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Cell type-preferred expression of maize *cab*-m1: Repression in bundle sheath cells and enhancement in mesophyll cells

(C4 photosynthesis/AT-1 sequence/light-harvesting chlorophyll a/b-binding proteins of photosystem II/*Zea mays*/ mesophyll-specifying region)

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ABSTRACT **Different sets of genes for photosynthesis are expressed in mesophyll cells (MCs) and bundle sheath cells (BSCs)—the two adjacent but morphologically and functionally distinct types of photosynthetic cells in leaves of maize and other C4 plants. For example, light-harvesting chlorophyll a/b-binding proteins of photosystem II, which are encoded by a family of *cab* genes, are 3- to 4-fold more abundant in maize MCs than in BSCs. Each maize *cab* gene is different from the others in its relative expression in MCs vs. BSCs and in its degree of photoresponsiveness. The gene *cab*-m1 is positively photoregulated and is highly preferentially expressed in MCs. A 159-bp sequence in the 5′ flanking region of this gene (−1026 to −868 relative to the translation start site) is required for MC-preferred expression of a reporter gene in greening maize leaves. Deletion as well as gain-of-function experiments have now shown that all of the sequence information required for MC-preferred expression resides within this mesophyll-specifying region and that *cab*-m1 is preferentially expressed in MCs because of the presence of two types of sequence elements: one is required for suppressing expression in BSCs and the other for promoting expression in MCs. One of the four cis-acting regions mapped within the mesophyll-specifying region resembles the AT-1 box of some plant gene negative regulatory elements. Various combinations of such MC-specific enhancing and BSC-specific repressing regions could make maize *cab* gene family members different from one another in their relative expression in MCs vs. BSCs.**

In maize, as in other C4 plants, different sets of photosynthetic genes are expressed in the morphologically and biochemically distinct adjacent leaf mesophyll cells (MCs) and bundle sheath cells (BSCs). A one-cell-thick cylinder of BSCs surrounds each vascular bundle; MCs lie outside of the BSCs. MCs and BSCs can be derived from sister cells (1). Some maize genes involved in photosynthetic carbon metabolism are expressed solely in MCs (2–6) but others are expressed exclusively in BSCs (7–10). Still other genes—e.g., *cab* genes for light-harvesting chlorophyll a/b-binding proteins of photosystem II (LHCPII)—are expressed at different levels in MCs and BSCs.

LHCPIIs are three to four times more abundant in maize MCs than in BSCs (11, 12). This results from differences in relative expression in MCs vs. BSCs of individual members of the *cab* family of nuclear genes (12). The maize genes *cab*-m1, *cab*-m5, *cab*-m6, and *cab*-m7 are expressed to different relative extents in MCs vs. BSCs but preferentially in MCs; *cab*-m2 and *cab*-m3 are expressed about equally in MCs and BSCs; *cab*-m4 is expressed preferentially in BSCs (12, 13). Maize *cab* gene family members also differ in how photoresponsive they are (12). The molecular bases for these differences are not known.

Transcripts of the highly photoregulated gene *cab*-m1 constitute about one-third of the total LHCPII mRNA in greened leaves; *cab*-m1 is expressed almost exclusively in MCs (12). Using a microprojectile-based *in situ* transient expression β-glucuronidase (GUS) assay system (14, 15), we found that a chimeric *cab*-m1 *promoter:GUS:nos* reporter gene (Fig. 1) is expressed from a 5′ flanking region (−1026 to +14 relative to the translation start site) of *cab*-m1 in a highly photoregulated manner and 10 times more in MCs than BSCs. A construct lacking 159 bp from the 5′ end (−1026 to −868) expressed GUS about equally in MCs and BSCs but sequences between −359 and +14 were required for photoregulation.

We have now found, through a series of deletion experiments and complementary gain-of-function experiments that a 177-bp fragment of *cab*-m1 that extends from −1026 to −850, and thus includes the 159-bp −1026 to −868 region, can confer MC-preferred expression on a chimeric reporter gene containing the −359 to +14 5′ segment of *cab*-m1. The 177-bp mesophyll-specifying region (MSR) contains at least four different controlling regions: some are involved in enhancement of expression of *cab*-m1 in MCs and others in the repression of its expression in BSCs. Differences in relative expression of the seven known maize *cab* genes in MCs and BSCs could result from various combinations of MC expression-enhancing and BSC expression-repressing elements.

MATERIALS AND METHODS

Plant Material. Second leaves of 10-day-old dark-grown maize (*Zea mays*; FR9^{CMS} × FR37; Illinois Foundation Seeds, Champaign, IL) plants were harvested. For bombardment, the upper half of each leaf was cut into two 3.5-cm-long segments and flattened on 0.8% agar Murashige and Skoog medium (GIBCO) in 50-mm Petri dishes with the lower epidermis facing upward.

Construction of Chimeric Genes. The plasmid pM1CAB1.1 (−1026 construct) (14) was used as the starting material for gene constructs employed in the present study. It contains 1026 bp of *cab*-m1 5′ flanking sequence plus the initial six codons of the transit peptide sequence fused upstream of the *Escherichia coli* GUS reporter gene (*uidA*) coding sequence and nopaline synthase (*nos*) terminator of *Agrobacterium tumefaciens* (16).

5′ → 3′ deletions within the 159-bp region (−1026 to −868) of cab-m1 promoter sequence. Two deletions with endpoints

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Abbreviations: MC, mesophyll cell; BSC, bundle sheath cell; MSR, mesophyll-specifying region; GUS, β-glucuronidase; LHCPII, light-harvesting chlorophyll a/b-binding proteins of photosystem II.

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at −953 and −897 were created using the polymerase chain reaction (PCR). The region extending from −976 or from −916 (both calculated on the basis of oligonucleotide start and end positions) through the GUS coding region and *nos* terminator was amplified using the "universal primer" in the vector (pUC19) and custom-synthesized primers (5′-TGAATTTAC AAAGAT TAG TCT AGA TTT AAT-3′) and (5′-ACG GGC AAA AGA GAT CTA GAC TAT TTC TGT-3′), respectively. A single base in the primer for the −953 construct was mutated (T → C), whereas two bases were mutated (C → T, A → G) for the −897 construct to create an *Xba* I site (TCTAGA). The PCR-amplified fragment in either case was double-digested with *Xba* I and *Eco*RI restriction endonucleases and cloned into pUC19 digested with *Xba* I and *Eco*RI.

Addition of defined DNA fragments from within the 177-bp region upstream of the −359 deletion construct. The 177-bp region (−1026 to −850) could be conveniently divided into three fragments: fragment A (−1026 to −956), fragment B (−953 to −899), and fragment C (−897 to −850). These fragments, individually or in combinations, were fused in front of the −359 construct.

(*i*) −1026 to −956/−359 construct (+A/−359). Using the plasmid pM1CAB1.1 as the template, "reverse primer" in the vector pUC19, and a custom-synthesized primer (MC2 = 5′-GCG ATA TTA AAG CTT AAC TAA TCT TTG TAA ATT CA-3′), the region between −1026 and −945 was amplified and, by mutating two bases (T → G and A → T), a *Hin*dIII site was created in the amplified fragment, which was then digested with *Hin*dIII and cloned into the *Hin*dIII-digested and dephosphorylated −359 deletion construct.

(*ii*) −953 to −899/−359 construct (+B/−359). Using the −953 deletion construct as the template, reverse primer in the pUC19 vector, and a custom-synthesized primer (MC4 = 5′-AAA ACA GAA AAA GCT TGG ATC TCT-3′), the region between −953 and −884 was amplified and a *Hin*dIII site was created in this region by mutating A → G and A → T in the primer sequence. The amplified region was digested with *Hin*dIII and cloned in front of the −359 construct as in *i* above.

(*iii*) −897 to −850/−359 construct (+C/−359). Using the −897 construct as the template, reverse primer, and a custom-synthesized primer (MC1 = 5′-CGA AAC CGG AAA GCT TTA CCG AAA ATA CGG-3′) with two bases mutated (C → A and G → C) to create a *Hin*dIII site, the region between −897 and −835 was amplified, digested with *Hin*dIII, and cloned in front of the −359 construct.

(*iv*) −930 to −850/−359 construct (+1/2 B+C/−359). Using the −953 construct as the template with the primers MC1 (see *iii* above) and MC7 (5′-TTT CTG AAA GCT TAC CGATTT CGG AAA CGG GCA-3′), the region between −942 and −835 was amplified, *Hin*dIII digested, and cloned in front of the −359 construct.

(*v*) −953 to −850/−359 construct (+B+C/−359). Using the −953 construct as the template with the reverse primer and the MC1 primer, the region between −953 and −835 was amplified, digested with *Hin*dIII, and cloned in front of the −359 construct.

(*vi*) −988 to −850/−359 construct (+1/2 A+B+C/−359). Using the plasmid pM1CAB1.1, the MC1 primer, and the MC6 primer (5′-CAG TTT CAT AAG CTT CTA AGT TAA GAT CAA TT-3′) with a *Hin*dIII site, the region between −1002 and −835 was amplified, digested with *Hin*dIII, and cloned in front of the −359 construct.

(*vii*) −1026 to −899/−359 construct (+A+B/−359). Using the plasmid pM1CAB1.1, the MC4 primer (see *ii* above), and the reverse primer, the region between −1026 and −884 was amplified, digested with *Hin*dIII, and cloned upstream of the −359 construct.

(*viii*) −1026 to −850/359 construct (+A+B+C/−359). Using the plasmid pM1CAB1.1, reverse primer, and the MC1 primer, the region between −1026 and −835 was amplified, digested with *Hin*dIII, and cloned in front of the −359 construct in normal and reverse orientation. In every case, the orientation was determined by restriction mapping and confirmed by sequencing (17).

Site-Directed Mutagenesis. The PCR-based megaprimer method (18) was used to amplify *in vitro* the mutated region using mutant primer (5′-TAG ATT TGA ACC CGG GTA GAA AAA AAT ACC GAT TTC GGA A-3′) and a primer in the 5′ flanking region of the *cab*-m1 gene (MC1 primer) for mutating the AT-1 box-like sequence in the A+B+C/−359 construct. The amplified mutated double-stranded DNA was then used as the megaprimer in the second round of PCR along with reverse primer in the vector region to amplify the complete fragment required. The amplified fragment was cloned before being used in the transient expression assay.

Transient *in Situ* Expression Assay. Plasmid DNA was precipitated onto 1.1-μm tungsten microprojectiles and used for shooting into leaf segments of dark-grown maize seedlings with the Biolistic Particle Delivery System-1000 (DuPont) (19) within 1 hr. One microgram of supercoiled DNA was used per 0.5 mg of tungsten particles per shot. The target was 9 cm from the stopping plate in the Biolistic apparatus. A *35S cauliflower mosaic virus promoter:GUS:nos* gene was used as a control in every bombardment as in our previous work (14); this gene is expressed about equally in BSCs and MCs under the conditions we used in the present experiments.

Localization of GUS Activity *in Situ* in MCs and BSCs. After being maintained at room temperature in the light (≈300 microeinsteins/m^2 per s) for 24 hr, leaf segments were incubated with the GUS enzyme histological assay substrate 5-bromo-4-chloro-3-indolyl glucuronide (Biosynth International, Skokie, IL) (14, 15, 20) in the dark for 48 hr. Then leaf segments were cleared by incubation with 70% ethanol for 24–28 hr at 65°C. The insoluble indigo dye deposit produced as a result of GUS activity was located in MCs or BSCs under a light microscope equipped with a phase-contrast system without sectioning. MCs and BSCs are readily distinguishable. As in the earlier work (14, 15), an individual spot was counted as a single expression event irrespective of the number of contiguous blue cells showing GUS activity. In our previous work (14), blue cells were located by microscopy of transversely sectioned leaf segments. More than 2000 "spots" have been examined in leaf sections to date; in no case have we seen expression in adjacent MCs and BSCs, although contiguous blue MCs occur frequently as do contiguous blue BSCs. Photographs of leaf sections appeared in ref. 14. Each gene construct was shot into at least four sets of leaf segments obtained from at least two different batches of plants. All blue spots present in the leaf segments were located. We determined the mean number of spots per shot in MCs or BSCs and calculated the SEM.

RESULTS

5′ → 3′ Deletion Analyses of the 159-bp Fragment (−1026 to −868) Required for MC-Preferred Expression in the Context of the Full-Length Promoter. Our first experiments were designed to determine, through deletion analyses, whether the BSC-suppressing and MC-enhancing activities in the region between −1026 and −868 of *cab*-m1 (14) (Fig. 1) are physically separable from one another or whether a single DNA sequence is involved in both activities. Two chimeric *cab*-m1 *promoter:GUS:nos* genes with 5′ ends at −953 and −897 (Fig. 2*B*) were made and each was analyzed for expression in MCs vs. BSCs by the *in situ* transient expression assay. The −867 deletion construct and the chimeric gene shown in Fig. 1 were used as controls. DNA of each

construct was precipitated onto microprojectiles that were shot into segments of dark-grown maize seedlings; after 24 hr of illumination, incubation with the GUS substrate, and clearing with 70% ethanol, blue spots resulting from GUS activity were located in MCs and BSCs (see *Materials and Methods*). It was found (Fig. 2) that (*i*) deletion of the region between −1026 and −954 (designated fragment A) enhanced expression in BSCs about 4-fold and reduced expression in MCs by about 20%, (*ii*) deletion of fragment B (−953 to −898) had no effect beyond that of deleting fragment A, and (*iii*) the deletion of fragment C (−897 to −850) further reduced expression in MCs by about half but had no effect on the level of expression in BSCs (Fig. 2). These experiments show that within the 177-bp MSR, containing the 159-bp sequence, there are separate elements that control repression in BSCs and the enhancement of reporter gene expression in MCs. The 177-bp (−1026 to −850) rather than the 159-bp (−1026 to −868) sequence was the basis for our manipulations in these and subsequent experiments for technical reasons.

The 177-bp Fragment (−1026 to −850) Is Enough to Confer MC-Specific Expression on a Truncated *cab*-m1 Gene Promoter Sequence. The 5′ flanking sequence extending from −867 to −359 does not influence the expression of the maize *cab*-m1 gene in MCs or BSCs (14) but we investigated whether sequences contained in the 177-bp fragment alone could confer MC specificity. The 177-bp fragment, also designated A+B+C, was fused in the normal or reverse orientation upstream of the −359 deletion construct (−359 to +14 of *cab*-m1 fused to *uidA* and *nos*) and the expression of each resultant construct—+A+B+C/−359 and the A+B+C fragment in the reversed orientation—i.e., +C+B+A/−359—was studied in the *in situ* transient assay. As had been seen in earlier experiments as well (14), expression of GUS from the −359 construct was about equal in MCs and BSCs (Fig. 3). In contrast, +A+B+C/−359 was expressed almost 10-fold more in MCs than in BSCs using standard conditions in which the *35S cauliflower mosaic virus promoter:GUS:nos* control gene was expressed about equally in the two cell types (data not shown). Expression from +A+B+C/−359 was about 2.5-fold higher in MCs than the basal level obtained with the −359 deletion construct, but expression in BSCs was about 4-fold lower than the basal level (Fig. 3). Thus, the 177-bp fragment contains all of the DNA sequence information required for MC-preferred expression. +C+B+A/−359 behaved like +A+B+C/−359 (Fig. 3).

Analyses of the MSR: Expression Specificity of Sequences Added to the −359 Construct. A series of experiments was performed to test which, if any, parts of the 177-bp MSR sequence would influence expression of GUS from the MC/BSC neutral −359 construct. Fragments of the MSR were produced (see *Materials and Methods*) and each was ligated upstream of the −359 construct. The expression pattern of each construct was compared with expression from

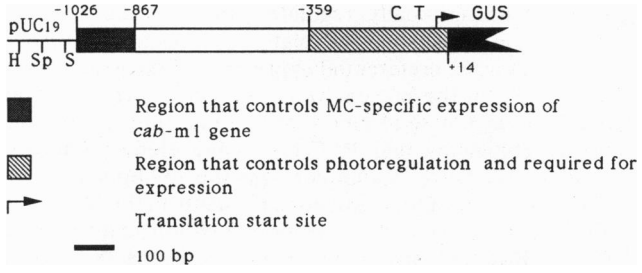

FIG. 1. Schematic representation of the chimeric gene in pM1CAB1.1 showing the locations of the regions required for strongly MC-preferred expression and for photoregulation of the maize *cab*-m1 gene (14). C, CCAAT box; T, TATAA box; H, *Hin*dIII; Sp, *Sph* I; S, *Sal* I.

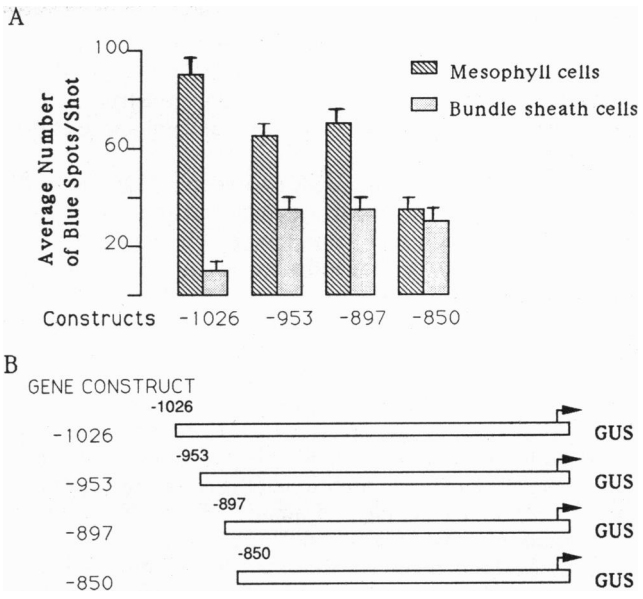

FIG. 2. *In situ* transient expression in MCs and BSCs of greening maize leaves of a set of 5′ → 3′ *cab*-m1 promoter deletions in *GUS:nos* chimeric genes. (*A*) The average number (±SEM) of blue spots per shot observed in MCs and in BSCs is shown for each deletion construct. (*B*) Diagrams of the chimeric gene constructs used.

A+B+C/−359 and the −359 reference chimeric genes in MCs vs. BSCs.

Addition of MSR fragment C (−897 to −850, see *Materials and Methods* and Fig. 7) to the −359 construct yielded a gene that was not expressed much differently from the control in BSCs but about twice as actively as the −359 control in MCs. Fragment C contains sequence(s) that approximately doubles GUS expression in MCs (Fig. 4). These observations are consistent with the differences observed in the behavior of the −897 vs. the −850 deletion constructs (Fig. 2). The addition of fragments B and C (−953 to −898 plus −897 to −850) 5′ to the −359 construct yielded a gene with an expression pattern similar to one carrying only fragment C.

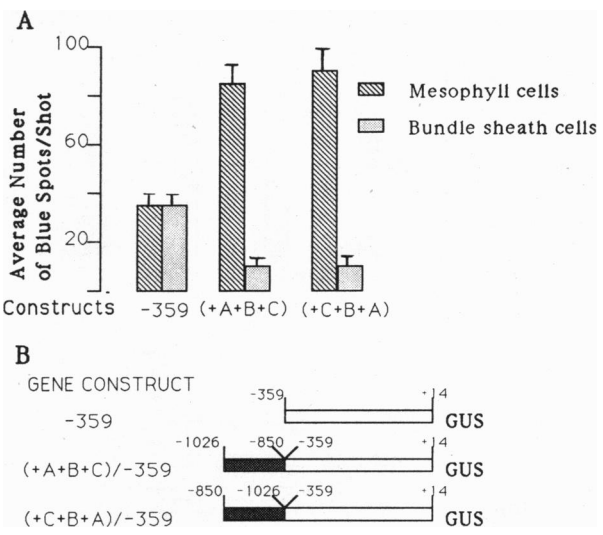

FIG. 3. *In situ* transient expression assays in MCs and BSCs of maize leaves of chimeric genes in which the 177-bp MSR was fused 5′ to the −359:*GUS:nos* construct in normal (+A+B+C) and reverse (+C+B+A) orientations. (*A*) The average number (±SEM) of blue spots per shot found in MCs and in BSCs is shown for each chimeric gene. (*B*) Diagrams of the chimeric gene constructs used (see text for details).

This result also corresponds to the observed difference in expression of the −953 deletion gene relative to the −897 deletion gene (Fig. 2). As already noted (Fig. 3), the addition of A+B+C (−1026 to −850) to the −359 construct resulted in a sharp decrease in BSC expression and enhanced expression in MCs (Fig. 4).

The 38-bp 5′ end of the MSR (between −1026 and −989) contains a sequence or sequences required for suppression of expression in BSCs and some part of the enhancement of expression in MCs. This is judged from the observation that expression of the chimeric gene +1/2A+B+C/−359, containing the −988 to −850 portion of the MSR, was not repressed in BSCs or enhanced in MCs to the levels attained from +A+B+C/−359 (Fig. 4). Subsequent experiments showed that sequences in the 38-bp region do not act alone.

The addition of fragment A or B alone to the 5′ end of the −359 construct did not alter MC/BSC expression from the latter (Fig. 5). However, addition of the A+B portion of MSR to the −359 construct had a dramatic effect. MC expression was enhanced substantially and BSC expression was strongly suppressed. Sequences in B thus appear to be required for the MC expression-enhancing and BSC expression-repressing activity for which the 38-bp 5′ region of fragment A is also required. Strong operational interactions between sequences in fragments A and B act in controlling MC-preferred expression and/or some regulatory element spans the boundary between A and B. As already noted, segment C alone has some MC expression-enhancing activity but, inasmuch as MC-enhanced expression from +A+B/−359 (Fig. 5) is about equal to that from A+B+C/−359 (Fig. 4) but fragment C alone (+C/−359; Fig. 4) roughly doubles MC expression over the base value, MC expression-enhancing elements in C and in A+B may work independently and/or hierarchically.

AT-1 Box-Like Sequence in the 5′ Flanking Region of *cab*-m1 Is Required to Repress Expression in BSCs. An (A+T)-rich negative regulatory element (NRE) that is present far upstream in the 5′ flanking region reduces the level of tobacco *cab* E gene expression in the light (21). The consensus sequence 5′AATATTTTTATT-3′, designated the AT-1 box, is present within the NRE of the tobacco *cab* E gene as well as in the promoter regions of some other *cab* and *rbcS* genes (22).

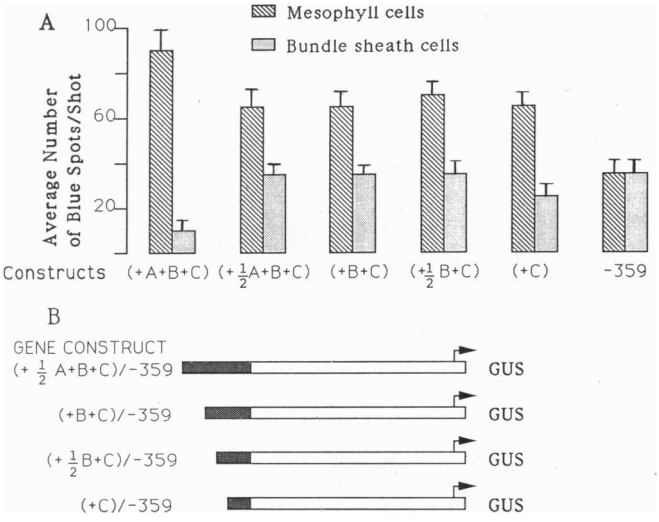

FIG. 4. *In situ* transient expression assays in MCs and BSCs of maize leaves of chimeric genes in which subfragments of the 177-bp MSR were fused to the 5′ terminus of the −359:*GUS:nos* construct. (A+B+C) = −1026 to −850; (+½A+B+C) = −988 to −850; (+B+C) = −953 to −850; (½B+C) = −930 to −850; (+C) = −897 to −850. The sequences are shown in Fig. 7. (*A*) The average number (±SEM) of blue spots per shot found in MCs and in BSCs is shown for each chimeric gene. (*B*) Diagrams of the chimeric gene constructs used.

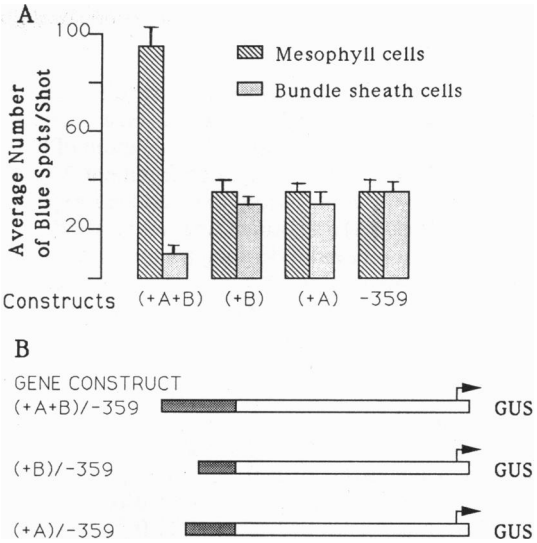

FIG. 5. *In situ* transient expression assays in maize leaf MCs and BSCs of GUS activity from chimeric genes in which MSR fragment A or B or A+B is fused 5′ to the −359 construct. (*A*) The average number (±SEM) of blue spots per shot found in MCs and in BSCs is shown for each construct. (*B*) Diagrams of the chimeric gene constructs used. (For details see Fig. 7 and legend to Fig. 4.)

We were interested in determining whether the AT-1 box-like sequence (5′-AATATTTTTTCT-3′) at −949 to −937 at the 5′ end of fragment B is involved in repressing *cab*-m1 gene expression in BSCs relative to MCs in maize leaves. Therefore, we extensively mutated the AT-1 box-like sequence in fragment B (18) in the +A+B+C/−359 construct and studied the expression pattern of the mutant gene in MCs vs. BSCs. Interestingly, expression was at about the control construct level in BSCs but expression in MCs remained as high as in the wild-type +A+B+C/−359 construct (Fig. 6), showing that the AT-1 box-like sequence is required for repressing expression in BSCs but not for promoting expression of the gene in MCs. However, some other sequence(s) downstream of the AT-1 sequence in fragment B is required, in concert with sequence(s) within fragment A, for enhancing expression in MCs.

DISCUSSION

We want to understand the mechanism(s) by which a single gene is expressed specifically or preferentially in MCs or BSCs in maize leaves and why members of the maize *cab* gene family differ in their patterns of expression. As a first step, we have mapped cis-acting regulatory elements in the 5′ flanking region of the maize gene *cab*-m1, which is expressed preferentially in MCs. MC-specific expression of the *cab*-m1 gene is transcriptionally regulated and a 5′ far-upstream 159-bp region (−1026 to −868 relative to translation start site) is required for the preferential expression of the gene *cab*-m1 in MCs (14). In the present work, we have further characterized this region and found that there are physically separate MC-enhancing and BSC-repressing elements located within the 177-bp MSR that contains the 159-bp sequence.

There are at least four sequence elements in the MSR (Fig. 7) that are involved in the regulation of *cab*-m1 expression in MCs and BSCs. Control region I lies within the 38 bp at the 5′ end (−1026 to −989) of fragment A; our experiments do not exclude the possibility that there are two separate functional sequences within this 38 bp (Fig. 4). Control region II is the AT-1-like element at −949 to −937 in fragment B, which, together with control region I, is necessary for suppression of

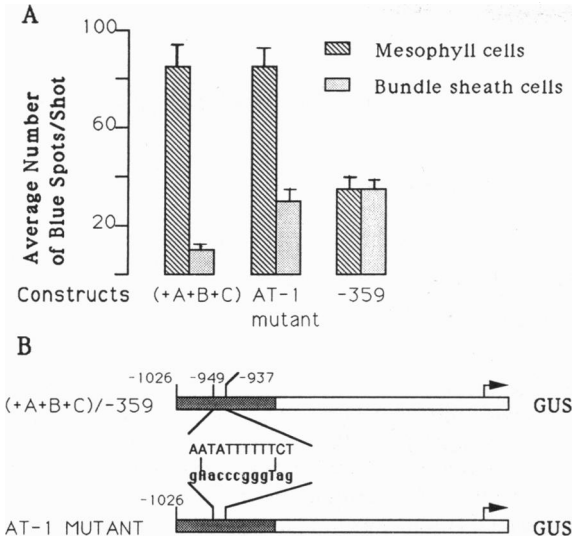

FIG. 6. Effect of mutations in the AT-1-like sequence in the construct (+A+B+C)/−359 on transient expression of GUS in MCs and BSCs of maize leaves. (*A*) The average number (±SEM) of blue spots per shot found in MCs and in BSCs is shown for each construct. (*B*) Diagrams of the wild-type and mutated chimeric gene constructs used.

cab-m1 expression in BSCs (Fig. 6). Control region III lies in fragment B between −937 and the 3′ end of fragment B at −897; its presence is required—together with control region I (i.e., the 38-bp 5′ end of fragment A)—for strongly enhanced expression of *cab*-m1 in MCs [note that addition of only fragment A to the −359 chimeric gene (Fig. 5) does not result in enhanced MC expression]. Control region IV lies in fragment C; it acts independently inasmuch as addition of fragment C alone to the −359/*GUS*/*nos* gene results in doubled expression in MCs (Fig. 4). In summary, *cab*-m1 expression in BSCs is suppressed by control regions I and II functioning cooperatively, and control regions I and III act together for maximal expression of *cab*-m1 in MCs. A lower level of MC-enhanced expression is conferred upon the −359 construct by control region IV alone.

It seems probable that the expression of *cab*-m1 in BSCs and MCs is regulated through negative and positive transcription factors that interact with specific DNA sequences. Models that can be imagined are (*i*) a single repressor protein

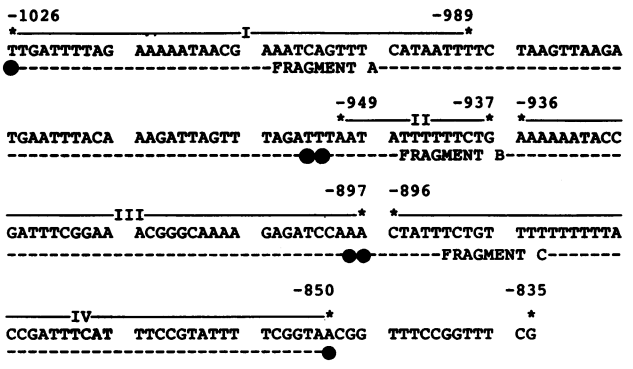

FIG. 7. Sequence of the 177-bp (−1026 to −850) MSR (14) showing locations of cis-acting control regions I–IV [see text; the DNA sequence shown has been deposited in the GenBank data base (accession no. M87020)]. Region IV is shown as ending at −850 but the observation of Bansal *et al.* (14) that deletion of the sequence from −1026 through −868 eliminates MC-preferred expression from the *cab*-m1 promoter suggests that the sequence between −867 and −850 may not be involved in this control.

associates with control regions I and II to suppress expression in BSCs or (*ii*) two regulatory proteins, one binding to region I and one to region II, interact to repress expression in BSCs. Enhancement of expression in MCs could require a MC transcription-enhancing factor that associates with regions I and III or a pair of interacting proteins, one that binds to region I and the other to region III. An independently acting factor could interact with region IV to enhance *cab*-m1 expression in MCs. It remains to be determined (*i*) whether these hypothetical positive factors exist and physically interact with one another, (*ii*) what the nature of the negative regulatory apparatus may be, (*iii*) whether control region I contains one or two different control sequences or whether a single sequence in the 38-bp distal region of the MSR is involved in enhancement of expression of *cab*-m1 in MCs and repression of its expression in BSCs, and (*iv*) whether MCs and BSCs differ in their complements of postulated effective regulatory proteins. The presence of multiple negative and positive regulatory elements within the MSR of *cab*-m1 suggests that the presence of various combinations of these elements could account for differences in the relative MC/BSC expression patterns of the other maize *cab* genes (23). It is particularly interesting that interaction of a protein with AT-1 sequences of certain pea nuclear genes is influenced by its state of phosphorylation (22).

A broader question is whether other nuclear genes for PSII components, such as those for proteins of the oxygen evolution apparatus, which like *cab*-m1, are preferentially expressed in MC (23), are controlled by *cab* gene mechanisms, and contain cis-acting sequences resembling those in *cab*-m1. The strictly MC-expressed maize genes for phospho*enol*pyruvate carboxykinase and pyruvate, orthophosphate dikinase do not contain AT-1-like sequences (4–6).

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