Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 2875–2879, May 1985
Genetics

Insertion of an unstable element in an intervening sequence of maize *Adh1* affects transcription but not processing

(Robertson's mutator/alcohol dehydrogenase 1)

L. J. ROWLAND AND J. N. STROMMER

Department of Genetics, University of Georgia, Athens, GA 30602

Communicated by Norman H. Giles, December 31, 1984

ABSTRACT **Three independently isolated and unstable mutants of the maize alcohol dehydrogenase 1 gene (*Adh1*) have arisen by insertion of the *Mu* transposing element into the first intervening sequence of the progenitor *Adh1* allele. The mutants have been selected for their decreased levels of alcohol dehydrogenase 1 activity. The original mutants were unstable, giving rise to both revertant alleles and altered mutant alleles. From one of the original mutants, two derivative mutants have been recovered and described. We analyzed the effect of *Mu* insertion in all five of these mutants by measuring relative levels of run-off transcripts from the progenitor and mutant alleles and by comparing levels of run-off transcripts corresponding to regions lying 5′ and 3′ to the insertion sites. In this paper we present evidence that early transcriptional events are affected, but that, in spite of the inclusion of a 1.4-kilobase transposing DNA element, processing of transcripts occurs normally.**

Robertson's mutator, or *Mu*, was initially described as a factor increasing the frequency of mutant phenotypes in maize seedlings 30- to 50-fold (1, 2). Freeling and co-workers (3, 4) crossed a *Mu* line bearing an alcohol dehydrogenase 1 (ADH1) allele, *Adh1-F*, to their standard *Adh1-S* line, which specifies a peptide of electrophoretic mobility distinguishable in starch gels. By allyl alcohol selection of pollen from F_1 plants, they were able to recover three unstable *Adh1-S* mutants, designated *Adh1-S3034*, *Adh1-S4477*, and *Adh1-S4478* (herein called *S3034*, *S4477*, and *S4478*). Each mutant allele was reported to produce 40% of the ADH1-S polypeptide produced by the progenitor. Mutants *S3034a* and *S3034b* were derived from *S3034* by subsequent allyl alcohol selection (3); the former produced no active ADH1-S polypeptide, whereas the latter produced 13% of that synthesized by the *Adh1-S* progenitor.

The dramatic increase in the synthesis of ADH1 accompanying the submersion of maize roots in water (5) provided a source of RNA enriched for ADH1-encoding sequences and, thus, permitted isolation of a cDNA clone for the *Adh1* allele (6). This probe allowed us to demonstrate that all of the mutants contain similar insertions of ≈1.5 kilobases (ref. 7; unpublished data). It also has been shown directly that submersion induces dramatic rises in ADH1 RNA levels (7, 8). Less ADH1 mRNA is produced by the mutant alleles, but its length, ≈1650 nucleotides, is indistinguishable from that produced by the progenitor (7).

Bennetzen *et al.* (9) have isolated genomic clones for both the progenitor and *S3034* alleles. The former has been completely sequenced (10), and the latter has been sequenced sufficiently to map the insertion site of *Mu* to the first intervening sequence (9). The entire sequence of the *Mu* element inserted in *S3034* has also been determined (11). By Southern mapping we have assigned the *Mu* insertions of *S4477* and *S4478* to the first intervening sequence as well but to a different region, 213–462 base pairs (bp) downstream from the insertion in *S3034* (unpublished data).

MATERIALS AND METHODS

Strains. Maize lines bearing *Adh1-S* and the mutant alleles in similar genetic backgrounds were originally obtained from M. Freeling. An inbred line of Boone County White, DM2, was the gift of D. Miles.

Recombinant plasmids pUGA1 and pUGA2 were derived from pBR322 by the subcloning of *Pst* I fragments from clones of *S3034* and *Adh1-S*, (9) respectively. The subclones contain 150 bp of DNA proximal to the transcriptional initiation site and 1960 bp of gene, representing 60% of the total transcript. The sequences lying 5′ to *Adh1* encode no transcript detectable in roots (unpublished data). The *Adh1-S* progenitor sequences subcloned in pUGA2 are depicted in Fig. 1. The ADH1 cDNA plasmid, pZmL84, obtained from J. Peacock, contains 879 bp, representing the 3′ half of the ADH1 mRNA (8).

Measurement of ADH1-S Activity. Homozygous *Adh1-S* progenitor and mutant lines were crossed to DM2, an inbred Boone County White line bearing an *Adh1-F* allele. Scutellar tissue was excised from several kernels of representative ears and ground in 0.1 M potassium phosphate (pH 8.3). After low-speed centrifugation at 4°C, the supernatant fraction was absorbed onto small discs of 3MM filter paper (Whatman), blotted gently, and loaded onto 11% starch gels. Electrophoresis was at 150 V for 17 hr at 4°C in the buffer system described by Scandalios (12). Gels were sliced and stained for ADH activity by the method of Ayala *et al.* (13) except that isopropanol was replaced with 95% ethanol. Relative activities of F/F (ADH1-F) homodimers, S/S (ADH1-S) homodimers, and F/S heterodimers were determined by integration of densitometric peaks by means of a Beckman DU8 spectrophotometer. Levels of activity of ADH1 in homodimers and heterodimers were used to estimate relative levels of active ADH1-S polypeptides (14). Activities assayed in this way have been expressed relative to activity of the progenitor ADH1-S polypeptide.

RNA Isolation and Hybridization. Seeds were washed in 1:1 (vol/vol) bleach and detergent, soaked in water for 1 hr, and then set on sand covered with damp paper towels. After 4–5 days at 20°C, seedlings were rinsed in water then submerged for 8 hr in water buffered with 10 mM Tris chloride (pH 7.5). Roots were harvested by quick-freezing in liquid nitrogen and stored at −70°C until used.

Total RNA was extracted from frozen roots by the method of Lizardi and Engelberg (15) and stored in liquid nitrogen. Twenty micrograms of this RNA in 5 μl of water was heated to 65°C and blotted directly onto dry 0.45-μm nitrocellulose membrane filters (Schleicher & Schuell). Blots were baked

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ADH1, alcohol dehydrogenase 1; bp, base pair(s); *Adh1*, ADH1 gene.

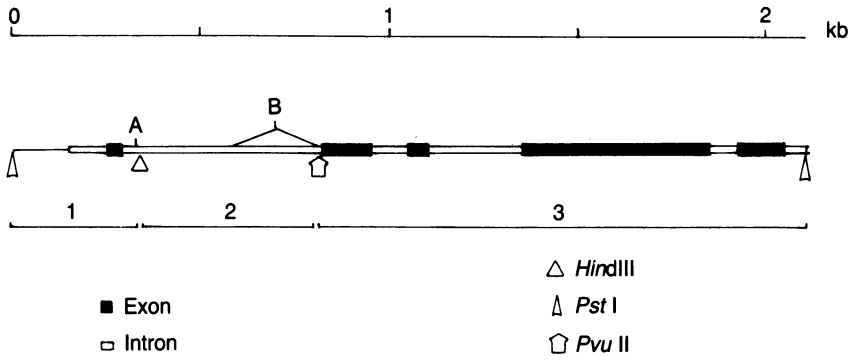

FIG. 1. Recombinant plasmid pUGA2, bearing 60% of the *Adh1-S* structural gene. Sites of *Mu* insertion are designed "A" (for the *S3034* family of mutants) and "B" (for *S4477* and *S4478*). Digestion sites used to separate 5′, intron, and 3′ regions are indicated, as are the fragments produced by digestion. The shortest fragment (7) represents the 5′ region; that of intermediate length (9), the intron; and the longest (3), the 3′ region. Fragment 1 contains 150 bp lying 5′ to the transcript; these are single-copy sequences not expressed in the induced root (unpublished data). kb, Kilobase(s).

under vacuum at 80°C for 2 hr and then stored at room temperature under vacuum.

Blots were hybridized with nick-translated pZmL84 (6) as described by Bruskin *et al.* (16) and then washed in 0.2× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate) containing 0.1% $NaDodSO_4$ at 68°C, conditions under which the probe is specific for *Adh1* (7). Filters were exposed to Kodak XAR-5 film for 1–7 days at −70°C by the method of Bonner and Laskey (17). Densitometric tracings of autoradiograms were obtained with a Beckman DU8 spectrophotometer.

Isolation of Run-Off Transcripts. Nuclei were isolated from roots by a method modified from that of Luthe and Quatrano (ref. 18; S. Berry-Lowe, personal communication). Modifications included the additional homogenization of tissue in a Polytron blender (Brinkmann) and elimination of the filtration through nylon mesh. Maize nuclei were recovered from the pellet after centrifugation through 80% Percoll.

Nuclear transcripts were labeled by a procedure of Luthe and Quatrano (19) in which transcripts elongate for a short distance and then terminate. For these experiments, 330 μCi (1 Ci = 37 GBq) of [^{32}P]UTP (New England Nuclear) were used with ≈10^7 nuclei. After 1 hr of incubation, RNA was extracted by the procedures of Harding *et al.* (20) and Glisin *et al.* (21). Nuclei were added to a solution of 5 M guanidinium isothiocyanate (Sigma)/50 mM Tris chloride, pH 8.5/50 mM EDTA/2% Sarkosyl/5 mM 2-mercaptoethanol to a final volume of 3 ml. After Vortex-mixing, the solution was heated to 68°C, mixed again, and layered over 1 ml of 5.7 M CsCl/0.1 M EDTA. The RNA was pelleted by ultracentrifugation in a Beckman SW 50.1 rotor at 40,000 rpm at 18°C. After resuspension of the pellet in water, 100 μg of *E. coli* tRNA was added, and the RNA was precipitated in 0.3 M sodium acetate/67% ethanol. This RNA was repelleted, resuspended in 10 mM Tris chloride, pH 7.5/40 mM NaCl/1 mM EDTA, extracted with an equal volume of buffered phenol, and again precipitated in ethanol. From 10^7 nuclei, we invariably recovered ≈3 × 10^6 dpm of RNA.

Gel Electrophoresis and Hybridization. The recombinant plasmid pUGA2, representing 60% of the progenitor *Adh1* gene, including 150 bp 5′ to the transcriptional start site (10), was digested with restriction enzymes *Pst* I, *Hin*dIII, and *Pvu* II (New England Biolabs) to yield 5′-, intron-, and 3′-specific fragments (Fig. 1). The plasmid pUGA1, containing both *Adh1* and *Mu*, was cleaved with restriction enzymes *Tth* I and *Bst*EII (New England Biolabs) to yield leftward (557 bp) and rightward (450 bp) halves of *Mu* as it lies in *S3034* oriented as in Fig. 1. Restriction fragments were separated by gel electrophoresis through a 1% agarose gel in Tris borate buffer (7). DNA fragments were transferred to nitrocellulose membrane filters by the method of Southern (22), utilizing 10× NaCl/Cit rather than 20× NaCl/Cit.

Equal amounts of radioactively labeled transcript from progenitor and mutants, 2.9 × 10^6 dpm, were hybridized to Southern blots of both pUGA1 and pUGA2 DNA digested as described above. Prehybridization and hybridization conditions were as described by Alwine *et al.* (23), with the addition of 100 μg of polyadenosine (Sigma) per ml to the prehybridization mixture. Washes, exposure, and analysis were as described for dot blots.

RESULTS

Protein extracts of scutellar tissue from progenitor and mutant *S/F* heterozygotes were resolved on starch gels and stained for ADH activity; typical patterns are shown in Fig. 2. Allozyme ratios permitted measurement of the proportion of active ADH1-S polypeptide in heterozygotes. If the level of ADH1-S activity in the progenitor/BCW heterozygote is defined as 100% ADH1-S, the mutant alleles produce 39% (*S3034*), <5% (*S3034a*), 35% (*S3034b*), 72% (*S4477*), and 44% (*S4478*) of the normal level of ADH1-S polypeptide.

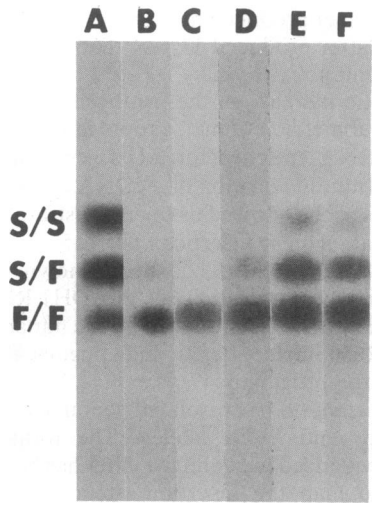

FIG. 2. Starch gels of scutellar tissue from *S/F* heterozygotes stained for ADH1 activity. In each case the *ADH1-F* allele was contributed by an inbred Boone County White line. S/S, ADH1-S homodimers; F/F, ADH1-F homodimers; S/F, heterodimers. *S* alleles were contributed from the progenitor *Adh1-S* (lane A), *S3034* (lane B), *S3034a* (lane C), *S3034b* (lane D), *S4477* (lane E), and *S4478* (lane F).

Preliminary experiments (data not shown) indicated that, under our induction conditions, ADH1 RNA synthesis in seedling roots is maximal after 8 hr of submersion. Therefore, roots of seedlings homozygous for the progenitor and mutant alleles were harvested after 8 hr of submersion and used as a source of RNA. Equal amounts of RNA in equal volumes were blotted onto nitrocellulose and hybridized to nick-translated pZmL84 containing the 3′ half of ADH1 RNA. An autoradiogram from one such experiment is illustrated in Fig. 3. From densitometric tracings provided from two such experiments, we determined the following relative levels of ADH1 RNA in the mutants: 26% (*S3034*), 12% (*S3034a*), 22% (*S3034b*), 72% (*S4477*), and 36% (*S4478*) of the ADH1 RNA seen in the progenitor. To assess the level of random hybridization, soybean shoot RNA was included as a negative control; there was no detectable hybridization to this RNA.

To compare transcriptional activities of the progenitor and mutant alleles, we relied on a measurement of transcription in isolated nuclei. The recombinant plasmid pUGA2, containing the transcriptional start site and 1960 bp representing 60% of the *Adh1-S* allele, was digested with restriction enzymes *Pst* I, *Hin*dIII, and *Pvu* II (Fig. 1). The digestion yielded fragments corresponding to sequences lying 5′ to the first intron (150 bp proximal to the transcript and 200 bp of transcribed region), the first intron (460 bp), and sequences lying 3′ to the intron (1300 bp). Identical blots of pUGA2 DNA were hybridized to the same amount of radioactive RNA isolated from each set of nuclei.

Autoradiograms obtained from such an experiment are reproduced in Fig. 4 *Upper*; in Table 1 we present relative levels of hybridization to the three *Adh1* regions for the progenitor allele and for the mutants. Data obtained directly from the integration of densitometric curves and corrected data expressed relative to the progenitor are listed. Transcription of all regions was decreased in the mutants, although the patterns varied. However, the relative levels of 3′ transcripts were very similar to relative levels of mature ADH1 RNA (Fig. 5).

To determine the level of *Mu* transcripts in the induced roots, run-off transcripts also were hybridized to blots of pUGA1 DNA containing *Mu*. The *Mu* element was separated from surrounding *Adh1* sequences and cut into leftward and rightward fragments, based on their positions in *S3034* as oriented in Fig. 1. The extent of hybridization to *Mu* sequences, illustrated in Fig. 4 *Lower*, was taken as a measurement of *Mu* transcription levels because no hybridizing sequences were found in non-*Mu* lines—i.e., lines not derived from the stock originally analyzed by Robertson (ref. 11; unpublished data). The hybridizing RNA represents transcripts of any of the 20–30 copies of *Mu* present in the DNA of these lines but not present in the progenitor (data not shown). Clearly all of the mutants contain significant levels

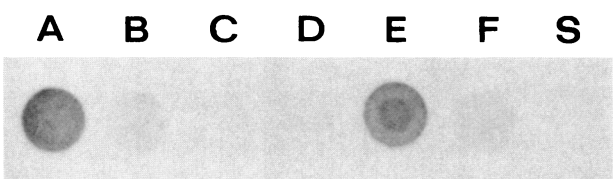

FIG. 3. RNA dot blot of total RNA extracted from submersed roots of homozygous seedlings. Blots were hybridized to pZmL84, a cDNA clone representing the 3′ half of ADH1 mRNA (6). RNA sources were *Adh1-S* (blot A), *S3034* (blot B), *S3034a* (blot C), *S3034b* (blot D), *S4477* (blot E), *S4478* (blot F), and soybean shoot RNA (blot S), a negative control to ensure specificity of the hybridization.

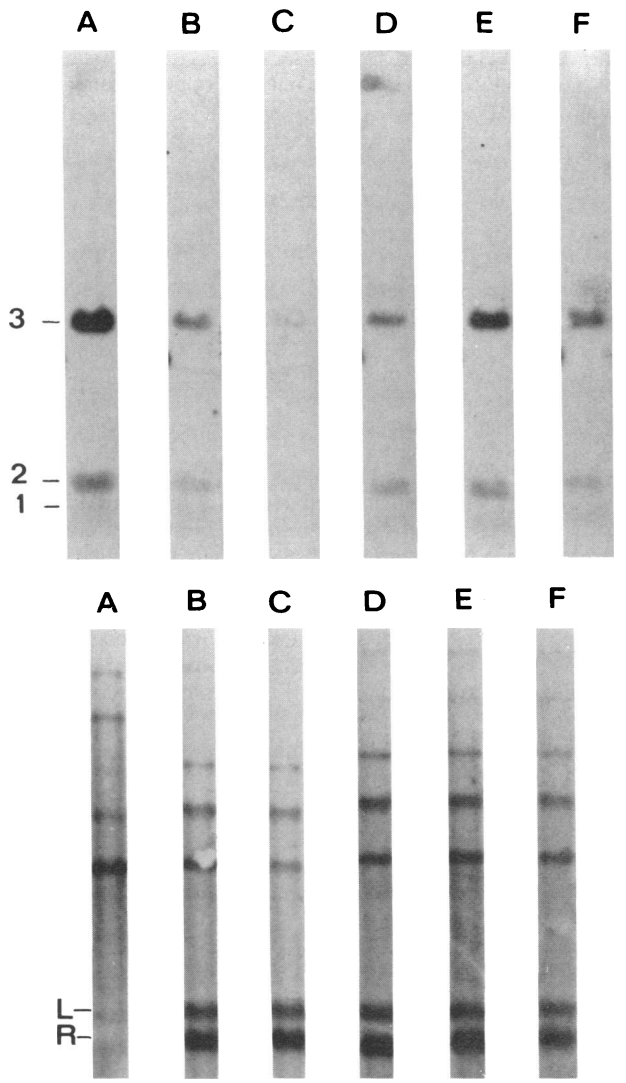

FIG. 4. (*Upper*) Hybridization of radioactively labeled run-off transcripts to cloned *Adh1-S* DNA. Recombinant plasmid pUGA2 was digested with restriction endonucleases to separate fragments representing the first 200 bp (fragment 1), the first intron (fragment 2), and the subsequent 1300 bp (fragment 3) of the *Adh1* transcript, representing in all 60% of the transcribed region. Lanes: A, hybridization to transcripts isolated from homozygous *Adh1-S*; B, from *S3034*; C, from *S3034a*; D, from *S3034b*; E, from *S4477*; and F, from *S4478*. pBR322 fragments, not detectable on the autoradiogram, served as a negative control. (*Lower*) Hybridization of run-off transcripts to *Mu*. Plasmid pUGA1 was digested to excise the *Mu* element and divide it into leftward (fragment L) and rightward (fragment R) segments, relative to its direction of insertion in *S3034* oriented as in Fig. 1. Lanes: A–F, the same mutants as defined by lanes A–F in *A*. Upper bands contain *Mu* termini and *Adh1* sequences.

of *Mu* run-off transcripts. The rightward region of *Mu* was represented more heavily in all cases.

DISCUSSION

Many *Mu*-Induced Mutations May be Highly Unstable. The levels of protein activity in some of our lines are clearly different from those originally reported by Freeling and co-workers (3, 4). While our measurements of ADH1 produced by *S3034*, *S3034a*, and *S4478* alleles are in good agreement, activities of *S3034b* (35% compared to 13%) and *S4477* (72% compared to 40%) alleles differ significantly. Our results, like those of Freeling and co-workers, are based on assays of several samples; in both cases, progenitor and

Table 1. Relative intensities of autoradiographic bands depicted in Fig. 4 *Upper*

Genotype	Relative intensity of hybridization		
	5′	i	3′
Adh1-S	1.15 (100%)	3.92 (100%)	9.86 (100%)
S3034	0.85 (74%)	1.88 (48%)	3.24 (33%)
S3034a	0.31 (27%)	0.54 (14%)	1.02 (10%)
S3034b	0.54 (47%)	1.65 (42%)	2.80 (28%)
S4477	0.82 (72%)	2.57 (66%)	6.55 (66%)
S4478	0.36 (32%)	1.10 (28%)	4.43 (45%)

Both raw data and data adjusted to the percentage of hybridization seen in the comparable region of the progenitor are included. 5′, transcripts hybridizing to sequences 5′ to the first intron; i, transcripts hybridizing to the first intron; and 3′, transcripts hybridizing to sequences 3′ to the intron.

mutants were assayed in similar genetic backgrounds. Two likely sources of difference remain. First, we have used different lines to provide the fast allele needed for a measurement of allozyme ratios. It is possible that *trans*-acting modifiers of *Adh1-S* expression exert variable effects in heterozygotes derived from different *Adh1-F* lines. But RNA levels measured in mutant homozygotes reflect ADH1-S activities in heterozygotes (see Fig. 5), a correlation arguing against variable *trans*-directed effects. A second possibility, which we deem more likely, is that these unstable elements have undergone rearrangements, below the limits of detection in our Southern blots, which alter their effects on *Adh1* gene expression. Support for this alternative has come from the analysis by L. P. Taylor and V. Walbot of a genomic clone for *S3034a* (personal communication). While the original *S3034a* of Freeling *et al.* was unstable (3), the allele analyzed by Taylor and Walbot appears to be a deletion-derivative of *S3034* in which the first exon–intron junction is missing, rendering phenotypic reversion unlikely even in the event of *Mu* excision.

The correlation between RNA levels measured in induced roots and protein activity assayed in scutellum also provides support for earlier evidence that there is no tissue specificity in *Mu*'s effect on *Adh1* expression (7). The one exception may be *S3034a*, for which we find RNA but no detectable protein activity. These results are consistent with those of Taylor and Walbot: deletion of an intron junction would make both reversion and the production of functional message unlikely.

Normal *Adh1* Transcripts Are Rarely Aborted. From the hybridization of progenitor run-off transcripts to the three *Adh1* regions represented, it is possible to estimate the relative transcriptional activity of each region. If we divide the intensity of hybridization to each region (listed in Table 1) by the length of transcript derived from each region, we obtain a measure of relative transcriptional activity (although we expect the 5′ terminus to be underrepresented slightly because we detect only elongation). Thus 5′ to the intron we find 8 units/200 bp = 0.04 unit/bp; within the intron, 26 units/460 bp = 0.06 unit/bp; and 3′ to the intron, 66 units/1300 bp = 0.05 unit/bp. These numbers are roughly equal, from which we conclude that transcripts once initiated proceed through at least the first 60% of the gene and that false starts are not typical of *Adh1* gene expression under these conditions.

***Mu* Insertion Affects Transcription but Not Maturation of Transcripts.** The densitometric data obtained from integrating the areas under absorption curves are listed in Table 1 and diagrammed along with protein and RNA data in Fig. 5. Open bars are used to indicate relative levels of transcripts representing regions lying 5′ to the first intron (bars 5), the intron (bars i), and regions lying 3′ to the intron (bars 3). Clearly levels of transcripts representing all regions are lower in mutants than in the progenitor. However, the pattern of the *S3034* family (Fig. 5, bar groups B–D) is different from that of *S4477* (Fig. 5, group E) and *S4478* (Fig. 5, group F). In the mutants *S4477* and *S4478*, with the *Mu* element near the downstream intron–exon border, transcript levels are fairly constant; that is, the transcription of each region of *S4477* occurs at about 70% of the rate seen for the progenitor, and for *S4478* the expression is decreased to about 35%. Whatever the effect of *Mu*, it is felt at the initiation of transcription or shortly thereafter. It appears most likely that the consequence of insertion has been to interfere with the initiation process.

In the *S3034* family, on the other hand, with *Mu* near the 5′ intron junction, transcripts hybridizing to 5′ regions are reduced, and levels of hybridization downstream are reduced further. There are several possible explanations for this phenomenon. Transcripts may tend to terminate prematurely

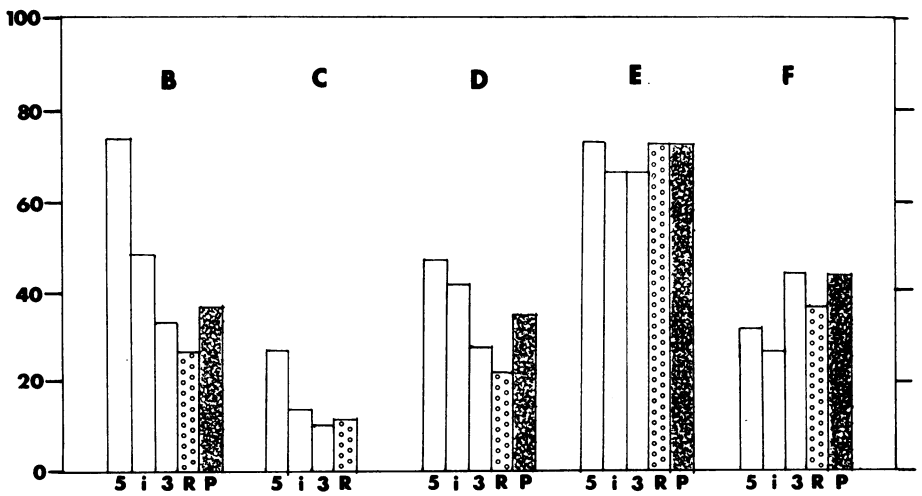

FIG. 5. Relative levels of run-off transcript, RNA, and polypeptide activity for the mutants. Each bar represents a measurement of mutant gene expression, either of run-off transcript levels [5′ fragment (bars 5), intron (bars i), and 3′ fragment (bars 3)], RNA level (bars R), or polypeptide activity (bars P). In each case the level is expressed relative to that seen in the progenitor, defined as 100%. Groups of bars: B, *S3034*; C, *S3034a*; D, *S3034b*; E, *S4477*; and F, *S4478*. These data were obtained from photographs of starch gels and from autoradiograms as described.

or to stall in the region around these *Mu* elements. Alternatively, the greater hybridization to 5′ regions may reflect the presence of *Mu*-directed transcripts reading back into the *Adh1* exon. Transcription promoted outward from *Mu* would be indistinguishable from *Adh1*-promoted transcription by our assay. Comparison to systems such as *IS10* of *E. coli* (24) suggests a model for disruption of gene expression through promotion of such a transcript. As described below, we do have evidence for discrete *Mu* transcripts, but we do not know their initiation or termination sites.

We conclude that our estimates of *Adh1* transcription are maximal. Furthermore, there are significant differences in the ways that *Mu* insertions affect *Adh1* gene expression in the two families of mutants. The difference may lie in the site of insertion or in minor variations in the sequence of the *Mu* elements.

In all mutants, on the other hand, relative levels of 3′ transcripts match the levels of total ADH1 RNA. That is, whatever differences there may be between progenitor and mutant alleles, lower levels of 5′ transcripts account for them totally. Transcription of distal sequences and maturation are apparently unaffected, and normal messages appear to be generated except in the case of *S3034a*.

Goldberg *et al.* (25) have reported on transcriptional inactivation resulting from the insertion of a DNA element into a gene for soybean lectin. In the case of this mutant, insertion totally represses transcription of lectin sequences upstream from the insertion site. *Mu* may act in a similar manner, but the mechanism remains a mystery. We are investigating the possibility that insertion is accompanied with structural alterations that render the gene less accessible for transcription.

The predilection of *Mu* for introns may be allele-specific or general. If general, *Mu* may define a class of quantitative mutators exerting their evolutionary influences largely through a modulation of gene expression such as that seen in the mutants described here. Analysis of other *Mu* sites and of the effects of other *Mu* elements on gene expression will permit us to address questions regarding the evolutionary consequences of *Mu*'s presence.

***Mu* Transcripts.** Given the 15–30 copies of *Mu*-like elements in the genomes of our mutant lines, the presence of *Mu* run-off transcripts, shown in Fig. 4 *Lower*, is not surprising. We cannot say to what extent such sequences represent adventitious transcription of *Mu* sequences inserted into active genes and to what extent they represent specific transcription of *Mu*. The greater hybridization of radioactive transcripts to the shorter fragment argues against purely adventitious transcription, however, and we have preliminary evidence of discrete RNA molecules homologous to *Mu* and short enough to be encoded within *Mu* (unpublished data).

We are grateful to M. Freeling for the push he gave us in this direction, to J. Bennetzen for sharing clones, to R. Barker for sequence data, and to E. Dennis and her colleagues for sharing probes and sequence data. We thank M. Haggart for skillful technical assistance and S. Berry-Lowe and R. Meagher for wise and friendly counsel. We are also grateful to R. Meagher, M. Case, R. Ivarie, and C. Findly for their critical reading of this manuscript. This research has been supported by National Science Foundation Grant PCM-8302591 to J.S.; L.R. is supported by National Institutes of Health Predoctoral Training Fellowship GM07103.

1. Robertson, D. S. (1978) *Mutat. Res.* **51,** 21–28.
2. Robertson, D. S. (1981) *Science* **213,** 1515–1517.
3. Freeling, M., Cheng, D. S.-K. & Alleman, M. (1982) *Dev. Genet.* **3,** 179–196.
4. Johns, M. A., Alleman, M. & Freeling, M. (1983) in *Genetic Engineering of Plants*, eds. Kosuge, T., Meredith, C. P. & Hollaender, A. (Plenum, New York), pp. 61–79.
5. Sachs, M. M., Freeling, M. & Okimoto, R. (1980) *Cell* **20,** 761–767.
6. Gerlach, W. L., Pryor, A. J., Dennis, E. S., Ferl, R. J., Sachs, M. M. & Peacock, W. J. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2981–2985.
7. Strommer, J. N., Hake, S., Bennetzen, J. L., Taylor, W. C. & Freeling, M. (1982) *Nature (London)* **300,** 542–544.
8. Ferl, R. J., Brennan, M. D. & Schwartz, D. (1980) *Biochem. Genet.* **18,** 581–691.
9. Bennetzen, J. L., Swanson, J., Taylor, W. C. & Freeling, M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4125–4128.
10. Dennis, E. S., Gerlach, W. L., Pryor, A. J., Bennetzen, J. L., Inglis, A., Llewellyn, D., Sachs, M. M., Ferl, R. J. & Peacock, W. J. (1985) *Nucleic Acids Res.*, in press.
11. Barker, R. F., Thompson, D. V., Talbot, D. R., Swanson, J. & Bennetzen, J. L. (1984) *Nucleic Acids Res.* **12,** 5955–5967.
12. Scandalios, J. G. (1969) *Biochem. Genet.* **3,** 37–116.
13. Ayala, F. J., Powell, J. R., Tracey, M. L., Mourao, C. A. & Perez-Salas, S. (1972) *Genetics* **70,** 113–139.
14. Schwarz, D. (1973) *Genetics* **74,** 615–617.
15. Lizardi, P. M. & Engelberg, A. (1979) *Anal. Biochem.* **98,** 116–122.
16. Bruskin, A. M., Tyner, A. L., Wells, D. E., Showman, R. M. & Klein, W. H. (1981) *Dev. Biol.* **87,** 308–318.
17. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 33–38.
18. Luthe, D. S. & Quatrano, R. S. (1980) *Plant Physiol.* **65,** 305–308.
19. Luthe, D. S. & Quatrano, R. S. (1980) *Plant Physiol.* **65,** 309–313.
20. Harding, J. D., MacDonald, R. J., Pryzbyla, A. E., Chirgwin, J. M., Pictet, R. L. & Rutter, W. J. (1977) *J. Biol. Chem.* **252,** 7391–7397.
21. Glisin, V., Crkvenjakov, R. & Byus, C. (1974) *Biochemistry* **13,** 2633–2637.
22. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
23. Alwine, J. C., Kemp, D. J., Parker, B. A., Resiser, J., Renart, J., Stark, G. R. & Wahl, G. M. (1979) *Methods Enzymol.* **68,** 220–242.
24. Simons, R., Hoopes, B. C., McCllure, W. R. & Kleckner, N. (1983) *Cell* **34,** 673–682.
25. Goldberg, R. B., Hoschek, G. & Vodkin, L. O. (1983) *Cell* **33,** 465–475.