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Genetics

Transposon-induced promoter scrambling: A mechanism for the evolution of new alleles

(maize *adh1*/DNA rearrangement)

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ABSTRACT We have studied a germinal revertant of the Mutator (*Mu3*)-induced mutation (*Adh1-3F1124*) of the maize alcohol dehydrogenase 1 gene (*adh1*). Transposon *Mu3* was inserted at the TATA box of the promoter. The excision of *Mu3* caused a complex, multibreakpoint DNA rearrangement with deletion, inverted duplication, and inversions affecting 430 nucleotides in the promoter region. These changes led to an unusual pattern of *adh1* gene expression: increased levels of enzyme activity in one organ, decreased levels in another, and almost unchanged levels in a third organ. The evolutionary impact of transposon-induced promoter scrambling on generation of allelic diversity is discussed. We present a fragmentation model to help explain how transposon excision could induce multiple breakpoint aberrations without involving a homologous chromosome.

Genetic variation in spatial and temporal regulation of alleles is undoubtedly important during the evolution of species and higher-order taxa. Goldschmidt (1) used the term macroevolution to distinguish cladogenic evolutionary processes from what he reasoned to be more fine-tuning, intraspecific evolutionary processes, which he called microevolution. Although unproved, this micro/macroevolutionary distinction is widely respected today (2, 3). In contrast, Goldschmidt's corollary idea, that different sorts of mutational lesions underlie micro- and macroevolution, remains both untested and particularly controversial today. Goldschmidt saw point mutation as just the sort of DNA-level change that might fuel microevolution and evoked a mysterious macromutational mechanism involving "changes in the intimate architecture of the chromosome." Later, McClintock's Nobel laureate address (4) presented the argument that any insult resulting in chromosomal breaks liberates quiescent transposons. Macroevolution might be particularly fueled by the production of intragenic chromosomal rearrangements that could result from such newly activated transposons. In weak support of this notion are the several cases of transposon insertion or excision causing new patterns of regulation (5–8). Point mutations in regulatory genes can also have pleiotropic consequences on development (9). If placed within the coding region of genes, such base substitutions might lead to altered product, while DNA rearrangements of coding sequence most likely would result in no product. If positioned within regulatory regions of the gene, DNA rearrangements would have a greater chance of leading to misexpression than base substitutions. If ectopic or untimely expression of transcription factors is important for macroevolution, then genetic diversity of promoter structure might be expected to be important as well.

This paper describes a transposon-induced *adh1* promoter showing a highly complex DNA rearrangement. We feel that the scrambling mechanism that generated this rearrangement and the new pattern of gene regulation that followed would, in theory, make a fine example of a macromutational mechanism and its consequence.

In plants, transposon activity has been most extensively studied in *Antirrhinum majus* (snapdragon) and *Zea mays* (maize). Three major mutagenic transposon systems have been described in maize: Activator/Dissociation (*Ac*/*Ds*) (for review, see ref. 10), *Spm* or *En* (11, 12), and Mutator (*Mu*) (13) (for review, see ref. 7). Characteristic of all these transposons is the generation of small direct duplications of host DNA at the site of insertion. Excisions usually result in complete removal of the transposon and partial removal of the direct duplication. Small deletions of the target site duplication or adjacent host DNA are often observed. Occasionally, inverted duplications are found that were copied either from sequences flanking the insertion site or located at some distance from the insertion site. Sometimes the host DNA sequences are perfectly restored upon excision of the element (7, 14–16). In an attempt to explain all of their *Mu* excision products, Doseff and coworkers (16) discuss the possibility of double-stranded breaks at the ends of Mutator transposon that might be followed by repair activities. The template used for repair would be provided by partially homologous sequences elsewhere in the gene since no wild-type homologue was present.

The particular Mutator element (*Mu3*) that we studied was inserted in the TATA box of the promoter of the *adh1* gene in maize and caused an organ-specific alteration of expression (17). Here we describe a complex reversion event, a transposon excision and DNA rearrangement, that led to distinct organ-specific expression of *adh1*.

MATERIALS AND METHODS

Genetic Analysis, Revertant Selection, Seedling Treatment Conditions, and Enzyme Activity Measurements. In an isolation plot, pollen from homozygous *Adh1-3F1124* plants in a *Mu*-active background was crossed to tester ears from homozygous *Adh1-2F11* plants carrying one copy of Activator (*Ac*). The phenotype of mutant *Adh1-2F11* has been described (18). The mutation is caused by insertion of the Dissociation transposon (*Ds2*) and carries characteristic polymorphisms in the untranslated leader region that allow clear distinction between the tester allele and the *Adh1-3F* and *Adh1-3F1124* alleles. In the scutellum, the level of ADH1 enzyme activity in *Adh1-2F11* is 5% that of the progenitor. The phenotype of mutant *Adh1-3F1124* has been described (17). This mutation is caused by insertion of the transposable element *Mu3* and is also unstable. ADH1 enzyme activity is ≈6% of normal in the scutellum and root. Heterozygous seeds (*Adh1-2F11*/*Adh1-3F1124*) were subjected to a hypoxic revertant selection scheme as described (19). Once identified, the revertant phenotype in seedling roots was studied after subjecting 7- to 10-day-old seedlings to the partial anaerobic environment of aerated water for 24 h to analyze ADH enzyme activity and for 16 h to analyze mRNA. The aerobic control seeds were germinated on wet paper towels on the bench top and

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Abbreviation: ARE, anaerobic regulatory element.

covered with aluminum foil. Forty-five minutes before harvesting roots, the foil was removed to allow maximum aeration. ADH1 enzyme activity was determined as described (8, 17, 19).

DNA Isolation and Cloning of the Revertant Allele. Preparation of maize genomic DNA, Southern blot hybridization, and description of *adh1*-specific probes, oligonucleotide primers, and PCR conditions were reported earlier (19). To clone the 12-kbp genomic *adh1 Bam*HI fragment, restricted genomic DNA from *r53* was fractionated on a 40–10% sucrose gradient. The 12-kbp fragment was ligated into Lambda DASH II vector (Stratagene) and the phage were packaged using the Gigapack II Plus packaging extract from Stratagene. A library of 80,000 plaques was screened with the plasmid clone pUCH10 containing the *adh1-3F* promoter region (19). Three positive clones yielded identical restriction patterns that matched those obtained from genomic Southern blots. Subcloning was performed in the Bluescript vector (Stratagene). Double-stranded or single-stranded DNA served as template for DNA sequencing. Single-stranded DNA was obtained by growing bacteria containing the plasmid of interest in the presence of helper phage. DNA sequencing was performed with a Sequenase kit (United States Biochemical) and sequencing products were separated on 6% acrylamide/7.5 M urea gels and exposed to Kodak X-AR film overnight. Genomic DNA from the counterallele *Adh1-2F11* was subjected to PCR under conditions described (19), cloned, and sequenced. Polymorphisms between *Adh1-2F* and *Adh1-3F* are at +69 (G) and +45 (T).

RNA Isolation and Analysis. RNA preparation methods, Northern and slot blot hybridization, and *adh1* and actin probes have been described (19). The probe for sucrose synthase 1, pCB16, was isolated as a cDNA clone from anaerobically treated seedling roots by D. C. Bennett (M.F.'s Laboratory). The hybridization signal was scanned with a PhosphorImage analyzer (Molecular Dynamics). Quantitative information was obtained with the IMAGE QUANT program.

RESULTS

Selection and Confirmation of the Revertant. ADH1 low or null seeds will not germinate under hypoxic conditions, such as immersion in 25°C water saturated with sea-level air (17). We immersed ≈40,000 seeds that were heterozygous for two different, readily distinguishable *Adh1* mutant alleles: the *Mu3* insertion mutant *Adh1-3F1124* and the *Ds2* insertion mutant (18) *Adh1-2F11* null tester. Nucleotide polymorphisms within the untranslated leader sequence at +69 and +45 further mark these alleles (M.F.'s laboratory, unpublished data). Four independent revertants were recovered as seedlings. Subsequent crosses and allozyme determination in scutellum, root, and pollen showed that all were at least partially restored in the ability to specify seedling ADH activity. One of them, *Adh1-3F1124r53*, displayed an interesting pattern of organ-specific *adh1* expression. We call this allele the "revertant" for the purposes of this paper. Similarly, *Adh1-3F* is called the "progenitor" wild-type allele, and *Adh1-3F1124* is called the "mutant." The progenitor generated the mutant that generated the revertant.

DNA Analysis of the Revertant Allele. Restricted genomic DNA from heterozygous revertant showed an *Adh1-3F*-type allele with *Mu3* being absent, and, as expected, the unchanged *Adh1-2F11* tester allele (data not shown). Use of DNA primers that flank the *Mu3* insertion site, and that had previously been used to successfully isolate other revertant alleles from the same mutant (19), did not yield any revertant PCR fragment, while amplification from progenitor DNA was successful. Using double restriction enzyme digestions, restriction fragment length polymorphisms between progenitor and revertant were found, suggesting some DNA rearrangement. A 10-kbp *Bam*HI genomic clone carrying the *Adh1-3F1124r53* revertant allele was isolated in a λ phage. DNA sequence information was obtained from the *Xba* I site to the *Hin*dIII site (Fig. 1). Starting at −266, several major changes affecting the promoter and untranslated leader region were found that evidenced an extensive rearrangement of these regulatory regions. First, the *Mu3* element and *adh1* sequences from −22 to −50, which include the TATA box, are deleted. Second, the sequence from −51 to −266 (shaded area) is inverted. This region includes the cis-acting sequences regulating the anaerobic response [anaerobic regulatory element (ARE); hatched arrow of Fig. 1; see ref. 21]. It also includes the position of one of the primers used in the PCR designed initially to clone the promoter region of the revertant allele. Due to the inversion, both primers point in the same direction and prevent synthesis of the expected DNA fragment in a PCR. Third, the sequence from −300 to −267 is found in inverted orientation downstream of the −51 to −266 inversion, resulting in a transposon-like structure (small arrows in Fig. 1). Fourth, abutting the −300 to −267 inverted duplication lies another duplication of the sequence (+131 to +88) in inverse orientation (large arrow in hatched box of Fig. 1). This sequence includes part of the untranslated leader sequence, the ATG translation initiation codon, and part of the first exon. Fifth, 6 bp, 5′-TGTTTT-3′, of unknown origin are located between the +131 to +88 duplication inversion and the native *adh1* sequence at −21. Sixth, the sequence 5′-GATC-3′ at −300, and at +129 in both progenitor and revertant, lies at the borders of the rearranged region and is also found at the junction of the two inversions (−300/+131) in the revertant.

The Organ-Specific Distribution of ADH1 Is Altered in the Revertant. *Analysis of enzyme activity.* The regulatory effects of the scrambled promoter were assayed by analyzing ADH1 enzyme activity in several organs: the scutellum, seedling

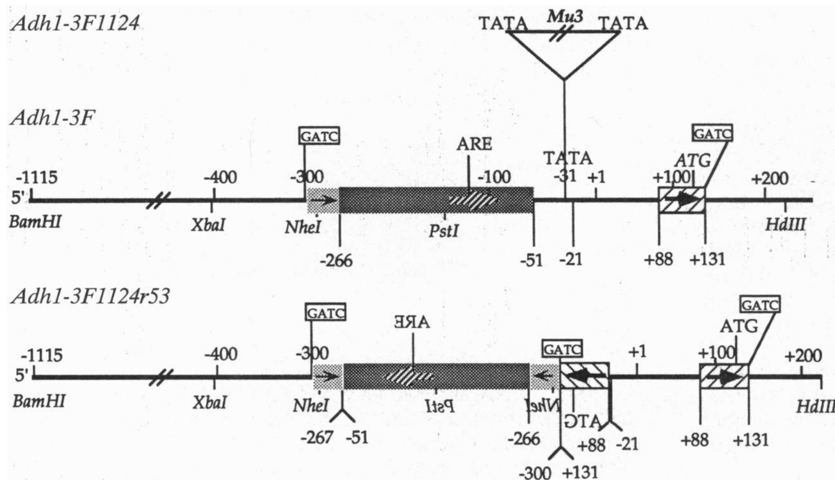

FIG. 1. Physical map and sequence of the *Adh1-3F, Adh1-3F1124*, and *Adh1-3F1124r53* alleles. The maps were constructed based on DNA sequence information. Numbering is based on the start of transcription (+1) in the *Adh1-3F* allele. TATA denotes the TATA box in the promoter; ATG is the translation initiation codon. ARE is symbolized as a hatched arrow. Altered regions in *Adh1-3F1124r53* are boxed in both alleles and, if inverted, the arrows point to the 5′ end. Between +88 and −21 in *Adh1-3F1124r53* the sequence 5′-TGTTTT-3′ is inserted. The *r53* promoter now has two pairs of inverted repeats, one pair on either side of the *Mu3* insertion site, as denoted by pairs of arrows. This structure might be genetically unstable (20).

roots, and pollen. In a wild-type maize seedling, ADH1 message accumulation and translation is induced under anaerobic conditions (22). Therefore we analyzed *adh1* expression in aerobic and partially anaerobic (hypoxic) revertant seedling roots and quantified the expression by comparing revertant and progenitor with the expression of a reference allele induced within the same cell. This was possible because the reference allele, *Adh1-1S*, encodes an ADH1 subunit with a different net surface charge than the subunit encoded by the revertant/progenitor (an ADH1-3F subunit). The S and F denote slower and faster migration rates toward the positive pole in native gels subjected to an electric field. Fig. 2 shows representative allozyme ratios with the various ADH dimers of revertant, mutant, and progenitor. Three bands of ADH1 enzyme activity (set I) can be seen in root extracts: the intermediately migrating heterodimer band (FS) composed of one fast and one slow migrating subunit, the fast migrating homodimers (FF) encoded by the *Adh1-3F* progenitor allele, and the slower migrating homodimers (SS) encoded by the reference allele *Adh1-1S*. Unlike the progenitor, the revertant expresses at a very low level; the binomial distribution of subunits so greatly favors ADH1-S that there is very little FS heterodimer, and no FF homodimer could be detected. This low level of revertant ADH1 expression is also reflected in those heterodimers formed with an ADH subunit encoded by *adh2* (set II). Since no FF homodimer activity was detectable in heterozygotes, we analyzed a highly concentrated extract from homozygous revertants and were able to demonstrate partial enzyme activity. This reduction of activity was observed in both aerobic and hypoxic roots, an indication that the ability to respond to low oxygen tension was not completely abolished.

Gels like the one displayed in Fig. 2 were densitometrically scanned, allozyme ratios were measured, and allele-specific enzyme activities were calculated (Table 1). In aerobic and partially anaerobic roots, the revertant expressed 12% and 18% of progenitor levels of ADH1 activity, respectively. These values are slightly elevated in comparison to the mutant allele (≈10% of progenitor; Table 1; see ref. 19). In the scutellum, revertant ADH1 activity amounts to 50% when compared to the progenitor (Table 1). Interestingly, the promoter rearrangement of the revertant has a less severe effect on *adh1* expression in the scutellum than the insertion of *Mu3* in the mutant (6% of the progenitor). ADH allozyme ratios were also analyzed in pollen extracts. Since pollen is haploid, each pollen grain from a heterozygote carries only one of the two alleles

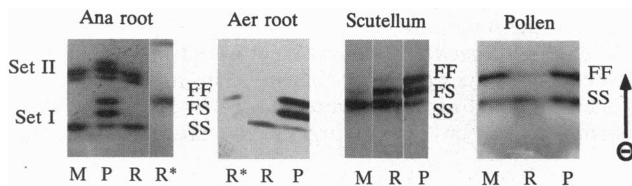

FIG. 2. ADH enzyme activity. Cytosolic extracts were obtained from root, scutellum, and pollen and separated by native gel electrophoresis; arrow indicates direction of migration toward the positive pole. Extracts were obtained from plants either homozygous (R*) or heterozygous at the *adh1* locus (M, mutant; P, progenitor; R, revertant). Heterozygous seeds were chosen because the wild-type *Adh1-1S* allele on one homologue serves as an internal control. The other homologue carries either the *Adh1-3F1124* allele (M), the *Adh1-3F* allele (P), or the *Adh1-3F1124t53* allele (R). Set I consists of ADH1 SS, FS, and FF dimers. Set II consists of heterodimers of ADH1 and ADH2 subunits. Partially anaerobic (Ana) root extracts from mutant, progenitor, and revertant were isolated after seedlings were treated hypoxically for 24 h; aerobic (Aer) root extracts were isolated from aerobic revertant and progenitor seedlings. The homozygous revertant Aer or Ana root samples (R*) were run on separate gels (note the longer distance of migration of set II in the anaerobic sample). Extracts from R* samples were 3-fold concentrated before loading on the gel.

Table 1. Quantitative analysis of ADH1 enzyme activity and mRNA accumulation

Genotype	Aer root	Ana root	Scutellum	Pollen
		Enzyme activity		
Mutant	<10	10	6	100
Revertant	12	18	50	57
		mRNA accumulation		
Mutant	10	10	ND	ND
Revertant	78	12	ND	ND

Zymograms from native starch gels were scanned in a densitometer. Progenitor values were taken as 100%. From each genotype (progenitor *Adh1-3F*, revertant *Adh1-3F1124r53*, and mutant *Adh1-3F1124*) and each tissue type, between 8 and 20 samples were analyzed. The values in this table were derived as follows: contribution of progenitor (F) activity was determined from heterozygous material. The values of F as percentage of total (F and S dimers) activity in the various organs are as follows: aerobic (Aer) root, 60%; anaerobic (Ana) root, 56%; pollen, 57%; scutellum, 58%. The amount of ADH1 mRNA was determined from densitometric analysis of slot blot hybridization to an *adh1* and sucrose synthase 1 probe and is given as percentage of the progenitor amount. Scutellum and pollen RNA were not analyzed (ND).

and only homodimer bands are expected (Fig. 2). Revertant enzyme activity (FF) is reduced to 57% when compared to either the progenitor or the mutant (Table 1).

Analysis of mRNA accumulation in roots. Poly(A)$^+$ mRNA from either aerobic or 16-h hypoxic roots was analyzed on Northern blots. Under both conditions, the expected 1.6-kbp *adh1*-specific transcript accumulates in progenitor roots while revertant ADH1 transcripts are heterogeneous in size (Fig. 3). The majority of these transcripts is larger by up to ≈80 nucleotides (Fig. 3). All poly(A)$^+$ mRNA samples were hybridized to an *adh1*-specific probe and compared to the level of hybridization to sucrose synthase. Sucrose synthase was chosen because its transcription, like that of *adh1*, is induced under hypoxic conditions (23). Under aerobic conditions, revertant ADH1 transcripts accumulate at a somewhat reduced level (80% of progenitor; Table 1), while a drastic reduction (12%) is seen under hypoxic conditions.

DISCUSSION

Excision of *Mu3* Can Be Explained by the Fragmentation Model. The mechanism of *Mu* excision is not known. Somatic and germinal excisions generally result in alterations of the host DNA sequences. These footprints are most commonly imprecise deletions (16, 24). Occasionally, however, sequence replacements were found, such that the substitutions and surrounding sequences are homologous to those distal to the insertion site and might be explained by recombination and gene conversion (16). Since we can distinguish both homologues, the involvement of the *Adh1-2F11* allele in our case can be excluded. The excision mechanism for *Ac*, *Spm*, and *Tam* families of transposons is thought to involve staggered nicks or blunt end cuts at both ends of the element. Either repair

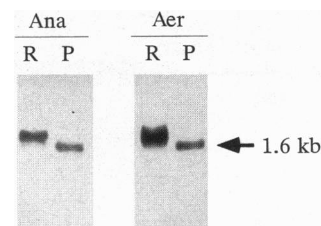

FIG. 3. RNA analysis. Poly(A)$^+$ mRNA was analyzed from partially anaerobic (Ana) and aerobic (Aer) roots obtained from progenitor (P) *Adh1-3F* and revertant (R) *Adh1-3F1124r53*. Northern blots were hybridized to an adh1-specific cDNA clone pZML793.

activities (14) or formation of hairpins, which would be resolved after further nicking, ligation, and DNA synthesis (15), have been proposed to explain the various excision products. Studies of transposon *Tc1* in *Caenorhabditis elegans* show that footprints are most likely due to imprecise repair procedures rather than imprecise excision (25). Imprecise gap repair may have also been involved in generation of the *Mu3* excision product that we present here. But the complexity of the DNA rearrangement with its inversions, inverted duplications, and deletion needs further explanation. The generation of large inverted duplications was reported in a *Tam*-induced mutation, *niv525*, at the nivea locus in *Antirrhinum*. Since this inversion duplication has its axis of symmetry at the site of excision, a hairpin model (26) might explain its generation. The inverted duplications seen in *r53* cannot be explained entirely by the Coen model (26) for several reasons: (*i*) the axis of symmetry is not at the site of insertion/excision; (*ii*) to generate the downstream inverted duplication, a staggered nick, separated by 110 bp, would be required; and (*iii*) if the −266 to −51 inversion had been generated through the postulated hairpin intermediate, a second coincidental event has to be invoked that resulted in deletion of the original sequences from −266 to −51.

None of the mechanisms used to explain the transposon-mediated rearrangements seen by others will explain our scrambled promoter. Thus, we present the fragmentation model, as diagrammed in Fig. 4 and explained in the legend. This model capitalizes on the replicative nature of Mutator transposition (7) and the possibility that Mutator uses double-strand gap repair mechanisms (16) and supposes that excisions are one outcome of transposition. *Mu* is released by double-stranded cuts at each side of the element. Double-stranded DNA breaks have been shown to activate nearby replication origins in yeast (27) and this might happen in maize as well. Migration of replication forks would result in loops of newly synthesized DNA bordering not yet duplicated DNA. Duplexes near the junctions between the new and old DNA may be more fragile and nick (arrow at −263 in Fig. 4) or break (arrows at −300, +88, and +131 in Fig. 4) easily. Exonucleolytic activity and rearrangement of the DNA fragments (A to B* to E* to D to E to F) could then result in the observed DNA structure in *Adh1-3F1124r53*. Whether the sequence 5′-GATC-3′ (Fig. 1) plays any mechanistic role during this process is not clear.

The Scrambled Promoter Drives an ADH Organ Specificity. The scrambled promoter causes a quantitative, organ-specific ADH expression pattern that is different from the original insertion mutant and different from the wild-type progenitor (Table 1). Since the DNA changes in the revertant are complex, it is not possible to assign a particular DNA region with a particular function. However, an interesting correlation between the drastic reduction of ADH1 transcripts (12% of wild type) and the fact that the ARE (21) is inverted (Fig. 1) is intriguing. This demonstrates the regulatory importance of the ARE region within the plant. The reduction of ADH1 transcript accumulation under aerobic conditions (≈80% of wild type) suggests that in addition to the ARE other cis-acting sequences that modulate aerobic transcription are also located within the affected promoter region.

In addition to sequence rearrangement, the TATA box region from −22 to −50 is deleted. We have shown previously that an allele at *adh1* in which an 18-bp deletion has removed the TATA box can accomplish transcription, although at reduced levels, and transcription initiates at multiple sites surrounding the normal start site (19). The accumulation of larger than normal transcripts described here supports this notion (Fig. 3).

A dramatic quantitative difference between transcript accumulation (≈80%) and enzyme activity (≈12%) is observed only in aerobic roots and not in hypoxic roots. One possibility is that only transcripts of wild-type size can be translated in aerobic roots and these are at relatively low levels. A second possibility is that translation might be affected by the DNA sequence changes within the untranslated leader region where an open reading frame of 23 amino acids (from −87 to −20) was generated due to the DNA rearrangement. These possibilities must be specific to aerobic gene expression. Short open reading frames within the untranslated leader sequence of other genes are known to alter translation quantitatively (28).

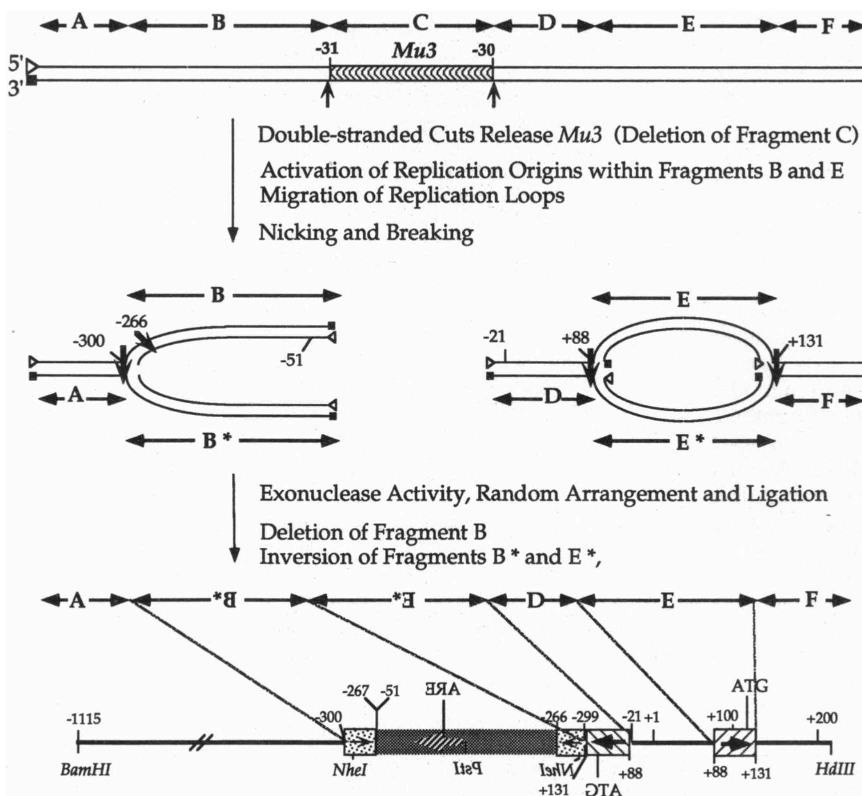

FIG. 4. Fragmentation model. *Mu3* is released due to double-stranded breaks bordering the element. Replication origins are activated somewhere within fragments B and E. Loops of newly synthesized DNA will form and at the junction between the newly synthesized and the old DNA, nicks (arrow at −266) or doubled-stranded breaks (arrows at +88 and +131) occur, resulting in further fragmentation of the affected promoter region (A, B, B*, D, E, E*, and F). Random arrangement of the available DNA fragments and ligations then led to inversions (fragments B* and E*) and deletions (fragment B). Exonucleolytic activity would result in the loss of sequences from −50 to −22. The model is used to explain the revertant *Adh1-3F1124r53* (as diagrammed in Fig. 1), but many other outcomes are possible. Fragments: A, *Bam*HI to −300; B, −299 to −31; C, *Mu3*; D, −30 to +87; E, +88 to +131; F, +132 and further downstream of *Adh1-3F*.

How Exceptional Is Our Case of a Scrambled Promoter? To generalize from exceptional occurrences is a well-known fallacy of deductive logic (often called converse accident). To our knowledge, revertant *r53* is the most complicated DNA rearrangement that has been reported to follow a single mutagenic event (i.e., transposon mutator excision). Scrambled promoters might be a reasonably common consequence of DNA excision within promoters, but the mutant assay systems generally used by others may miss them. What makes our case unique is the mutant selection screen permitting recovery of quantitative elevations of ADH activity and a quick allozyme screen by which we determine quantitative organ-specific activity. Without the exceptionally interesting organ-specific expression phenotype, we would not have pursued sequencing this allele. *r53* initially appeared wild type in a Southern blot, and only when we could not amplify an expected PCR fragment did we suspect a chromosomal aberration. Therefore, it would be wrong to dismiss *r53*'s multibreakpoint nature as merely a rare occurrence and thus overlook scrambling when cataloging the mutational lesions induced by transposon excision.

Transposon-Facilitated Generation of Allelic Variability. Transposon activity causes changes in DNA sequence and gene expression. These changes are the result of transposon insertions and the resultant small genomic duplications of host DNA, as well as the small and large deletions associated with transposon excisions. Such lesions can lead to the activation of cryptic genes (29) or the repression of neighboring genes by trans-acting suppression mechanisms (for review, see ref. 30). All of these changes become particularly effective when the transposon preferentially inserts into promoters (31). *Mu* transposons are thought to preferentially insert into unique-sequence DNA, possibly favoring 5′ regions of genes (for review, see ref. 32). Insertions into the 5′ regulatory region can alter cis-acting sequences and consequently the pattern of gene expression. For example, in the plant *Antirrhinum*, a *Tam3*-induced derivative of the *niv:531* allele carries a large insertion of unlinked *Antirrhinum* DNA within the nivea promoter; the expression of nivea is now under the control of a different gene (33). Similarly, in *Drosophila*, the Antennapedia mutant allele $Antp^{73b}$ resulted from an inversion event that was due to reciprocal exchange of promoter sequences with another gene (*rfd*), which is now controlling the expression of $Antp^{73b}$ (34); this inversion was likely facilitated by the presence of two *Doc* elements flanking the inversion.

Evolutionary Implications. Our single case of a scrambled *aAdh1* promoter following *Mu3* excision has led us to propose a new mechanism for the generation of genetic diversity. Independent assortment of chromosomes into the gametes and genetic recombination have been long established as general mechanisms for generating diversity in taxa that reproduce sexually. Perhaps localized, intrachromosomal scrambling—fragmentation—will prove to be another general mechanism and one not dependent on heterozygosity or sex. As promoter lineages become better understood, our fragmentation model can be put to the test. For example, analysis of *adh1* promoter sequence phylogenies in flies would provide a great test. However, such sequences are unavailable as yet. Goldschmidt's idea (1) that there should be a macromutational mechanism generating macromutations, and that these should fuel macroevolution, is certainly not proved. Nevertheless, promoter scrambling certainly would generate regulatory diversity that could result in macromutations.

B. DeFrancisi performed the screening procedure and the initial characterization of the revertants. D. Foreman participated in the quantitative assessment of ADH1 activity in pollen of the *r53* revertant. We thank B. Lane, B. Rotz, and J. Watkins for excellent greenhouse and field supervision. We thank all members of M.F.'s laboratory for stimulating discussions and Lisa Harper, Damon Lisch, and especially David S. Sullivan for critical comments on the manuscript. This work was supported by grants from the Department of Energy and the National Science Foundation.

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