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Genetics

Inactivation of maize transposon *Mu* suppresses a mutant phenotype by activating an outward-reading promoter near the end of *Mu1*

(suppression/initiator)

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ABSTRACT **We described previously a mutation in maize, *hcf106*, caused by the insertion of a *Mu1* transposon. When the *Mu* transposon system is in an active phase, *hcf106* conditions a nonphotosynthetic, pale green phenotype. However, when the *Mu* system is inactive (a state correlated with hypermethylation of *Mu* elements), the plant adopts a normal phenotype despite the continued presence of the transposon within the gene. The molecular mechanisms that mediate this suppression of the mutant phenotype have now been investigated. We show here that the *Mu* element responsible for the *hcf106* lesion lies within sequences encoding the 5′-untranslated leader of the *Hcf106* mRNA. When the *Mu* transposon system is active, this insertion interferes with the accumulation of mRNA from the *hcf106* allele. However, when *Mu* is inactive, mRNA similar in size and abundance to that transcribed from the normal allele accumulates. These transcripts initiate at many sites throughout a 70-base-pair region, within and immediately downstream of the *Mu1* insertion. Thus, an unusual promoter spanning the downstream junction between *Mu1* and *Hcf106* substitutes for the normal *Hcf106* promoter but only when *Mu* is inactive. The pattern of mRNA accumulation in different organs and in response to light suggests that the activity of this promoter is conditional not only upon the phase of *Mu* activity, but also upon signals that regulate the normal *Hcf106* promoter.**

The insertion of transposable elements can subvert gene expression in a variety of ways, including activation or repression of target genes. The phenotypes of some transposon-induced mutations can be modified by unlinked genetic loci or by epigenetic factors that alter transposon activity. In maize, the phenotypic effects of insertions of members of the *Spm* transposon family are in some instances exaggerated and in others reduced by the presence of an active *Spm* element elsewhere in the genome (for review, see refs. 1–3). In yeast and *Drosophila*, mutations resulting from insertion of the retrotransposons *Ty*, *gypsy*, or *copia* can also be enhanced or suppressed by unlinked genetic factors (for reviews, see refs. 4–6).

We described (7) genetic evidence that another maize transposon family, Robertson's Mutator (*Mu*), can cause mutations whose phenotypes depend upon epigenetic changes in the transposon insertion. The *Mu* family consists of a set of elements found in active *Mu* lines that share a common 220-base-pair (bp) terminal inverted repeat (8–10). The photosynthetic mutant *hcf106* was caused by the insertion of *Mu1*, a member of this family (11). *hcf106* is a nuclear, recessive mutation that conditions a defect in the assembly of the chloroplast electron-transport chain (12). Mutant seedlings are pale green and are seedling lethal, due to their lesion in photosynthesis. A molecular clone of the *hcf106* locus was obtained by taking advantage of the *Mu1* insert as a molecular tag (11).

We reported (7) that the activity of the *Mu* transposon system modulates the phenotypic effect of the *Mu1* insert at the *hcf106* locus. *Mu* can adopt one of two phases: the "active" phase is defined by a high forward-mutation rate, somatic reversion of reporter alleles containing a *Mu* insertion, and transposition of *Mu* elements to different genomic locations. The "inactive" phase lacks these properties (13–15) and is usually marked by hypermethylation of sequences within the terminal inverted repeats of *Mu* transposons (14, 16). The *hcf106* phenotype is expressed only when the *Mu* transposon system is active. When *Mu* is inactive, the mutant phenotype is suppressed, and plants homozygous for the *hcf106* lesion exhibit a normal phenotype. Thus, the same nucleotide sequence can confer either of two phenotypes, depending upon the phase of *Mu* activity. The normal allele, lacking a *Mu* insert, will be referred to below as *Hcf106*, the mutant allele as *hcf106*, and the mutant allele in a *Mu*-inactive background (and therefore with no phenotypic effect) as *hcf106*(*Mu*-Off).

Here, we demonstrate that the *Mu1* element in the *hcf106* allele lies within sequences encoding the 5′-untranslated leader of the *Hcf106* mRNA. This insertion interferes with the accumulation of *Hcf106* mRNA when *Mu* is active. However, when *Mu* becomes inactive, the mutant phenotype is suppressed due to the activation of a promoter near the end of *Mu1* that directs transcription outward, into the adjacent *hcf106* gene. The activity of this promoter is conditional not only upon the phase of *Mu* activity but also upon signals that regulate the normal allele. This scheme adds to a growing list an additional mechanism by which maize transposons minimize their impact on the viability of the host (2). We suspect that *hcf106* is the prototype for what may be a frequent class of *Mu*-induced mutations whose phenotypes are modulated by the phase of *Mu* activity.

MATERIALS AND METHODS

Plant Material. The inbred line B73 (Pioneer Hi-Bred) was used as the "normal" line because the normal progenitor of *hcf106* is not known. Phenotypically normal plants homozygous for *hcf106* were obtained by self-pollinating heterozygous plants that had lost *Mu* activity after three outcrosses to B73. Their genotype was defined by genetic tests and confirmed by DNA analysis (data not shown).

Recombinant DNAs. The plasmid p106A is a molecular clone of a 3.7-kilobase-pair (kbp) *Sst* I fragment containing the *Mu1* element and flanking host sequences of the *hcf106* allele (11). p106D contains the 600-bp *Mlu* I–*Sst* I fragment of p106A (the

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right end of *Mu1* and the flanking *Hcf106* sequence) in a Bluescript SK vector. pB73106B encodes the 5′ portion of the *Hcf106* locus from B73, cloned into a Bluescript vector. This DNA was obtained by amplification of B73 DNA in a PCR using primers flanking the site of *Mu* insertion in *hcf106*. A full-length cDNA clone of the *Hcf106* mRNA was purified from a library representing B73 seedling leaf mRNA, constructed with a Unizap X-R cDNA cloning kit (Stratagene). p106cDNA 2-3 is the corresponding plasmid clone, in a Bluescript SK vector; its nucleotide sequence will be reported elsewhere. The plasmid pcDNA2-3Δ3′ is identical to p106cDNA2-3, except that polylinker sequences between the *Xho* I and *Kpn* I sites were removed to eliminate the signal resulting when radiolabeled transcripts of this region hybridized to a plastid rRNA species on Northern (RNA) blots. pHMG106 2-3, a modified form of p106cDNA2-3, was constructed for use in RNase protection experiments. A 380-bp *Sst* I fragment of an unrelated cDNA from tomato (from Jon Narita, University of California) was substituted for the 250-bp *Sst* I fragment at the 5′ end of the *Hcf106* cDNA insert, and the 3′ terminal *Acc* I–*Xho* I fragment (250 bp) of the *Hcf106* cDNA was removed. The RNA probe derived from this clone was 900 bp long, with a 600-bp region of homology with *Hcf106* mRNA, a length we found to give optimal results in RNase protection experiments.

Hybridization Probes and Control Transcripts. Radiolabeled RNA probes were transcribed *in vitro* by standard procedures, treated with DNase I, and gel-purified. The location of each probe on the *Hcf106* map is diagrammed in Fig. 3. The RNA probe used in Fig. 2 was transcribed by SP6 polymerase from p106A that had been digested with *Mlu* I at a site 194 bp inside *Mu1*. The probe used in Fig. 4*A* was transcribed by T7 RNA polymerase from pHMG106 2-3 that had been cleaved in the polylinker distal to the T7 promoter. The probe used in Fig. 4*B* was transcribed by T7 RNA polymerase from pcDNA2-3Δ3′ that had been digested with *Acc* I. This 250-nucleotide probe was specific for the *Hcf106* locus; a gene related to *Hcf106* exists within the maize genome but bears no homology with this probe (unpublished results). The probes for the S1 protection experiments were synthesized by using the 5′-end-labeled oligonucleotide used in the primer extensions to prime second-strand synthesis on single-stranded forms of pB73106B (Fig. 1) or p106D (Fig. 2). The 3′-end of each probe was then defined by restriction digestion in the distal polylinker sequence. The single-stranded probes were purified on a denaturing polyacrylamide gel. The unlabeled transcripts used as controls were synthesized by T3 RNA polymerase from p106D (T3/hcf), pB73106B (T3/WT), or p106cDNA 2-3 (T3/Hcf106).

Transcript Analyses. RNA was purified by guanidine thiocyanate extraction, phenol extraction, and DNase treatment, as described (11). Northern analysis was as described (17), except that ethidium bromide (40 μg/ml) was added to the sample buffer. Filters were washed at 65°C in 0.1× SSC/0.3% SDS, treated with RNase T1 (BRL) at 100 units/ml for 1 hr at 20°C to eliminate residual binding of the RNA probe to rRNA and washed briefly in 0.1× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.3% SDS at 65°C. Primer extensions were done as described by McKnight (18). The primer was a 5′-end-labeled 24-mer homologous to the *Hcf106* mRNA between 37 and 61 nucleotides downstream of the translation start codon. S1 nuclease protection experiments were done with single-stranded probes as described (17), except that probes were labeled specifically at their 5′ ends. RNase protection experiments were done essentially as described in ref. 19. Hybrids were treated with either RNase T1 (BRL) at 300 units/ml and RNase A at 40 μg/ml (Fig. 2) or RNase T1 alone (Fig. 4).

RESULTS

The *hcf106* Mutation Is Caused by the Insertion of a *Mu1* Transposon Immediately Downstream of the Normal Transcription Start Sites. Results of Northern hybridization experiments using strand-specific probes suggested that the *Mu* element was inserted near the normal transcription start site in the *Hcf106* gene (ref. 11 and data not shown). To precisely map the *Hcf106* transcription start sites, the 5′ ends of transcripts derived from the normal allele in the inbred line B73 were determined by primer extension and S1 nuclease protection. The same 5′-end-labeled oligonucleotide was used to prime the synthesis of *Hcf106* cDNA by reverse transcriptase, to prime the synthesis of a probe for S1 nuclease protection experiments, and to prime a sequencing ladder from a clone of the B73 *Hcf106* allele (Fig. 1).

Three primer-extension products of approximately equal abundance were obtained. These comigrated with three S1

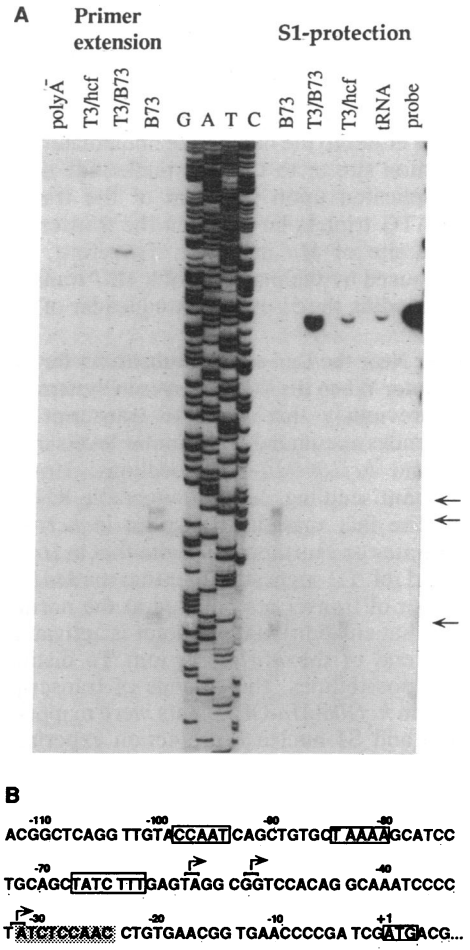

FIG. 1. Mapping the 5′ termini of transcripts derived from the *Hcf106* promoter. (*A*) Primer-extension and S1 nuclease protection assays. One-half microgram of poly(A)$^+$ leaf RNA from greenhouse-grown B73 seedlings [or an equivalent amount of poly(A)$^-$ leaf RNA or tRNA] was analyzed. T3/B73 and T3/hcf are T3 transcripts of the normal and mutant genomic clones, respectively, in the same sense as the Hcf106 mRNA. One-twentieth of the amount of probe used in each hybridization is shown. The map locations of the primer, S1 nuclease probe, and control transcripts are shown in Fig. 3. (*B*) Nucleotide sequence of the 5′ region of the *Hcf106* gene in B73. The putative CAAT box, TATA boxes, and start codon are enclosed in boxes. The 9-bp sequence into which *Mu1* is inserted in *hcf106* is shaded. This sequence was duplicated upon *Mu* insertion, flanking the *Mu* element on both sides in the mutant allele. Arrows indicate the 5′ ends mapped in the experiment shown in *A*. Because the precise 5′ nucleotide is ambiguous, two nucleotides corresponding to each terminus are bracketed.

nuclease-resistant products. A control experiment was performed in which a transcript synthesized *in vitro* by using the *Hcf106* genomic clone as template was substituted for leaf mRNA. This transcript (T3/B73) is colinear with the 5′ region of the authentic mRNA, except that it extends further upstream into the 5′-flanking region of the *Hcf106* gene (see Fig. 3). Just one primer extension or S1 nuclease-resistant product, of the length expected, was obtained from T3/B73 RNA. There was no evidence of premature termination in the primer-extension reaction or internal cleavage in the S1 nuclease protection reaction to give rise to reaction products that comigrate with those obtained from leaf mRNA. Therefore, the products resulting from the analysis of leaf mRNA are not artifacts particular to the *Hcf106* RNA sequence and so must represent true 5′ termini. The probe fragment protected by T3/hcf, a T3 transcript of the *hcf106* genomic clone (see Fig. 3), marks the site of *Mu* insertion in *hcf106* because the sequences of the normal and mutant alleles first diverge at that point.

The three transcription start sites lie ≈30 nucleotides downstream of two TATA-like sequences (Fig. 1*B*). The two upstream start sites lie a short distance upstream of the site into which the *Mu1* element is inserted in *hcf106*. The third start site maps either to the nucleotide immediately preceding the *Mu* insertion site or to the first nucleotide of the target sequence duplicated upon insertion of the transposon in *hcf106*. No ATG triplets lie between the transcription start sites and the site of *Mu* insertion. Therefore, the *hcf106* mutation is caused by the insertion of a *Mu1* transposon into sequences encoding the 5′-untranslated leader of the *Hcf106* mRNA.

A Promoter Near the End of *Mu1* Substitutes for the Normal *Hcf106* Promoter When the *Mu* Transposon System Is Inactive. We found previously that a 1.2-kb transcript containing *Hcf106* sequences accumulates to similar levels in the leaves of *Hcf106* and *hcf106*(*Mu*-Off) seedlings. However, the leaves of mutant seedlings lacked detectable RNA from the locus (11). The fact that the transcript in *hcf106*(*Mu*-Off) plants comigrates on Northern gels with that in *Hcf106* plants (see Fig. 4 and ref. 11) suggests either that the *Mu1* sequences are spliced out of transcripts initiated at the normal *Hcf106* promoter or that an additional promoter is activated near the downstream end of the *Mu1* insertion. To distinguish between these possibilities, the 5′ ends of transcripts of the *hcf106* locus in *hcf106*(*Mu*-Off) plants were mapped by primer-extension and S1 nuclease protection experiments. The experimental design was identical to that shown in Fig. 1, except that the S1 nuclease probe and sequencing ladder were derived from a clone of the mutant rather than of the normal allele.

The results of these experiments (Fig. 2) show that the 5′ ends of these transcripts are heterogeneous, mapping to ≈13 different sites. The termini span a 70-nucleotide region, starting within the *Mu1* terminus and extending into the flanking *Hcf106* sequences downstream. The similarity between the patterns obtained by the S1 nuclease protection and primer-extension assays shows that the heterogeneity is not an artifact of the experimental procedures. This conclusion is supported by the results of control reactions in which the T3/hcf transcript was substituted for leaf RNA. This transcript, which contained the same sequences as the *in vivo* transcripts, except that it extended further upstream into *Mu1* (see Fig. 3), yielded only full-length primer extension and S1 nuclease-resistant products. Therefore, the products obtained from leaf mRNA are not artifacts particular to the RNA sequence. Furthermore, a similar heterogeneous pattern of transcripts was detected by using an RNase protection assay (Fig. 2*A*), although these results cannot be placed side by side for comparison due to differences in the migration

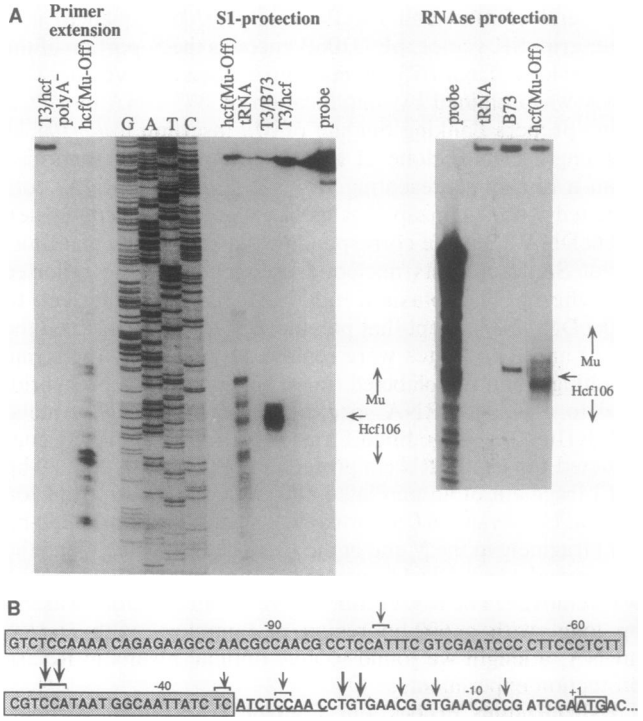

FIG. 2. Mapping the 5′ termini of transcripts from the *hcf106*::*Mu* promoter. (*A*) Primer-extension and S1 nuclease protection assays were analogous to those in Fig. 1. The sequencing ladder (derived from the *hcf106* genomic clone), primer extension, and S1 nuclease probe synthesis were all primed with the same oligonucleotide. The probe for the RNase protection experiment is diagrammed in Fig. 3. Two micrograms (*hcf-Mu* OFF) or 0.5 μg (B73) of poly(A)$^+$ leaf RNA from greenhouse-grown seedlings was analyzed. The site of *Mu* insertion is marked by the bands in lanes labeled T3/B73 or B73 because the probe sequence diverges in sequence from the B73 sequence where *Mu* is inserted. (*B*) Nucleotide sequence of the 5′ region of the *hcf106* allele. Sequences of the *Mu1* transposon are shaded. Arrows indicate the 5′ termini mapped in the primer-extension experiment. The length and boldness of the arrows vary in approximate proportion to the relative abundance of transcripts initiating at that site.

rates of RNA and DNA. RNA preparations from four different genetic families gave similar results (data not shown).

These results indicate that a cryptic promoter mapping near the end of the *Mu1* element in the *hcf106* allele is activated when the *Mu* transposon system becomes inactive. This "*hcf106*::*Mu*" promoter substitutes for the normal *Hcf106* promoter, generating a set of transcripts very close to the normal size and containing the entire protein-coding region. However, when *Mu* is active this promoter is not used, little mRNA from the locus accumulates, and the plant adopts a mutant phenotype.

The *Hcf106* and *hcf106*::*Mu* Promoters Are Regulated Similarly. It was of interest to determine whether the unusual *hcf106*::*Mu* promoter was regulated only by the phase of *Mu* activity or by other factors as well. RNA was purified from dark-grown leaves, leaves illuminated for 24 hr, roots, and immature cobs of plants homozygous for *Hcf106* or *hcf106*(*Mu*-Off). The abundance of the *Hcf106* transcript as a proportion of total RNA was then determined by Northern hybridization and RNase protection assays (Fig. 4). Relative transcript abundance was estimated by comparison to dilutions of the *Hcf106* "light" RNA sample (Fig. 4*A* and data not shown). The results of the two assays agree. Transcripts from both promoters are ≈4-fold more abundant in leaf tissue exposed to light for 24 hr than in leaves of the same age grown in the absence of light. Transcripts from both promoters are

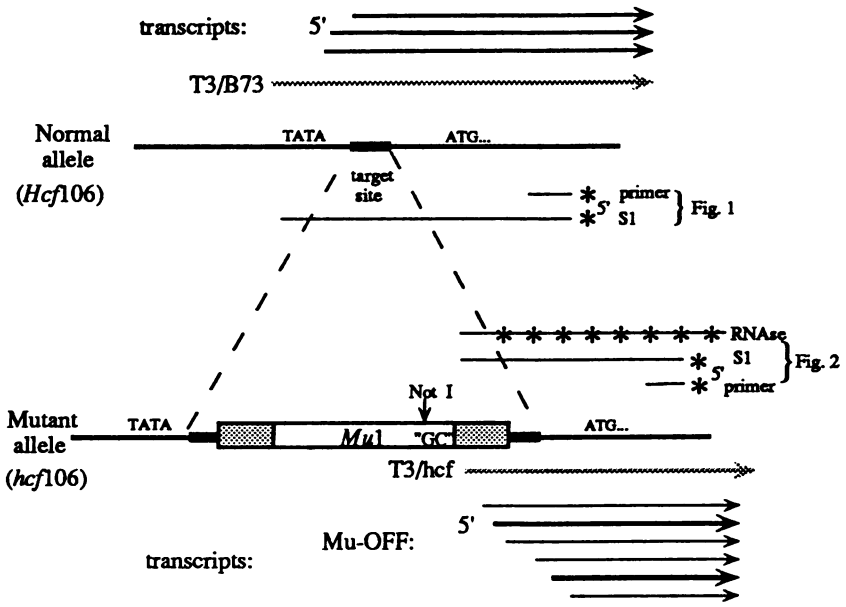

FIG. 3. Organization of alleles of the *Hcf106* locus. Features are not drawn to scale. The target site is the 9-bp host sequence into which *Mu* is inserted in *hcf106*. The 220-bp terminal inverted repeats of *Mu1* are represented by speckled boxes. A G+C-rich region begins immediately at the junction between the internal region of *Mu1* and the right terminal repeat. A unique *Not* I restriction site in *Mu1* defines its orientation. The three 5'-termini mapped in Fig. 1 are indicated by solid arrows. The heterogeneous termini mapped in Fig. 2 are indicated by arrows of various thickness to indicate relative abundance. Control transcripts, generated *in vitro* from genomic clones of the normal (T3/B73) and the mutant (T3/hcf) alleles, are shown as textured arrows. Asterisks represent radiolabeled residues in probes used for transcript mapping.

approximately twice as abundant in immature cobs as in roots. The *Hcf106* promoter, however, directs a higher level of leaf mRNA than the *hcf106*::*Mu* promoter. These results suggest that the regulation of the *hcf106*::*Mu* promoter by the phase of *Mu* activity is superimposed upon signals that control the expression of the normal *Hcf106* promoter. Because the genetic backgrounds of the *hcf106*(*Mu*-Off) and *Hcf106* plants are not identical, the slight differences in their expression patterns may reflect genetic differences at modifying loci.

DISCUSSION

We have shown that the *hcf106* mutation is caused by the insertion of a *Mu1* transposon into sequences encoding the 5'-untranslated region of the *Hcf106* mRNA. In *Mu*-active leaf tissue, this insertion decreases the accumulation of mRNA from the locus 10- to 20-fold (ref. 11 and unpublished results) resulting in a nonphotosynthetic phenotype. However, when *Mu* switches to the inactive phase, the plant adopts a normal phenotype due to the activation of a promoter near the downstream end of *Mu1*. The regulation of this "*hcf106*::*Mu*" promoter resembles that of the normal *Hcf106* promoter in terms of light-induction and tissue specificity, suggesting that *Hcf106* cis-acting regulatory signals can interact with the *hcf106*::*Mu* promoter.

In *Mu*-active leaf tissue, the *Mu* insertion in *hcf106* interferes with accumulation of *Hcf106* mRNA. This interference might be at the level of transcript initiation, premature termination, or mRNA stability. *Mu1* insertions in *Adh1* interfere with the initiation of transcription, cause transcriptional attenuation, and contain polyadenylylation signals (20–23). It is not possible, however, to extrapolate these results to *hcf106* because the *Mu1* element is in the opposite orientation relative to target gene transcription.

Members of the *Ac/Ds* and *Spm* transposon families sometimes function as introns, thereby permitting expression of a gene they have interrupted (for review, see ref. 24). Functional splice donors and acceptors have also been identified in *Mu1* (22, 23). We can, however, eliminate the possibility that removal of *Mu* sequences by splicing accounts for the accumulation of a transcript of the same size as the *Hcf106* mRNA because (*i*) the 5'-end mapping by primer extension confirmed the results of nuclease protection experiments, indicating that 5' ends, rather than splice junctions, map to the *Mu1* terminus; (*ii*) the splice acceptor sites identified previously in *Mu1* are not near the ends mapped here; and (*iii*) no AG dinucleotides, found universally at splice acceptor sites (25, 26), occur in this 70-bp region.

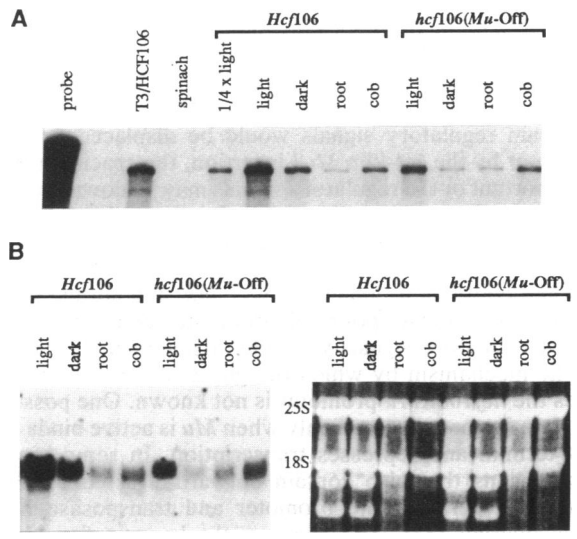

FIG. 4. Pattern of accumulation of mRNA transcribed from the *Hcf106* and *hcf106*::*Mu* promoters. Leaf RNA was purified from the apical half of leaves of seedlings grown for 7 days in the dark followed by 24 hr of light (light) or grown for 8 days in the dark (dark). Root RNA was prepared from seedlings grown for 10 days. Cob RNA was purified from 2-inch-long cobs, harvested prior to silk emergence. Samples of total RNA containing equal amounts of cytoplasmic rRNA (≈15 μg) were analyzed. (*A*) RNase-protection assay. A 1/4× dilution of light-grown leaf RNA was analyzed to aid in quantification. Two picograms of RNA transcribed *in vitro* from the *Hcf106* cDNA clone (T3/HCF106) was analyzed as a positive control. This transcript mimics the *in vivo* mRNA. Fifteen micrograms of spinach RNA was assayed as a negative control. One twentieth of the amount of probe used in each hybridization is shown. (*B*) Northern hybridization. RNAs were hybridized with a radiolabeled RNA probe specific for the *Hcf106* locus. The UV fluorescence of the ethidium-stained RNA bound to the filter, pictured to the right of the autoradiograph, illustrates that samples contained an equal amount of cytoplasmic rRNA. The two additional stained bands in the leaf RNA samples are chloroplast rRNAs; these are slightly more abundant in light-grown than in dark-grown leaves.

The Nature of the *hcf106*::*Mu* Promoter. The *hcf106*::*Mu* promoter does not contain a distinct TATA box upstream of each transcription start site, although some short A-T stretches may serve this function. The sequences surrounding the four most upstream start sites do, however, resemble the "initiator" element (27), a pyrimidine-rich sequence that can by itself define transcription start sites and promote transcription. Transcription from an initiator is stimulated by the presence of TATA or G+C-rich elements upstream. The sequence of *Mu1* becomes extremely G+C-rich ≈200 bp upstream from the clustered transcription start sites arising from the *hcf106*::*Mu* promoter (28). It is possible that this G+C-rich region serves as part of the promoter. Heterogeneous and TATA-independent transcript initiation has also been reported for the *Ac* transposase gene in maize (29, 30).

The transcription start sites arising from the *hcf106*::*Mu* promoter span the junction between *Mu1* and *Hcf106* sequences. It is not clear, therefore, whether the functional promoter lies entirely within *Mu1*, is a chimera between *Mu* and *Hcf106* sequences, or lies entirely within the transcribed region of *Hcf106*. Examples have been described of mammalian polymerase II promoters requiring sequences downstream of the transcription initiation site (for review, see ref. 31), but in these cases downstream sequences are not sufficient for transcription.

Regulation of the *hcf106*::*Mu* Promoter. The regulation of the *hcf106*::*Mu* and *Hcf106* promoters appears similar, except that *hcf106*::*Mu* responds to *Mu* activity. RNA transcribed from the two promoters is light-induced to a similar extent; the ratio of mRNA levels in immature cobs relative to roots is also comparable. While the increased resolution obtainable by *in situ* hybridization methods will be required to precisely determine the cell-type specificities, these results suggest that some of the normal *Hcf106* cis-acting regulatory signals can interact with the *hcf106*::*Mu* promoter. Because upstream regulatory signals would be displaced from this promoter by the 1.4-kbp *Mu1* insertion, the spacing may not be important or the regulatory signals may lie downstream, in the transcribed region. Alternatively, the ends of the *Mu1* element may be brought together by the binding of a factor to both termini. Trans-acting factors bound upstream of the *Mu* element could then "reach across" *Mu*, and normal spacing between the transcription initiation sites and upstream cis-acting regulatory signals would be maintained.

The mechanism by which the phase of *Mu* activity regulates the *hcf106*::*Mu* promoter is not known. One possibility is that a factor expressed only when *Mu* is active binds to the *Mu* termini and represses transcription. In some bacterial transposons that also contain outward-directed promoters near their termini, the promoter and transposase-binding sites overlap (32). The same might be true for *Mu1*. A DNA-binding activity present only in *Mu*-active plants, and another present in both *Mu*-active and -inactive plants, interact with sequences in the *Mu* terminal inverted repeats (Z.-Y. Zhao and V. Sundaresan, personal communication). These binding activities might represent factors that regulate the *hcf106*::*Mu* promoter. Methylation of the *Mu* termini in the *Mu*-inactive background might also play a role in the activation of this promoter.

The transcriptional activity of retrotransposons such as *Ty* and *gypsy* can have a profound effect upon the expression of nearby genes (for review, see refs. 4 and 6). By analogy, transcription within *Mu1* might modulate the activity of the *hcf106*::*Mu* promoter. However, there is, as yet, no evidence for transcription within *Mu1* that correlates with the phase of *Mu* activity.

***Mu1* May Frequently Induce Mutants Whose Phenotypes Are Suppressible.** *hcf106* may be the prototype for what is a rather common class of *Mu*-induced mutations. The phenotypes of a *Mu*-induced dwarf mutant (J. Colasanti and V. Sundaresan, personal communication) and an allele of *vp1* (R.A.M. and D. McCarty, unpublished work) are modified by *Mu* activity. The *al-mum2* allele, caused by the insertion of a *Mu1* element near the normal *A1* transcription start site (33), prevents anthocyanin biosynthesis in the plant when *Mu* is active but not when *Mu* is inactive (P. Chomet, personal communication). Similarly, a *Mu1* element inserted into the promoter region of *Kn1-0* prevents the expression of the dominant knotted phenotype but only when *Mu* is active (S. Hake, J. Mathern, and B. Lowe, personal communication). The fact that *Mu* insertions can cause phenotypes that disappear when *Mu* becomes inactive should be considered in transposon-tagging experiments. The absence of an insertion from phenotypically normal plants is not a good criterion for genetic linkage to a mutation, unless it is certain that the mutant phenotype is not suppressible.

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