

The Tc3 Family of Transposable Genetic Elements in *Caenorhabditis elegans*

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ABSTRACT

We describe genetic and molecular properties of Tc3, a family of transposable elements in *Caenorhabditis elegans*. About 15 Tc3 elements are present in the genomes of several different wild-type varieties of *C. elegans*, but Tc3 transposition and excision are not detected in these strains. Tc3 transposition and excision occur at high frequencies, however, in strain TR679, a mutant identified because of its highly active Tc1 elements. In TR679, Tc3 is responsible for several spontaneous mutations affecting the *unc-22* gene. Tc3-induced mutations are unstable, and revertants result from precise or nearly precise excision of Tc3. Although Tc3 is very active in TR679, it is not detectably active in several other mutator mutants, all of which exhibit high levels of Tc1 activity. Tc3 is 2.5 kilobases long, and except for sequences near its inverted repeat termini, it is unrelated to Tc1. The termini of Tc3 are inverted repeats of at least 70 base pairs; the terminal 8 nucleotides of Tc3 are identical to 8 of the terminal 9 nucleotides of Tc1.

TRANSPOSABLE genetic elements are present in the genomes of most, if not all, organisms. Because of their ability to insert into and excise from the chromosomes of their hosts, transposons are a significant source of spontaneous mutations in organisms as diverse as bacteria, fungi, maize, soybeans, snapdragons, nematodes, insects, mice, and humans (KLECKNER 1979; ROEDER and FINK 1983; FINNEGAN 1985; FINK, BOEKE and GARFINKEL 1986; DORING and STARLINGER 1986; COEN and CARPENTER 1986; HERMAN and SHAW 1987; KAZAZIAN *et al.* 1988; MORSE *et al.* 1988). Insertion and excision of transposable elements can alter the quantity, size, activity, tissue specificity, or developmental pattern of expression of an altered gene product (McCLINTOCK 1965; DOONER and NELSON 1977; ERREDE *et al.* 1980; REYNOLDS, FELTON and WRIGHT 1981; MCGINNIS, SHERMOEN and BECKENDORF 1983; SHURE, WESSLER and FEDEROFF 1983; TSUBOTA and SCHEDL 1986; COEN, CARPENTER and MARTIN 1986).

Activity of transposable elements can be regulated in response to factors as diverse as temperature (HARRISON and FINCHAM 1964; PAQUIN and WILLIAMSON 1984; ROBERTSON *et al.* 1988), genetic background (ENGELS 1983; DORING and STARLINGER 1986), tissue or cell type (FINNEGAN 1985), developmental timing (FINNEGAN 1985), and "genomic stress" (McCLINTOCK 1984). *Drosophila P* elements, for example, are normally active only in the germ line and only in strains having appropriate "cytotypes" (ENGELS 1979a, b). Many eukaryotic transposons encode proteins that are required in *trans* for transposition and excision. Such activities are known collectively as "transposases," but their roles in the transposition process are generally

unknown. The activity of an entire family of transposable elements is often governed by expression of its transposase. For example, the strain-specific and tissue-specific activity of *Drosophila P* elements is due to regulation of transposase expression (ENGELS 1984; LASKI, RIO and RUBIN 1986).

In the nematode *Caenorhabditis elegans*, transposition and excision of the element Tc1 is regulated in a strain-specific manner. Multiple copies of Tc1 are present in the genomes of all *C. elegans* varieties that have been collected from nature (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983), but Tc1 is genetically active only in certain of these strains (MOERMAN and WATERSTON 1984; EIDE and ANDERSON 1985a, b; GREENWALD 1985; MOERMAN, BENIAN and WATERSTON 1986). In *C. elegans* variety "Bristol," Tc1 is quiescent, but in variety "Bergerac," Tc1 is responsible for most spontaneous mutations. Tc1 is regulated in a tissue-specific manner as well. In variety Bergerac, for example, Tc1 excision in somatic cells occurs about 1000-fold more frequently (per cell) than in the germ line (EMMONS and YESNER 1984; EIDE and ANDERSON 1988).

We previously described mutants in which the frequencies of Tc1 transposition and excision in the germ line are greatly elevated (COLLINS, SAARI and ANDERSON 1987). Because of their high frequencies of Tc1 transposition, such mutants exhibit a mutator phenotype. Considering the diversity of transposons that are present in the genomes of most species, it seemed unlikely to us that Tc1 is the only such element in *C. elegans*. We investigated this possibility by looking more closely among mutator-induced mutations for those that contain insertions of elements other than

Tc1. We reasoned that, like Tc1, other families of transposable elements might be more active in mutator mutants than in wild-type genetic backgrounds. We describe here the genetic and molecular properties of a new family of transposable elements, which we designate the Tc3 family.

MATERIALS AND METHODS

Genetic procedures: *C. elegans* wild-type strains Bristol (N2), Bergerac (EM1002), and DH424 have been previously described (BRENNER 1974; EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983). TR403 is a wild-type strain that we collected from the stockyards of the University of Wisconsin-Madison campus. Spontaneous *unc-22* mutants were isolated by establishing independent cultures of strain TR679 [genotype *mut-2(r459)*; see COLLINS, SAARI and ANDERSON (1987)] using inocula of 3–5 worms. After several generations of growth, we identified spontaneous *unc-22* mutants in each culture using the selection described by MOERMAN and BAILLIE (1979). Only a single *unc-22* mutant was retained from each culture.

Biochemical procedures: Our methods for nematode growth, DNA extraction, and Southern blot hybridization have been described (EIDE and ANDERSON 1985a). We cloned *unc-22(r750::Tc3)* as described previously (EIDE and ANDERSON 1985a), except that we used lambda-EMBL3 as the cloning vector (FRISCHAUF *et al.* 1983) and plasmid TR#12 (see below) as the radiolabeled hybridization probe. One hybrid phage, designated TR#51, was retained.

Restriction fragments to be subcloned were purified from agarose gels (WEISLANDER 1979), ligated to appropriately digested plasmid DNA, and transformed into *E. coli* strain JM83 (VIEIRA and MESSING 1982). Tc3 was subcloned from lambda phage TR#51 into plasmid pIB176 (International Biotechnologies, Inc.) as a 5.1 kilobase (kb) *Bgl*II restriction fragment, yielding plasmid TR#10. This clone includes the entire Tc3 element (2.5 kb) inserted into a 2.6 kb *unc-22* fragment. The equivalent region of the wild-type *unc-22* gene was subcloned from phage lambda-DM20 (MOERMAN, BENIAN and WATERSTON 1986) into pIB176 as a 2.6-kb *Bgl*II fragment, yielding plasmid TR#12. A complete Tc1 element was subcloned from a bacteriophage lambda clone of *unc-54(r323::Tc1)* (EIDE and ANDERSON 1985b) into pUC9 (VIEIRA and MESSING 1982) as a 3.1-kb *Hind*III/*Bam*HI restriction fragment, yielding plasmid TR#30.

We derived a restriction map of Tc3 by comparing the sizes of restriction fragments generated by digestion of TR#10 and TR#12 with various single- and double-enzyme combinations. We sequenced the Tc3 insertional junctions of TR#10 and the corresponding wild-type region of TR#12 using the method of SANGER, NICKLEN and COULSON (1977), as modified for double-stranded plasmid DNA (ZAGURSKY *et al.* 1985). We used the SP6 Promoter Primer (New England Biolabs) to sequence the wild-type empty site and the “right” junction (as shown in Figure 2) of Tc3. Based on the sequence of the empty site, we designed a synthetic oligonucleotide (18-mer; obtained from the University of Wisconsin Biotechnology Center) complementary to a region of the wild-type *unc-22* gene about 20 nucleotides “left” of the insertion site. We used this oligonucleotide as a primer to sequence the left insertional junction of Tc3 and to sequence the opposite strand of the wild-type *unc-22* region. Comparisons of these three sequences unambiguously identified the termini of Tc3.

RESULTS

Isolation of Tc3-induced mutations: We discovered Tc3 by analyzing a large number of mutator-induced mutations affecting the *unc-22* gene. *unc-22* was chosen for this analysis for several reasons: (1) *unc-22* mutants are easy to identify and the gene is non-essential. *unc-22* mutants are muscle-defective, and they exhibit a distinctive “twitch” of their body-wall muscle (WATERSTON, THOMSON and BRENNER 1980). Rare *unc-22* mutants can be identified in large populations of phenotypically wild-type animals using a simple method that exploits this twitcher phenotype (MOERMAN and BAILLIE 1979). (2) The *unc-22* gene is a preferred target for Tc1 insertion (MOERMAN and WATERSTON 1984; MOERMAN, BENIAN and WATERSTON 1986); it might be a preferred target for other transposons as well. (3) The *unc-22* gene has been cloned (MOERMAN, BENIAN and WATERSTON 1986), providing hybridization probes for molecular analysis of mutant alleles.

We isolated 60 spontaneous *unc-22* mutants in the mutator strain TR679 [genotype *mut-2(r459)*; see COLLINS, SAARI and ANDERSON 1987]. Our methods of isolation ensure that each mutant represents an independent mutational event (see MATERIALS AND METHODS). We analyzed the gene structure of each *unc-22* mutant using genomic Southern blots. The hybridization probes for these experiments, clones lambda-DM17 and lambda-DM18 (MOERMAN, BENIAN and WATERSTON 1986), include approximately 25 kb of the *unc-22* gene region. These probes detect all Tc1 insertions that cause a strong *unc-22* mutant phenotype (D. MOERMAN, personal communication). Of 60 spontaneous *unc-22* mutants, 43 contained insertions of Tc1. We inferred that Tc1 caused an *unc-22* mutation if the allele contained a $1,600 \pm 50$ base pair (bp) insertion and if the inserted DNA was cut with restriction enzyme *Eco*RV but not with enzymes *Sac*I, *Bgl*II, or *Bam*HI. These criteria do not prove rigorously that an insert is Tc1. However, such restriction sites are predicted from the sequence of Tc1 (ROSENZWEIG, LIAO and HIRSH 1983), and all similar mutations affecting the *unc-54* gene are caused by Tc1 (11 of 11; see EIDE and ANDERSON 1985b).

Of the remaining 17 mutants, three of them [*unc-22(r735)*, *unc-22(r743)* and *unc-22(r750)*] contained insertions of approximately 2.5 kb within *unc-22*. The DNA inserted in these mutants represents a new family of transposable elements, which we designate Tc3. Genomic Southern blots of these three mutants are presented in Figure 1, lanes 4–6. For comparison, an additional mutant, *unc-22(r745::Tc1)*, that contains a Tc1 insertion in the same region of *unc-22* is shown in lane 3. The probe for Figure 1 (plasmid TR#12, see MATERIALS AND METHODS) hybridizes only to a small region of *unc-22* that includes these four inser-

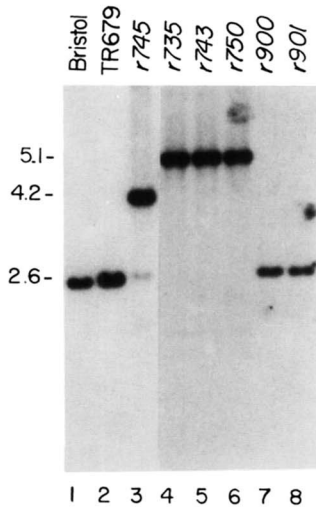


FIGURE 1.—Southern blot analysis of *unc-22::Tc3* mutants and revertants. Strains Bristol and TR679 are *unc-22*⁺. *r735*, *r743*, *r745*, and *r750* are spontaneous *unc-22* mutants isolated from strain TR679. *r900* and *r901* are spontaneous wild-type revertants of *r743* and *r750*, respectively. Genomic DNA from each strain was digested with *Bgl*II and separated on a 0.8% agarose gel. The radiolabeled probe was plasmid TR#12 (see MATERIALS AND METHODS). Sizes, in kilobases, are shown to the left of the blot.

tions. The restriction enzyme used (*Bgl*II) does not cut within Tc1 or Tc3. We performed similar Southern blots with several different restriction enzymes. These experiments allowed us to map more precisely the sites of Tc3 insertion within *unc-22* and to estimate the degree of similarity among the three independent insertions. *r735*, *r743* and *r750* each resulted from Tc3 insertion at a different site in *unc-22*, although the three insertions are clustered within a 500-bp region of the gene. Restriction maps of the three elements that we could deduce from Southern blots were indistinguishable from each other, but very different from that of Tc1.

Germline excision of Tc3: Both *unc-22(r743::Tc3)* and *unc-22(r750::Tc3)* revert spontaneously to *unc-22*⁺ in the TR679 background at a frequency of approximately 10^{-3} . We were unable to test rigorously for reversion of *unc-22(r735::Tc3)*. This mutant exhibits a very mild twitcher phenotype, making it difficult to distinguish revertants from mutants. We examined the gene structures of two revertant alleles. *unc-22(r900)* and *unc-22(r901)* are wild-type revertants of *unc-22(r743::Tc3)* and *unc-22(r750::Tc3)*, respectively. Figure 1, lanes 7 and 8, show that *r900* and *r901* resulted from precise or nearly precise excision of Tc3. The resolution of these experiments is ± 50 bp.

Somatic excision of Tc3: We investigated whether Tc3 exhibits a high frequency of somatic excision, similar to that of Tc1. Excision of Tc1 from its sites of insertion generates “empty sites” which, because of the very high frequency of somatic excision, can be

detected on genomic Southern blots as restriction fragments of wild-type mobility (EMMONS and YESNER 1984). In a typical DNA sample, empty sites derived from somatic DNA represent several percent of the total DNA molecules (EMMONS and YESNER 1984; EMMONS, ROBERTS and RUAN 1986). Using genetic methods, we estimate that the rate of Tc1 excision (excisions per cell division) is 1000-fold higher in somatic cells than in the germ line (EIDE and ANDERSON 1988).

An example of Tc1 somatic excision is evident in Figure 1, lane 3. *unc-22(r745::Tc1)* is a Tc1-induced allele of *unc-22*. The faint band of wild-type mobility represents somatic excision DNA. Lanes 4–6 are DNAs from the three Tc3-induced *unc-22* mutants. No DNA of wild-type mobility is detectable in these lanes. Even using increased amounts of genomic DNA or following prolonged autoradiography, we did not detect a somatic excision band for these Tc3 insertions. We conclude that somatic excision of Tc3 does not occur at frequencies comparable to Tc1, or that it occurs in a manner unlike Tc1. For example, if somatic excision resulted in empty sites of heterogeneous size, we might fail to detect their presence.

Tc3 does not hybridize to Tc1: To investigate the relatedness of Tc3 and Tc1, we cloned the element responsible for *unc-22(r750::Tc3)*. We prepared a genomic library of strain *unc-22(r750::Tc3)* in a bacteriophage lambda vector and identified clones that hybridized to plasmid TR#12, a clone of the wild-type *unc-22* gene. We then subcloned the Tc3-containing restriction fragment from one such phage into a plasmid vector. The resulting clone, designated TR#10, contains the complete Tc3 element (2.5 kb) inserted within a 2.6 kb restriction fragment of the wild-type *unc-22* gene. A restriction map of the Tc3 element contained in TR#10 is shown at the top of Figure 2.

We tested whether TR#10 hybridizes to TR#30, a plasmid clone of the complete Tc1 element (see MATERIALS AND METHODS). We prepared a Southern blot in which restriction fragments from 200 ng of TR#10 were transferred to nitrocellulose and hybridized with radiolabeled TR#30. We detected no hybridization between the two elements. The vector sequences of TR#30 hybridized intensely to those of TR#10. Because of their inability to hybridize, we conclude that Tc1 and Tc3 share little or no base sequence similarity.

Tc3 is a dispersed repetitive element: We investigated the distribution of Tc3 elements in the genomes of several different wild-type *C. elegans* varieties. Each of these varieties represents a geographically separate isolate from nature and, hence, a unique wild-type genetic background. We digested genomic DNA of each strain with an enzyme that does not cut within Tc3 (*Bgl*II) and probed Southern blots with plasmid

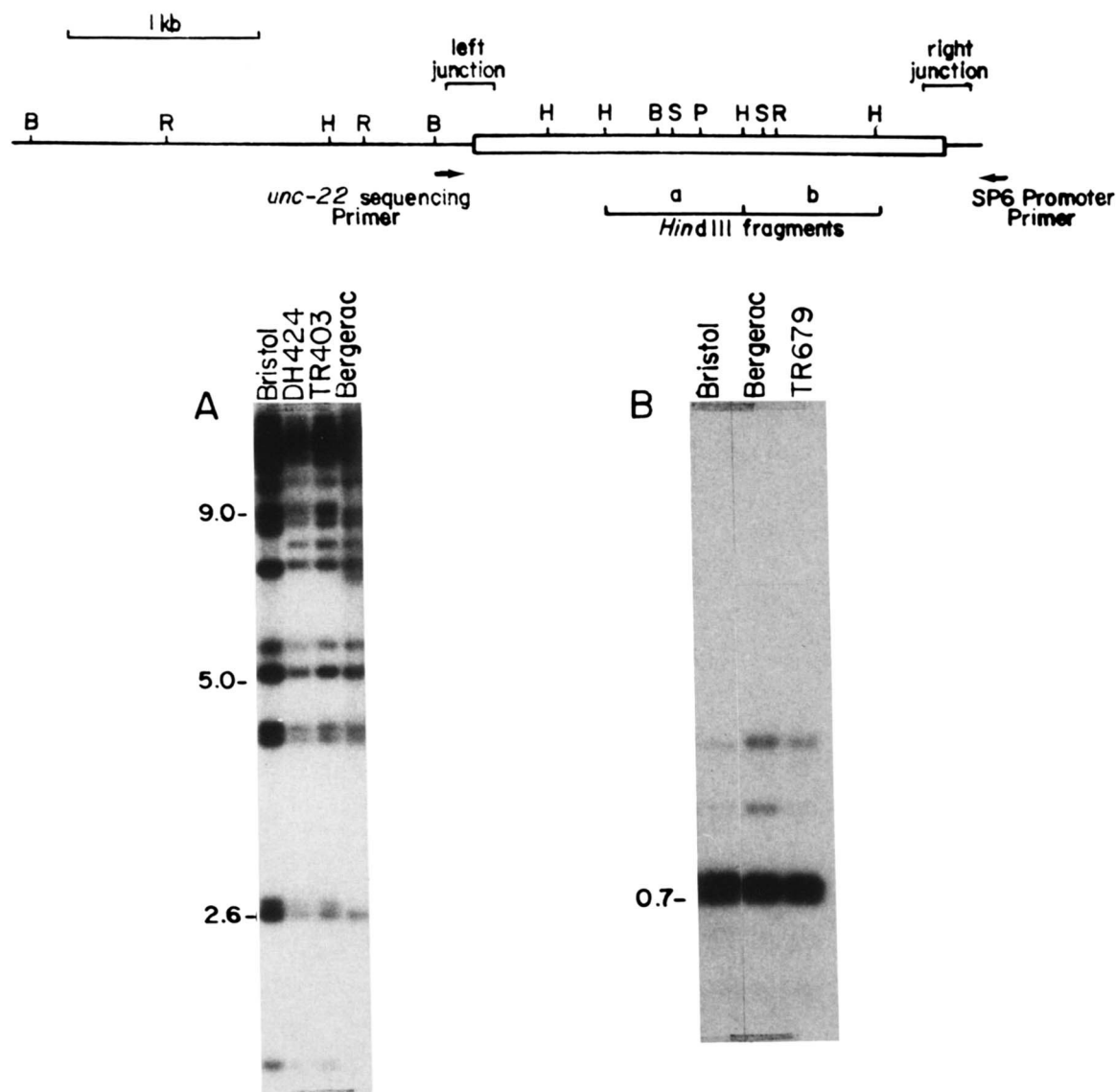


FIGURE 2.—Genomic organization of Tc3 elements. A restriction map of the Tc3 element and flanking *unc-22* DNA contained in plasmid TR#10 is shown for the restriction enzymes *Bam*HI, B; *Hind*III, H; *Pst*I, P; *Eco*RI, R; and *Sac*I, S. Restriction enzymes that were tested and do not cut within Tc3 are: *Bgl*II, *Eco*RI, *Sal*I, and *Xba*II. The arrows below the restriction map indicate the positions and orientations of primers used to sequence the insertional junctions of Tc3, shown as the “left junction” and “right junction” above the map. Sequences of these junctions are presented in Figure 3. (A) Southern blot analyses of wild-type strains Bristol, DH424, TR403, and Bergerac. Genomic DNAs were digested with *Bgl*II and separated on a 0.8% agarose gel. The radiolabeled probe was plasmid TR#10 (see MATERIALS AND METHODS). (B) Southern blots of Bristol, Bergerac and TR679. Genomic DNAs were digested with *Hind*III and separated on a 1% agarose gel. The radiolabeled probe was an equal mixture of gel-purified *Hind*III fragments “a” and “b,” shown below the Tc3 restriction map. Sizes, in kilobases, are shown to the left of each blot.

TR#10. The results for four different wild-type strains are presented in Figure 2A. Each of these strains contained approximately 15–18 copies of Tc3; individual strains differed from each other by, at most, two or three copies. The exact copy number is difficult to determine because for all enzymes tested, the hybridizing bands of high molecular weight were not resolved from one another. The similarity of Tc3 copy number and position in these strains indicates that Tc3 elements have remained relatively stable since the strains diverged. We examined four other *C. elegans* varieties in similar experiments (wild-type

strains N3, N62, GA3, and HA8). Each variety contained 12–18 dispersed copies of Tc3, and most of the hybridizing restriction fragments were the same as those shown in Figure 2A.

We investigated the degree of length heterogeneity among the genomic copies of Tc3. Tc3 contains four *Hind*III sites internal to the element (see Figure 2). We digested genomic DNAs of Bristol, Bergerac, and TR679 with *Hind*III and hybridized Southern blots with two radiolabeled *Hind*III fragments from the interior of Tc3. Each of these probe fragments is about 700 bp in length. Thus, only sequences from

A.

"Left"
Junction
TGGTTTCGTTGGCTTGCTTTTACCTTGATATTGAT**ACAGTGTGGGAAAGTTCTATAGGACCCCCCTAATTTG**
.....
WT *unc-22* TGGTTTCGTTGGCTTGCTTTTACCTTGATATTGAT**AGTGACACTGTCAGTTCCATTAATGTTGGTTGCAGTGA**
.....
"Right"
Junction
AATTAGGGGGGTCCTATAGAACTTTCCACACTGTAGTGACACTGTCAGTTCCATTAATGTTGGTTGCAGTGA

B.

Tc1: CAGTGCTGGCCAAAAAGATATCCACTTTTG... 1.6 kb ... CAAAAGTGGATATCTTTTGGCCAGCACTG
||||| |||
Tc3: CAGTG-TGGGAAAGTTCTATAGGACCCCC... 2.5 kb ... GGGGGTCTATAGAACTTTCCCA-CACTG
||||| |||

FIGURE 3.—The termini of Tc3 are inverted repeats. (A) The insertional junctions of *unc-22*(*r750::Tc3*) are shown above and below the wild-type *unc-22* target site sequence. Tc3 DNA is shown in boldface. (B) The sequences of the termini of Tc1 (ROSENZWEIG, LIAO and HIRSH 1983) and Tc3 are aligned for comparison. All sequences in panels A and B are shown 5'-3'.

this internal 1.4 kb region of Tc3 were detected in this experiment. Heterogeneity in the length of hybridizing fragments would indicate structural heterogeneity among individual Tc3 elements in the genome. The results are shown in Figure 2B. For each strain, the probe hybridized almost exclusively to *Hind*III fragments of 700 bp. We occasionally detected faint hybridization to fragments larger than 700 bp, but we believe that these represent incomplete digestion of genomic DNA. Their intensity was variable and the restriction map of Tc3 predicts partial digestion fragments of these sizes. It is also possible that they represent restriction site heterogeneity among different Tc3 elements. We conclude that most copies of Tc3 in the genome are similar in structure to the cloned copy.

The termini of Tc3 are inverted repeats similar to those of Tc1: In order to determine the nature of Tc3 termini, we sequenced the Tc3 insertional junctions and the insertion site of the wild-type *unc-22* gene. Our sequencing strategy is described in Materials and Methods and illustrated in Figure 2. The sequences of the Tc3 termini are shown in Figure 3A. Tc3 termini are perfect inverted repeats of at least 70 base pairs, which is the extent of our current sequencing data. Two features of these sequences are notable: (1) The terminal eight nucleotides of Tc3 are identical to eight of the terminal nine nucleotides of Tc1. This sequence similarity is shown in Figure 3B. Other than these terminal nucleotides, we detect no further similarity between the inverted terminal repeats of Tc1 and Tc3. (2) Like all sequenced sites of Tc1 insertion, Tc3 inserted into a TA dinucleotide of the target DNA. A single TA is present in the wild-type gene,

and TA dinucleotides flank the Tc3 insertion. Thus, Tc3 generates at most a two base pair target site duplication upon insertion. Since the sequence TA is an inverted repeat, and since one copy of the TA could be part of the element itself, these data are also consistent with Tc3 inserting without a target site duplication. Other than the TA dinucleotide, the *unc-22*(*r750::Tc3*) insertion site is not similar to the insertion site consensus sequence of Tc1 (EIDE and ANDERSON 1988; MORI *et al.* 1988).

Tc3 activity is *mut-2* specific: The copy number and genomic positions of Tc3 elements are relatively constant in the wild-type varieties (Figure 2A). Thus, very little Tc3 transposition and excision have occurred since these strains diverged. Tc3, furthermore, is not responsible for any of over 200 spontaneous mutations that have been isolated in these wild-type backgrounds and analyzed molecularly. Sixty-five spontaneous mutations in *unc-54* of Bristol (EIDE and ANDERSON 1985a), 90 in *unc-54* or *unc-22* of DH424 (EIDE and ANDERSON 1985b; D. EIDE and P. ANDERSON, unpublished data), and 56 in *unc-54*, *unc-22*, or *lin-12* of Bergerac (EIDE and ANDERSON 1985b; GREENWALD 1985; MOERMAN, BENIAN and WATERSTON 1986; COLLINS, SAARI and ANDERSON 1987) have been tested; none are caused by Tc3. In contrast, we found that three of 60 spontaneous *unc-22* mutations that occurred in TR679 are caused by Tc3. This suggested to us that Tc3 is genetically active in TR679 but not in wild-type strains.

To investigate this further, we examined the Tc3 elements present in individual animals isolated from a population of TR679. We isolated single animals from TR679 and propagated them independently for

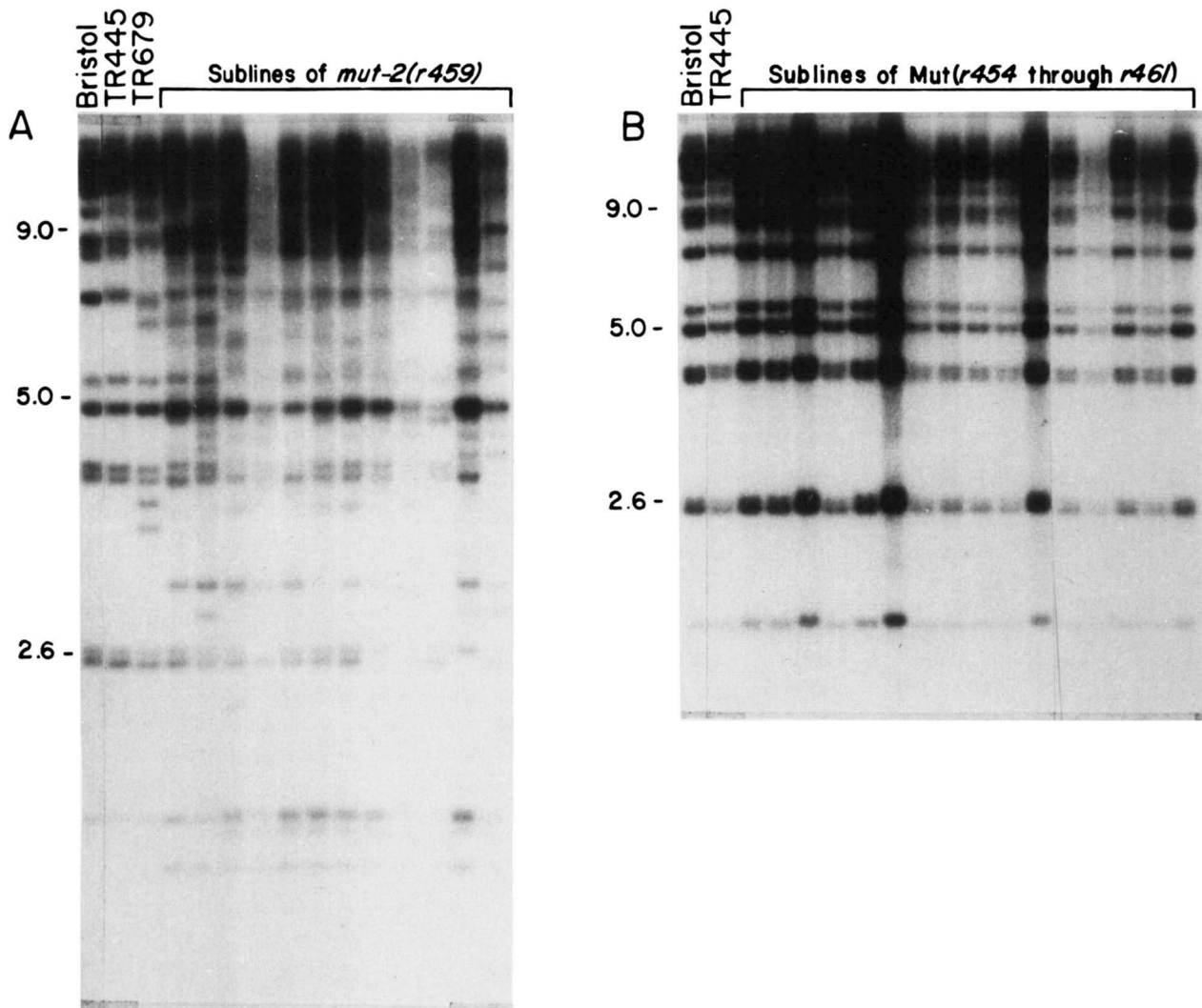


FIGURE 4.—Tc3 is highly activated only in the *mut-2* genetic background. Genomic DNAs were digested with *Bgl*II and separated on 0.8% agarose gels. The radiolabeled probe was plasmid TR#10. Strain TR445 is the parent strain from which the eight independent mutator mutants [*Mut(r454)* through *Mut(r461)*] were isolated. (A) Southern blots of Bristol, TR445, TR679 [genotype *mut-2(r459)*], and 12 independent sublines derived from TR679. (B) Southern blots of Bristol, TR445 and 16 independent sublines of the remaining seven mutators mutants.

several generations prior to harvesting their DNA. We then performed genomic Southern blots using a Tc3 hybridization probe. Differences among these sublines concerning the Tc3 copy number or position must reflect heterogeneity among the TR679 population from which the sublines were established. Since the sublines were unselected with regard to Tc3 activity, any differences would indicate a relatively high level of Tc3 transposition or excision. The results are shown in Figure 4A. The number and pattern of Tc3-hybridizing restriction fragments is highly polymorphic among these strains. We estimate that the Tc3 copy number varies from 17 to 25 for individual sublines. Furthermore, each subline exhibits an almost unique collection of Tc3 elements. We interpret these results to indicate that Tc3 elements transpose and excise at high frequency in TR679. We believe that the changes we observe are due to Tc3 transposition

and excision for two reasons: (1) The copy number of Tc3 in these strains is *greater than* the copy number in the wild-type strains (compare Figures 2A and 4A). Such an increase in copy number could not be caused by chromosome rearrangements or transposition of Tc1 within or near copies of Tc3. (2) Using the same method, we detect no evidence for activation of Tc3 in several other mutator mutants, all of which are highly active for Tc1 transposition and excision (see below).

mut-2(r459) is one of eight independent mutator mutants that we have isolated (COLLINS, SAARI and ANDERSON 1987). Each of these mutants was identified because it exhibits elevated frequencies of Tc1 transposition and excision. We investigated whether Tc3 is activated along with Tc1 in all of these mutants. We isolated single animals from populations of the remaining seven mutator mutants and analyzed them

as described above. We tested a total of 34 sublines, including at least four sublines for each mutator mutant. Examples of these results are shown in Figure 4B. The number and pattern of Tc3 elements in these strains are almost invariant and are similar to those of the wild-type varieties (compare Figures 2A and 4B). We conclude that Tc3 is activated to high levels in TR679, but not in the remaining seven mutator mutants.

DISCUSSION

We describe genetic and molecular properties of Tc3, a newly discovered family of transposons in *C. elegans*. We conclude that Tc3 is a transposable element for several reasons: (1) Three spontaneous *unc-22* mutations contain Tc3 insertions within the gene. (2) Tc3-induced mutations are unstable; revertants result from precise or nearly precise excisions of Tc3. (3) Tc3 is a dispersed, repetitive sequence; there are about 15 copies of Tc3 in the genome of most wild-type *C. elegans* isolates. (4) The termini of Tc3 are perfect inverted repeats. (5) The terminal inverted repeats of Tc3 are related to those of the *C. elegans* transposable element Tc1. (6) Like Tc1, Tc3 inserts into target DNA at TA dinucleotides, and TA sequences flank the inserted Tc3 element.

Eukaryotic transposable elements that exhibit short, inverted terminal repeats have been described in many species. For example, the *P*, *Hobo*, *HB*, and *Mariner* elements of *Drosophila* species (O'HARE and RUBIN 1983; JACOBSON, MEDHORA and HARTL 1986; MCGINNIS, SHERMOEN and BECKENDORF 1983; BRIERLEY and POTTER 1985), the *Ac/Ds*, *Spm/En*, *Mu*, *Tam1*, *Tam2*, *Tam3*, and *Tgm1* elements of plant species (POHLMAN, FEDEROFF and MESSING 1984; MULLER-NEUMANN, YODER and STARLINGER 1984; PERIERA *et al.* 1986; BARKER *et al.* 1984; BONAS, SOMMER and SAEDLER 1984; UPADHYAYA *et al.* 1985; SOMMER *et al.* 1985; VODKIN, RHODES and GOLDBERG 1983), the *Tx1* and *1723* elements of *Xenopus* (GARRETT and CARROLL 1986; KAY and DAWID 1983), the Tc1 element of *C. elegans* (ROSENZWEIG, LIAO and HIRSH 1983), the *sigma* element of yeast (DEL REY, DONAHUE and FINK 1982), and the *DIRS-1* element of *Dictyostelium* (ZUKER *et al.* 1984) all exhibit inverted repeats ranging from a few to a few hundred nucleotides. These elements comprise a general class of transposons and are distinct from other classes, such as the retroviruses (VARMUS 1983) or transposable elements without terminal repeats (WEINER, DEININGER and EFSTRATIADIS 1986).

A surprising number of elements that have short, terminal inverted repeats share common sequences at their termini. Figure 5 shows a comparison of the inverted repeat termini of 9 different transposons isolated from 6 different species. Each of these ele-

Element	Species	5'-Inverted Repeat-3'
<i>Mariner</i>	<i>D. mauritiana</i>	CCAGGTGTAC
Tc1	<i>C. elegans</i>	CAG-TGCTG
Tc3	<i>C. elegans</i>	CAG-TGTGG
<i>P</i>	<i>D. melanogaster</i>	CA--TGATG
<i>Hobo</i>	<i>D. melanogaster</i>	CAGAGAACT
<i>Spm/En</i>	<i>Z. mays</i>	CACTACAAG
<i>Tam1</i>	<i>A. majus</i>	CACTACAAC
<i>Tam2</i>	<i>A. majus</i>	CACTACAAC
<i>Tgm1</i>	<i>G. max</i>	CACTATTAG

FIGURE 5.—A comparison of inverted repeat termini of nine different transposable elements. The terminal inverted repeats of these elements are aligned to show their similarities.

ments begins with the sequence CA at or near one terminus and ends with the sequence TG at or near the other terminus. For certain of these elements, similarities extend even farther into the element, as noted previously (DORING and STARLINGER 1986; STRECK, MACGAFFEY and BECKENDORF 1986). The similarity of their termini might reflect common ancestry, cross-species transmission, or convergent evolution, and their mechanisms of transposition and excision might be similar.

A common feature of transposons having inverted repeat termini is the heterogeneous size of individual elements. "Defective" elements that contain deletions and other internal rearrangements are widespread (FINNEGAN 1985). For example, about two-thirds of *P* elements contain internal deletions and, therefore, are nonautonomous (O'HARE and RUBIN 1983). In contrast, most or all copies of Tc3 are similar to the cloned element (Figure 2B). Similarly, the Tc1 family in *C. elegans* is remarkably homogeneous in structure (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983). Perhaps mechanisms for maintaining the homogeneity of multigene families (*e.g.*, gene conversion) are especially active in *C. elegans*. Alternatively, perhaps *C. elegans* transposons are recent arrivals in the genome and have not had sufficient time to accumulate deleted copies.

Although Tc1 and Tc3 do not hybridize to each other, they are clearly related. The terminal eight nucleotides of Tc3 are identical to eight of the terminal nine nucleotides of Tc1 (Figure 3B). Like Tc1, Tc3 inserts into a TA dinucleotide of the target site, and TA sequences flank the inserted Tc3 element. The similarities between termini of Tc1 and Tc3 and between their sites of insertion suggest an explanation for how Tc1 and Tc3 are both activated in TR679: both of these elements are probably substrates for the same transposase functions, and the mutation *mut-2(r459)* likely affects a component that is common to both transposons. The origins of this transposase are unknown. Tc1 contains a long open reading frame (ROSENZWEIG, LIAO and HIRSH 1983), but there is no direct evidence that this gene encodes transposase. Both Tc1 and Tc3 would now appear to be possible

sources of transposase. By characterizing additional mutator-induced mutations, we and others have recently discovered two additional families of transposons (Tc4 and Tc5; J. COLLINS and P. ANDERSON, unpublished data; J. YUAN, M. FINNEY and R. HORVITZ, personal communication). Which, if any, of these elements encodes proteins required for transposition is unknown.

Tc1 and Tc3 are both activated in TR679 (COLLINS, SAARI and ANDERSON 1987) (Figure 4A), but it is clear that activities of these two elements are not always correlated: (1) Tc1 copy number varies over 10-fold in different wild-type varieties (EMMONS *et al.* 1983), but the copy number and genomic positions of Tc3 are relatively constant (Figure 2A). The frequencies of Tc3 transposition and excision, therefore, are relatively low in these genetic backgrounds. (2) Consequently, Tc3 is not responsible for any of over 200 spontaneous mutations that have been isolated in the wild-type varieties (EIDE and ANDERSON 1985a, b; GREENWALD 1985; MOERMAN, BENIAN and WATERSTON 1986; COLLINS, SAARI and ANDERSON 1987; D. EIDE and P. ANDERSON, unpublished data). Tc1 is very active in some of these strains. (3) Tc1 exhibits a very high frequency of somatic excision (EMMONS and YESNER 1984), but we cannot detect such excisions for Tc3 (Figure 1). (4) Transposition and excision of Tc1 are activated to high levels in each of eight different mutator mutants (COLLINS, SAARI and ANDERSON 1987), but Tc3 is activated only in *mut-2(r459)* (Figure 4). Collectively, these observations indicate that activity of Tc3 is regulated in a manner similar, but not identical, to that of Tc1.

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LITERATURE CITED

- BARKER, R. F., D. V. THOMPSON, D. R. TALBOT, J. SWANSON and J. L. BENNETZEN, 1984 Nucleotide sequence of the maize transposable element *Mu1*. *Nucleic Acids Res.* **12**: 5955–5967.
- BONAS, U., H. SOMMER and H. SAEDLER, 1984 The 17 kb *Tam1* element of *Antirrhinum majus* induces a 3 bp duplication upon integration into the chalcone synthase gene. *EMBO J.* **3**: 1015–1019.
- BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* **77**: 71–94.
- BRIERLEY, H. L., and S. S. POTTER, 1985 Distinct characteristics of loop sequences of two *Drosophila* foldback transposable elements. *Nucleic Acids Res.* **13**: 485–500.
- COEN, E. S., and R. CARPENTER, 1986 Transposable elements in *Antirrhinum majus*: generators of genetic diversity. *Trends Genet.* **2**: 292–296.
- COEN, E. S., R. CARPENTER and C. MARTIN, 1986 Transposable elements generate novel spatial patterns of gene expression in *Antirrhinum majus*. *Cell* **47**: 285–296.
- COLLINS, J., B. SAARI and P. ANDERSON, 1987 Activation of a transposable element in the germ line but not the soma of *Caenorhabditis elegans*. *Nature* **328**: 726–728.
- DEL REY, F. J., T. F. DONAHUE and G. R. FINK, 1982 *Sigma*, a repetitive element found adjacent to tRNA genes of yeast. *Proc. Natl. Acad. Sci. USA* **79**: 4138–4142.
- DOONER, H. K., and O. E. NELSON, 1977 Controlling element-induced alterations in UDP glucose: flavonoid glucosyltransferase, the enzyme specified by the *bronze* locus in maize. *Proc. Natl. Acad. Sci. USA* **74**: 5623–5627.
- DORING, H.-P., and P. STARLINGER, 1986 Molecular genetics of transposable elements in plants. *Annu. Rev. Genet.* **20**: 175–200.
- EIDE, D., and P. ANDERSON, 1985a The gene structures of spontaneous mutations affecting a *Caenorhabditis elegans* myosin heavy chain gene. *Genetics* **109**: 67–79.
- EIDE, D., and P. ANDERSON, 1985b Transposition of Tc1 in the nematode *C. elegans*. *Proc. Natl. Acad. Sci. USA* **82**: 1756–1760.
- EIDE, D., and P. ANDERSON, 1988 Insertion and excision of the *C. elegans* transposable element Tc1. *Mol. Cell. Biol.* **8**: 737–746.
- EMMONS, S. W., and L. YESNER, 1984 High-frequency excision of transposable element Tc1 in the nematode *Caenorhabditis elegans* is limited to somatic cells. *Cell* **36**: 599–605.
- EMMONS, S. W., S. ROBERTS and K. S. RUAN, 1986 Evidence in a nematode for regulation of transposon excision by tissue-specific factors. *Mol. Gen. Genet.* **202**: 410–415.
- EMMONS, S. W., L. YESNER, K. RUAN and D. KATZENBERG, 1983 Evidence for a transposon in *Caenorhabditis elegans*. *Cell* **32**: 55–65.
- ENGELS, W. R., 1979a Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. *Genet. Res.* **33**: 219–223.
- ENGELS, W. R., 1979b Extrachromosomal control of mutability in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **79**: 4570–4574.
- ENGELS, W. R., 1983 The P family of transposable elements in *Drosophila*. *Annu. Rev. Genet.* **17**: 315–344.
- ENGELS, W. R., 1984 A *trans*-acting product needed for P factor transposition in *Drosophila*. *Science* **226**: 1194–1196.
- ERREDE, B. T., T. CARDILLO, F. SHERMAN, E. DUBOIS, J. DESCHAMPS and J. WAIME, 1980 Mating signals control expression of mutations resulting from insertion of a transposable repetitive element adjacent to diverse yeast genes. *Cell* **25**: 427–436.
- FINK, G. R., J. D. BOEKE and D. J. GARFINKEL, 1986 The mechanism and consequences of retrotransposition. *Trends Genet.* **2**: 118–123.
- FINNEGAN, D. J., 1985 Transposable elements in eukaryotes. *Int. Rev. Cytol.* **93**: 197–206.
- FRISCHAUF, A.-M., H. LEHRACH, A. POUSTKA, and N. MURRAY, 1983 Lambda replacement vectors carrying polylinker sequences. *J. Mol. Biol.* **170**: 827–842.
- GARRETT, J. E., and D. CARROLL, 1986 Tx1: a transposable element from *Xenopus laevis* with some unusual properties. *Mol. Cell. Biol.* **6**: 933–941.
- GREENWALD, I., 1985 *lin-12*, a nematode homeotic gene, is homologous to a set of mammalian proteins that includes epidermal growth factor. *Cell* **43**: 583–590.
- HARRISON, B. J., and J. R. S. FINCHAM, 1964 Instability at the *Pal* locus in *Antirrhinum majus*. I. Effects of environment on frequencies of somatic and germinal mutation. *Heredity* **19**: 237–258.
- HERMAN, R. K., and J. E. SHAW, 1987 The transposable genetic element Tc1 in the nematode *C. elegans*. *Trends Genet.* **3**: 222–225.
- JACOBSON, J. W., M. M. MEDHORA and D. L. HARTL, 1986 Molecular structure of a somatically unstable transpos-

- able element in *Drosophila*. Proc. Natl. Acad. Sci. USA **83**: 8684–8688.
- KAY, B. K., and I. B. DAWID, 1983 The 1723 element: a long, homogeneous, highly repeated DNA unit interspersed in the genome of *Xenopus laevis*. J. Mol. Biol. **170**: 583–596.
- KAZAZIAN, H. H., JR., C. WONG, H. YOUSSEF, A. F. SCOTT, D. G. PHILLIPS and S. E. ANTONARAKIS, 1988 Haemophilia A resulting from de novo insertion of L1 sequences represents a novel mechanism for mutation in man. Nature **332**: 164–166.
- KLECKNER, N., 1979 Transposable elements in prokaryotes. Annu. Rev. Genet. **15**: 341–404.
- LASKI, F. A., D. C. RIO and G. M. RUBIN, 1986 Tissue specificity of *Drosophila* P element transposition is regulated at the level of mRNA splicing. Cell **44**: 7–19.
- LIAO, L. W., B. ROSENZWEIG and D. HIRSH, 1983 Analysis of a transposable element in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **80**: 3585–3589.
- MCCLINTOCK, B., 1965 The control of gene action in maize. Brookhaven Symp. Biol. **18**: 162–184.
- MCCLINTOCK, B., 1984 The significance of responses of the genome to challenge. Science **226**: 792–801.
- MCGINNIS, W., A. W. SHERMOEN and S. K. BECKENDORF, 1983 A transposable element inserted just 5' to a *Drosophila* glue protein gene alters gene expression and chromatin structure. Cell **34**: 75–84.
- MOERMAN, D. G., and D. L. BAILLIE, 1979 Genetic organization in *C. elegans*: Fine-structure analysis of the *unc-22* gene. Genetics **91**: 95–104.
- MOERMAN, D. G., and R. H. WATERSTON, 1984 Spontaneous *unc-22* IV mutations in *C. elegans* var. Bergerac. Genetics **108**: 859–877.
- MOERMAN, D. G., G. M. BENIAN and R. H. WATERSTON, 1986 Molecular cloning of the muscle gene *unc-22* in *Caenorhabditis elegans* by Tc1 transposon tagging. Proc. Natl. Acad. Sci. USA **83**: 2579–2583.
- MORI, I., G. M. BENIAN, D. G. MOERMAN and R. H. WATERSTON, 1988 Transposable element Tc1 of *Caenorhabditis elegans* recognizes specific target sequences for integration. Proc. Natl. Acad. Sci. USA **85**: 861–864.
- MORSE, B., P. G. ROTHBERG, V. J. SOUTH, J. M. SPANDORFER and S. M. ASTRIN, 1988 Insertional mutagenesis of the *myc* locus by a LINE-1 sequence in a human breast carcinoma. Nature **333**: 87–90.
- MULLER-NEUMANN, M., J. I. YODER and P. STARLINGER, 1984 The DNA sequence of the transposable element *Ac* of *Zea mays*. Mol. Gen. Genet. **198**: 19–24.
- O'HARE, K., and G. M. RUBIN, 1983 Structures of P transposable elements and their sites of insertion and excision in the *Drosophila melanogaster* genome. Cell **34**: 25–35.
- PAQUIN, C. E., and V. M. WILLIAMSON, 1984 Temperature effects on the rate of Ty transposition. Science **226**: 53–54.
- PERIERA, A., H. CUYPERS, A. GIERL, Z. SCHWARTZ-SOMMER and H. SAEDLER, 1986 Molecular analysis of the En/Spm transposable element system of *Zea mays*. EMBO J. **5**: 835–841.
- POHLMAN, R. F., N. V. FEDEROFF and J. MESSING, 1984 The nucleotide sequence of the maize controlling element *Activator*. Cell **37**: 635–643.
- REYNOLDS, A., J. FELTON and A. WRIGHT, 1981 Insertion of DNA activates the cryptic *bgl* operon in *E. coli* K12. Nature **293**: 625–629.
- ROBERTSON, H. M., C. R. PRESTON, R. W. PHILLIS, D. JOHNSON-SCHLITZ, W. K. BENZ and W. R. ENGELS, 1988 A stable genomic source of P element transposase in *Drosophila melanogaster*. Genetics **118**: 461–470.
- ROEDER, G. S., and G. R. FINK, 1983 Transposable elements in yeast. pp. 300–328. In: *Mobile Genetic Elements*, Edited by J. A. SHAPIRO. Academic Press, New York.
- ROSENZWEIG, B., L. W. LIAO and D. HIRSH, 1983 Sequence of the *C. elegans* transposable element Tc1. Nucleic Acids Res. **11**: 41–4209.
- SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**: 5463–5467.
- SHURE, M., S. WESSLER and N. FEDEROFF, 1983 Molecular identification and isolation of the *Waxy* locus in maize. Cell **35**: 225–233.
- SOMMER, H., R. CARPENTER, B. J. HARRISON and H. SAEDLER, 1985 The transposable element Tam3 of *Antirrhinum majus* generates a novel type of sequence alteration upon excision. Mol. Gen. Genet. **199**: 225–231.
- STRECK, R. D., J. E. MACGAFFEY and S. K. BECKENDORF, 1986 The structure of hobo transposable elements and their insertion sites. EMBO J. **5**: 3615–3623.
- TSUBOTA, S., and P. SCHEDL, 1986 Hybrid dysgenesis-induced revertants of insertions at the 5' end of the *rudimentary* gene in *Drosophila melanogaster*: transposon-induced control mutations. Genetics **114**: 165–182.
- UPADHYAYA, K. C., H. SOMMER, E. DREBBERS and H. SAEDLER, 1985 The paramutagenic line *niv-44* has a 5 kb insert, Tam2, in the chalcone synthase gene of *Antirrhinum majus*. Mol. Gen. Genet. **199**: 201–207.
- VARMUS, H. E., 1983 Retroviruses. pp. 411–503. In: *Mobile Genetic Elements*, Edited by J. A. SHAPIRO. Academic Press, New York.
- VIEIRA, J., and J. MESSING, 1982 The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19**: 259–268.
- VODKIN, L. O., P. R. RHODES and R. B. GOLDBERG, 1983 cA lectin gene insertion has the structural features of a transposable element. Cell **34**: 1023–1031.
- WATERSTON, R. H., J. N. THOMSON and S. BRENNER, 1980 Mutants with altered muscle structure in *Caenorhabditis elegans*. Dev. Biol. **77**: 271–302.
- WEINER, A. M., P. L. DEININGER and A. EFSTRATIADIS, 1986 Nonviral retrotransposons: genes, pseudogenes, and transposable elements generated by the reverse flow of genetic information. Annu. Rev. Biochem. **55**: 631–661.
- WEISLANDER, L., 1979 A simple method to recover intact high molecular weight RNA and DNA after electrophoresis in low gelling temperature agarose gels. Anal. Biochem. **98**: 305–309.
- ZAGURSKY, R., N. BAUMEISTER, N. LOMAX and M. BERGMAN, 1985 Rapid and easy sequencing of large linear double-stranded DNA and supercoiled plasmid DNA. Gene Anal. Techn. **2**: 89–94.
- ZUKER, C., J. CAPPELLO, H. F. LODISH, P. GEORGE and S. CHUNG, 1984 *Dictyostelium* transposable element DIRS-1 has 350-base-pair inverted terminal repeats that contain a heat shock promoter. Proc. Natl. Acad. Sci. USA **81**: 2660–2664.

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