in MC. They also demonstrated that the suppression system develops during the

first 24 h that dark-grown seedlings are illuminated. In the present work, we have shown that stimulation of *rbcS*-m3 expression in BSC is under the control of a phytochrome system, whereas the two photoregulated steps, which occur in MC and result in suppression of *rbcS*-m3 reporter gene expression in MC, are mediated by a B-requiring photoperception and signal transduction system. Phytochrome regulation of *rbcS* expression has been reported for a number of species (e.g., see refs. 11–16); it can be imagined that all photosynthetic cells in the leaf of the C3 progenitor of maize had the phytochrome photocontrol system now present only in BSC but that this is absent from MC because a photoreceptor or a component(s) of a signal transduction chain was lost.

Coaction of phytochrome- and UVA/B-mediated systems on physiological and biosynthetic processes have been studied extensively and various models have been proposed (e.g., see ref. 33). The expression of *rbcS*-m3 in maize leaves depends on the action of the two photoperception systems acting through different parts of the same gene (33).

During the first 24 h after reporter gene DNA has been introduced into segments of dark-grown maize leaves, MC express *rbcS*-m3 reporter genes to the same extent regardless of whether the tissues are or are not illuminated. In this respect, MC behave like *Arabidopsis* mutants of the classes *cop, det*, and *fus*, which even in darkness exhibit some characteristics of light-grown seedlings; they appear to lack some elements of phytochrome-driven signal transduction chains (reviewed in refs. 6, 34, and 35). Again in relation to *rbcS*-m3 expression, MC resemble *Arabidopsis hy* mutants that are deficient in the bile pigment chromophore of phytochrome and have etiolated phenotypes in light or darkness, but MC respond to FR during hours 0–24 and thus must contain some active phytochrome systems (Fig. 5). Finally, inasmuch as BSC lack all or part of the UVA/B photoreceptor signal transduction system that acts in MC for development of the suppression apparatus and for suppression itself, BSC resemble, with regard to *rbcS*-m3 expression, the *hy4* mutant of *Arabidopsis*, which lacks a blue photoreceptive pigment (36, 37).

We cannot say that the results presented in Fig. 5 do or do not show that phytochrome-mediated as well as UVA/B-mediated processes are involved directly in the suppression of *rbcS*-m3 reporter gene expression in MC. Expression of the *rbcS*-m3 reporter gene could be reduced in BSC by the exposure to FR (also see Fig. 2*B*) and that the comparatively smaller reduction in expression in MC could be the "normal" response to B, perhaps with a further direct or indirect effect of FR.

We have noted (4) that *rbcS*-m3 reporter gene expression is not suppressed in MC as much as would be expected from the very low levels of *rbcS*-m3 mRNA found in MC of green maize leaves (3, 38). Transcription may be suppressed more and/or mRNA may be degraded more rapidly in intact plants than in leaf segments. Also, the GUS mRNA-containing transcripts produced from the reporter genes may be inherently more stable than transcripts of *rbcS*-m3.

The present experiments show that the maize gene *rbcS*-m3 carries sequences that appear to respond independently to MC- and BSC-specific phytochrome and UVA/B photoperception signal transduction systems. It remains to be determined whether such cell-type-specific photoperception and signal transduction systems are used to regulate *rbcS* expression in MC and BSC in the numerous C4 species that evolved independently of maize and whether phytochrome and UVA/B systems are used to control expression of a single gene differently in adjacent cells in C3 plants.

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