Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 2579–2583, April 1986
Genetics

Molecular cloning of the muscle gene *unc-22* in *Caenorhabditis elegans* by Tc*1* transposon tagging

(mutator system/nematode/transposable element)

D. G. MOERMAN, G. M. BENIAN, AND R. H. WATERSTON

Washington University School of Medicine, Department of Genetics, 660 South Euclid Avenue, St. Louis, MO 63110

Communicated by Melvin I. Simon, December 20, 1985

ABSTRACT **The previously described mutator system of *Caenorhabditis elegans* var. Bergerac has as one of its targets *unc-22*, a previously uncloned gene on chromosome IV important in assembly and function of the body wall musculature. By assuming that the mutator activity involved transposition of the repetitive element Tc*1* into the *unc-22* gene we have succeeded both in cloning the *unc-22* gene and in demonstrating that Tc*1* transposition is the principal basis of the mutator activity in the Bergerac strain. Although germ-line excision of Tc*1* is sensitive to genetic background, somatic excision appears to be less so, suggesting that Tc*1* movement is controlled differently in germ-line and somatic tissue. The availability of a transposon-based mutator system should aid in the cloning of additional genes in *C. elegans*, and the particular properties of this Tc*1* system may provide information about the control of transposable element activity more generally.**

Transposable elements are probably ubiquitous in living organisms (1). A practical application to emerge from studies of transposable elements has been "transposon tagging," in which the elements are used to recover DNA from mutationally defined genes for which there is no known molecular correlate (2–6).

We have described a mutator system in the nematode *Caenorhabditis elegans* that is active in the Bergerac strain BO, but inactive in the more commonly used Bristol strain (N2) (7). This mutator system has properties similar to those of known transposable element systems, including high spontaneous mutation frequency, genetic instability of alleles, and sensitivity of both the forward mutation frequency and the reversion frequency to genetic background. The association of a specific transposable element with the Bergerac mutator system would provide a means of monitoring the mutator system at the molecular level as well as a tool for cloning DNA of interesting genes in *C. elegans*.

A favored target of this mutator system is *unc-22* on chromosome IV (7). Mutants of the *unc-22* locus have an abnormal organization of the fibrillar components of the body wall muscle cells and exhibit an almost continuous fine twitch of these muscles (8–10). The *unc-22* mutants are uniquely suppressed by a class of *unc-54* myosin heavy chain mutants that may involve myosin ATPase activity (11, 12). Selection methods exist both for and against *unc-22* mutations in heterozygotes; an intragenic map has been constructed and several deficiencies for the region have been recovered (10, 13, 14). Despite these efforts, the protein product of this locus has remained unknown and no DNA probes for the gene were available. Thus we could not directly assay for changes in *unc-22* sequences as a result of mutator activity; rather we began with the hypothesis that the repetitive element Tc*1* is responsible for the mutator activity.

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.

The Tc*1* element was initially identified as a repetitive sequence responsible for restriction fragment length polymorphisms in DNA of Bergerac and Bristol strains (15). It is present in more than 300 copies in the former strain and in only 30 copies in the latter strain (16, 17). Sequencing of Tc*1* reveals that it is structurally similar to a transposable element; it is 1.6 kilobases (kb) in length, contains a 54-base-pair perfect inverted repeat at its ends, and has a large open reading frame (18). As well, there is evidence for excision of Tc*1* in somatic cells (16, 19, 20), and free linears and circles of Tc*1* have been identified in the Bergerac strain (21, 22). More recently, Eide and Anderson (20) demonstrated that Tc*1* can insert into *unc-54* a gene encoding the major body wall myosin heavy chain (23–25). We show here that Tc*1* is responsible for the mutator activity in the Bergerac strain BO that yields unstable *unc-22* mutations.

MATERIALS AND METHODS

Growth, Maintenance, and Description of Nematode Strains. All nematode strains used in this study were derived from the N2 strain of *C. elegans* var. Bristol or the BO strain var. Bergerac (8, 26, 17, 7). Strains used are listed and described in Table 1 (also see ref. 7). Stock maintenance and strain growth were as described (8, 27).

Bacterial Strains, Cloning Vectors, and Media. *Escherichia coli* strain C600 or Q364 was used to grow the bacteriophage λ1059 (28). The plasmid pBR322 (29) and recombinant plasmid derivatives were grown in the *E. coli* strain RR1. *E. coli* was grown on either L broth or CY broth (28).

General Biochemical Methods. Methods for isolation of nematode genomic DNA, growth of phage and plasmids, recovery of DNA, gel electrophoresis, and Southern blotting have all been described (28, 30–32). Nick-translated probes were prepared and hybridization conditions were essentially as described (33, 34). An N2 genomic library was constructed by using the vector λ1059 (28) and screened by the Benton and Davis method (35).

Preparation of DNA Probes. The cloning of pGBc2, containing a segment of *unc-22*, and the isolation from this plasmid of a 1.1-kb *Eco*RV fragment to use as a probe is described in *Results*. The Tc*1* probe was initially pCe2001, a pBR322 derivative containing Tc*1* in an 8.7-kb segment isolated from a Bergerac (BO) λ1059 library (16). Later, the Tc*1* probe was pDM4, which is a pBR322 derivative containing Tc*1* in a 2.4-kb *Hin*dIII fragment isolated from strain RW1461.

RESULTS

Tc*1* Elements Associated with *unc-22* Mutations. To test the hypothesis that a Tc*1* element was associated with spontaneous *unc-22* mutations recovered in Bergerac-derived

Abbreviation: kb, kilobase(s).

Table 1. *C. elegans* strains used

Strain	Genotype	Chromosomal background	Comments
RW7002	*unc-22(st136)*	Bergerac	Spontaneous unstable mutation
RW7012	*unc-22(st136)*	Primarily Bristol	Stable mutation
RW1461	*daf-14(m77) unc-22(st136) dpy-4(e1166)*	Primarily Bristol	Reduces BO region to <<5 map units
RW1462	*unc-43(e408) dpy-4(e1166)*	Primarily Bristol	Recombinant replacing DNA left of and into *unc-22*
RW1463	*daf-14(m77)*	Primarily Bristol	Recombinant replacing DNA right of and into *unc-22*
RW7008	*unc-22(st137)*	Mixed (Bristol LG IV)	Spontaneous unstable mutation
RW7018	*unc-22(st137)*	Primarily Bristol	Stable mutation
RW1464	*daf-14(m77) unc-22(st137) dpy-4(e1166)*	Primarily Bristol	
RW1465	*unc-43(e408) dpy-4(e1166)*	Primarily Bristol	Recombinant replacing DNA left of and into *unc-22*
RW1466	*daf-14(m77)*	Primarily Bristol	Recombinant replacing DNA right of and into *unc-22*
RW2370	*unc-22(+)*	Primarily Bristol	Intragenic revertant from RW7018
RW2371	*unc-22(+)*	Primarily Bristol	Intragenic revertant from RW7018
RW2372	*unc-22(+)*	Primarily Bristol	Intragenic revertant from RW7018
RW2373	*unc-22(+)*	Primarily Bristol	Intragenic revertant from RW7018
RW6103	*sDf19/nT1*	Bristol	

Ref. 7 describes in detail the isolation and construction of these strains.

strains, we looked for a Tc*1* polymorphism associated with a spontaneous *unc-22* mutation. The Bergerac mutator strain (BO) has several hundred copies of the element (16, 17). Before we could examine the spontaneous *unc-22* mutant alleles directly for Tc*1* polymorphisms it was necessary to reduce the overall Tc*1* copy number by placing the spontaneous *unc-22* mutation, *st137*, of the Bergerac-derived strain RW7008 into a largely Bristol genetic background (see ref. 7 for details). This reduced the overall Tc*1* copy number to less than 40 copies and stabilized the spontaneous *unc-22* mutation.

This reduced copy number made it feasible to identify on Southern transfers of genomic DNA the restriction enzyme fragments carrying individual Tc*1* elements. Multiple Tc*1*-containing fragments were present in the mutant strains that were absent from the congenic wild-type strains. Because of the crossing procedure used to reduce the Tc*1* copy number, these mutant strains likely contained several Tc*1* copies linked to *unc-22*, which were not present in the Bristol strain, in addition to the Tc*1* that represented the mutational event.

To distinguish between Tc*1* elements that were simply linked to *unc-22* and the element responsible for the *unc-22* mutation, we examined DNA from coisogenic revertant strains and intragenic recombinants. In one mutant strain, RW7018, containing the *unc-22(st137)* mutation in a largely Bristol background, we found four rare wild-type revertants (see ref. 7). DNA from the coisogenic strains RW7018 and RW2370, mutant and wild type for *unc-22*, respectively, was cleaved to completion with different restriction enzymes, transferred to nitrocellulose, and probed with a nick-translated (33) pBR322 clone containing Tc*1* flanked by single-copy nematode DNA (Fig. 1). Of several restriction enzymes used, *Bgl* II and *Hpa* I most clearly showed a band present in digests from the mutation-bearing strain, RW7018, that was absent in digests from the wild-type revertant strain RW2370. For *Bgl* II, this band was 3.8 kb in size, and we postulated that this band corresponded to an *unc-22* fragment containing the Tc*1* insertion.

The DNA of a second spontaneous mutation, *unc-22(st136)*, was also examined after the mutation was stabilized by placing it in a primarily Bristol background (7). This strain, RW7012, also has a 3.8-kb *Bgl* II fragment that hybridizes to Tc*1* (Fig. 2). We were unable to recover wild-type revertants from RW7012, but we were nevertheless able to confirm by recombination analysis that the 3.8-kb *Bgl* II fragment derives from *unc-22*.

The 3.8-kb *Bgl* II band remains in RW1461, a triply mutant strain (*daf-14 unc-22 dpy-4*) derived by recombination from RW7012, in which all of the fourth chromosome is Bristol except for the immediate region around *unc-22* (*daf-14* is 0.8–1.2 map units left of *unc-22* and *dpy-4* is about 4.5 map units right of *unc-22*) (36). A few other polymorphic Tc*1* fragments present in RW7012 are lacking in the triple mutant and are presumably outside of the immediate *unc-22* vicinity. The RW1462 and RW1463 strains were derived by intragenic recombination within *unc-22* using *S7* and *S14*, respectively, two ethylmethanesulfonate-induced alleles of *unc-22* (7, 13). Bands present in one strain that are not present in the other (Fig. 2) are Tc*1* elements within *unc-22* or either left (if in RW1463) or right (if in RW1462) of *unc-22*. The 3.8-kb fragment is the only band detected in RW1461 that is absent in both RW1462 and RW1463. This particular Tc*1* element is associated with the mutated state of *unc-22*, and the intragenic mapping and restriction fragment analysis demonstrate that this element is located within the genetic interval defined as *unc-22*.

Cloning of the 3.8-kb *Bgl* II Fragment and of the *unc-22*

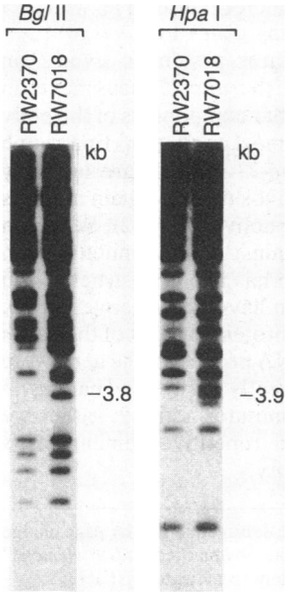

FIG. 1. A Tc*1* dimorphism associated with the *unc-22(st137)* mutation. Strain RW7018 contains the spontaneous mutation *unc-22(st137)* crossed into a largely Bristol background, and strain RW2370 is a wild-type revertant of *unc-22* coisogenic with RW7018. DNA (5 μg per sample) from RW7018 and RW2370 was digested to completion with either *Bgl* II or *Hpa* I, fractionated on a 0.7% agarose gel, and transferred to a nitrocellulose filter as described (32). The nick-translated probe used for the hybridization was pDM4, a pBR322 derivative containing Tc*1*. In both strains several identical fragments are observed, but in each digest there is at least one band present in RW7018 that is absent in RW2370.

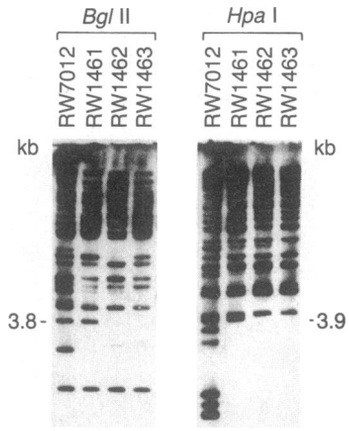

FIG. 2. A Tc*1* dimorphism associated with *unc-22(st136)*. Strain RW7012 contains the spontaneous mutation *unc-22(st136)* crossed into a largely Bristol background. The strain RW1461 is a triple mutant *daf-14 unc-22(st136) dpy-4* that replaces most of Bergerac chromosome IV by the Bristol homolog except for the region near *unc-22*, and RW1462 and RW1463 are *unc-22(+)* intragenic recombinants (see Table 1 and ref. 7). DNA from these strains was prepared and analyzed as described in *Materials and Methods* and Fig. 1. The plasmid pDM4, which contains Tc*1*, was used as a hybridization probe. In both the *Bgl* II and *Hpa* I digests RW7012 and RW1461 contain a fragment that is absent in the other two strains.

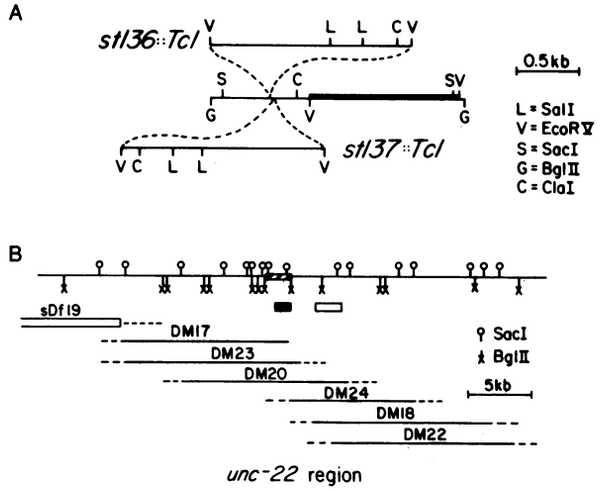

FIG. 3. (*A*) Restriction map of insertion sites of *st136*::Tc*1* and *st137*::Tc*1*. The insertion sites of *st136* and *st137* are about 40 base pairs apart. Using the asymmetric *Cla* I restriction site present in Tc*1*, we determined that the element is oriented in opposite directions in *st136* and *st137*. (*B*) Restriction map of the *unc-22* region. A unique 1.1-kb *Eco*RV fragment (black rectangle in *A*) derived from pGBc2 (see text for details) was used as a probe to screen a λ1059-N2 genomic library. Over 20 positive λ clones (not all shown) were isolated and used to construct a restriction map of the region, which covers about 35 kb. The striped box delineates the 2.2-kb *Bgl* II fragment into which Tc*1* transposed in *st136* and *st137*. Separated by a few kilobases is a second region (the open box) that is weakly homologous to the 1.1-kb *Eco*RV probe. Also shown on the map is the deficiency *sDf19* which orients the restriction map relative to the genetic map.

Region. To recover this 3.8-kb *Bgl* II fragment, which is well separated from most other Tc*1*-containing fragments, we ligated size-fractionated *Bgl* II-digested RW7018 DNA into the *Bam*HI site of pBR322 and transformed *E. coli*. Among several clones recovered by their hybridization to a Tc*1* probe, (37) one plasmid, pGBc2, was selected that had an insert of appropriate size. *Eco*RV digestion of pGBc2 DNA yielded the expected 1.6-kb fragment from Tc*1* (Tc*1* has *Eco*RV sites near the distal ends of the inverted repeats) (18), as well as other fragments. A 1.1-kb fragment near the Tc*1* insertion site was recovered to use as a probe specific for putative *unc-22* sequences (black rectangle in Fig. 3*A*).

We isolated a series of overlapping λ clones from a λ 1059-N2 genomic library by using this specific sequence probe. The λ clones (>20) were of two classes: those that contained a strongly hybridizing sequence and those that had a more weakly hybridizing sequence. Construction of a restriction map for the two sets of clones, surprisingly, showed the two sets to overlap, with some clones containing both weakly and strongly hybridizing sequences (Fig. 3*B*). The weakly homologous sequence corresponds to a weakly hybridizing signal seen on Southern transfers of genomic digests (see below and Fig. 4). The clones cover a total of about 30 kb around the initial 1.1-kb region. One of these clones, λ DM17, was used to obtain overlapping cosmid clones for the *unc-22* region (J. Sulston, personal communication), thus extending the amount of continuous cloned DNA to about 70 kb, which is likely to include all the *unc-22* gene.

Confirmation that the Cloned Sequences Come from the *unc-22* Region. To confirm that the 1.1-kb *Eco*RV fragment comes from the *unc-22* region, DNA transfers from several strains wild type and mutant for *unc-22* were probed with the nick-translated 1.1-kb *Eco*RV fragment (Fig. 4). The 3.8-kb *Bgl* II fragment seen with a Tc*1* probe is observed with this unique sequence probe in the *st137* strains, RW7008 and RW7018, and in the *st136* strains, RW7002 and RW7012. Neither the Bristol parental strain, N2, nor the Bergerac parental strain, BO, contains a 3.8-kb piece, but instead each has a 2.2-kb *Bgl* II fragment expected for the sequence lacking Tc*1*. Four wild-type revertants of RW7018 (RW2370, RW2371, RW2372, and RW2373) all have a 2.2-kb fragment but lack a signal at 3.8 kb. (The other prominent bands in some of these lanes we attributed to partial digestion.) The strain RW1464 contains the triple mutations *daf-14 unc-22(st137) dpy-4* and has the 3.8-kb fragment, while the intragenic recombinant strains RW1465 and RW1466, which are wild type for *unc-22*, have the fragment at 2.2 kb (see Table 1 and ref. 7). Analogous results were found for the *st136* strains RW1461, RW1462, and RW1463. Thus for the *st136* and *st137* mutations, all the *unc-22* mutant strains contain a 3.8-kb fragment and all the strains wild type for *unc-22* contain a 2.2-kb fragment.

Two minor bands observed on this gel deserve comment. There is a band visible at 2.8 kb whether *unc-22* is mutated or not. This band is due to the region within *unc-22* that has weak cross-homology with the 1.1-kb probe. A second minor band of 2.2 kb is present in all the *unc-22* mutant strains and probably is the result of somatic excision of Tc*1* from the 3.8-kb fragment, a property of Tc*1* also observed by others (16, 19). Somatic excision occurs in all *st136* and *st137* strains regardless of genetic background, which is in marked contrast to the dependence of Tc*1* germ-line excision on genetic background.

Orientation of the Physical Map on the Genetic Map. As part of studies on the genetic organization of *unc-22* and its surrounding region, a deficiency map centering on *unc-22* has been constructed (14). One deficiency, *sDf19*, with a breakpoint in *unc-22*, extends left of *unc-22* to at least *let-56*, a nearby essential gene (14, 38). If the cloned DNA were from the *unc-22* region, then probing restriction-digested and fractionated DNA on filters from a strain containing *sDf19* (maintained as a heterozygote over the translocation *nT1*; see legend to Fig. 5 for details) should reveal alterations in the restriction fragment profile relative to N2. Because the position of the deficiency breakpoint is known relative to the *st136* and *st137* insertion sites on the genetic map (7), the

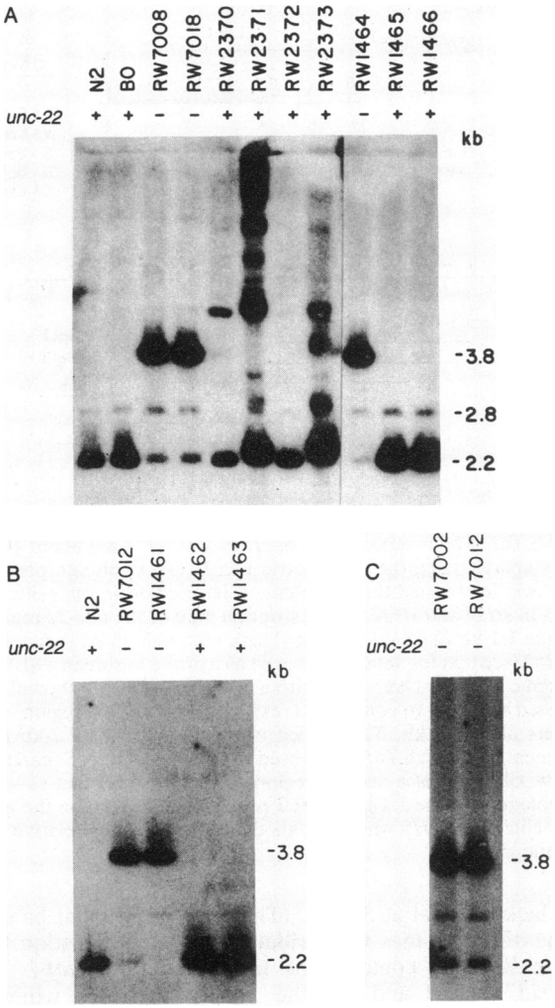

FIG. 4. Analysis of several mutator-induced *unc-22* strains with the unique 1.1-kb *Eco*RV probe. Southern blots were prepared as described in the text. The + or − at the top of each lane denotes the wild-type state or the mutant state, respectively, of the *unc-22* locus. All strains having *unc-22* in the + state have the 2.2-kb *Bgl* II fragment, while strains having *unc-22* in the − state have the 3.8-kb fragment. The 2.8-kb band present in these strains is a second region within *unc-22* that has homology to the hybridization probe. (See Fig. 3 and text.) (*A*) *st137*::Tc*1*-containing strains and derivatives. The strains are described in Table 1 and in ref. 7. (*B*) *st136*::Tc*1*-containing strains and derivatives. The strains are described in Table 1 and in ref. 7. (*C*) Comparison of somatic excision in Bergerac and Bristol strain backgrounds. The BO-derived strain RW7002 containing *st136*::Tc*1* and the multiply out-crossed strain RW7012 are shown. Note that the ratios of intensity of the 3.8-kb *Bgl* II fragment to that of the 2.2-kb fragment are similar in the two lanes.

identification of fragments present in haploid amounts would allow us to orient the physical map on the genetic map.

A clone, λDM17, containing the leftward region was used as a probe to Southern transfers of restricted wild-type and *sDf19/nT1* DNA (see Fig. 5) and an extra band, about half the intensity of most of the other major bands, was observed in several digests of the deficiency heterozygote DNA. This is probably the fusion fragment generated by the *sDf19* deficiency joining *unc-22* to an adjacent region. The small doublet in the *Eco*RI *sDf19/nT1* lane also appears to be of lower intensity (Fig. 5) and likely represents fragments present in haploid amounts. All the restriction fragments affected are located at the left end of the restriction map shown in Fig. 3*B*. These results with *sDf19* confirm that the DNA cloned is from the *unc-22* region and includes at least part of the *unc-22* gene.

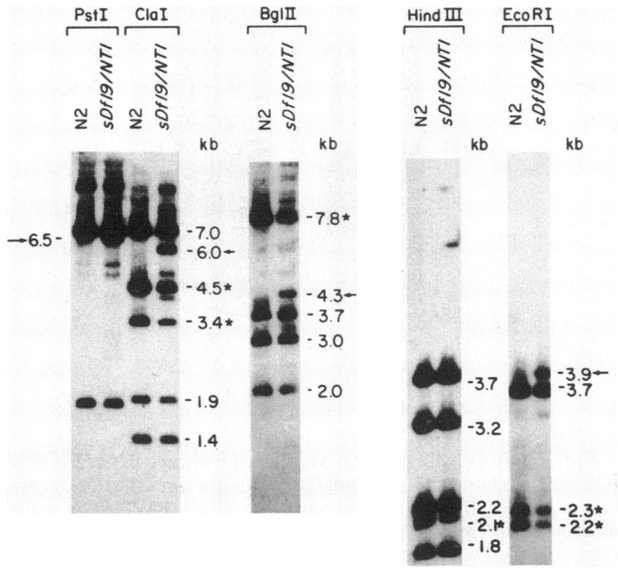

FIG. 5. Restriction pattern differences between N2 and *sDf19* DNA. DNA was isolated and Southern blots were prepared; the nick-translated hybridization probe in each case was λDM17 (see Fig. 3). The deficiency *sDf19* when homozygous is an early larval lethal (ref. 38; D.G.M., unpublished results) and therefore was balanced using the IV:V reciprocal translocation *nT1* (39). In most digests the *sDf19/nT1* lane contains bands of reduced intensity relative to the same bands in the N2 lane (marked by asterisks). As well, in some digests a band is present in the *sDf19/nT1* lane that is not observed in the N2 lane (marked by an arrow).

Placing the deficiency breakpoint at the left of the cloned region, and to the left of the insertion sites for *st136* and *st137* on the map in Fig. 3, orients the cloned region on the genetic map.

Independently Isolated Spontaneous *unc-22* Alleles Contain Tc*1* at Different Sites. The initial characterization of *st136* and *st137* showed that they are in the same *Bgl* II fragment, but genetic data suggested that they are distinct sites since they could be separated by recombination (7). Higher-resolution restriction mapping of plasmid clones of the mutant sequences demonstrated that the mutations are at separate sites, about 40 base pairs apart (Fig. 3*A*). A *Cla* I digest that cuts Tc*1* at an asymmetric site revealed that these two phenotypically similar mutants resulted from Tc*1* elements that inserted in opposite orientations (Fig. 3*A*).

We have subsequently examined a further 12 spontaneous *unc-22* mutations from either the Bergerac strain or mixed Bristol-Bergerac strains. All 12 exhibited changes in the hybridizing fragments consistent with 1.6-kb inserts in the region encompassed by the λ and cosmid clones (data not shown), suggesting that Tc*1* insertion is the basis for all of these mutations. By restriction map analysis these 12 new mutations could be resolved into at least 10 separate sites spanning approximately 30 kb in the *unc-22* region.

DISCUSSION

Our results demonstrate unequivocally that the mutator system generating spontaneous *unc-22* mutants in the BO strain involves transposition of the repetitive element Tc*1*. This mutator system does not require crosses to other strains to activate it, but is continuously active in the BO strain. The high rates of mutation and reversion of *unc-22* in this Bergerac strain make it the gene of choice for genetic studies of Tc*1* transposition, and the cloning of *unc-22* DNA will permit the molecular analysis of the transposition events.

Ultimately it may be possible to control more closely the transposition of this element.

An important question is whether transposon tagging can be used as a general method for recovering DNA sequences of other genes in the nematode. Our results, and the results of others, suggest that Tc*1* transposition is responsible for a significant fraction of spontaneous mutations in the Bergerac strain. For example, Tc*1*-induced alleles of *unc-54* have been recovered (20), and a second gene, *lin-12*, has been isolated by Tc*1* transposon tagging (40). The methods we used to identify the DNA fragment containing the insertion should be generally applicable, but coisogenic revertants and fine structure mapping may not always be available to confirm the identity of the relevant polymorphism. For smaller genes, however, it may be possible to identify several independent mutants that all have the same restriction fragment affected as was the case for *lin-12* (40). Further research may allow us to develop more direct approaches for using Tc*1* to recover tagged genes.

Finally, the isolation of DNA from *unc-22* by using transposon tagging provides us with a means of determining the product of this gene. The almost constant twitch exhibited by *unc-22* mutants suggests control of the contractile events is altered. The findings that certain alleles of *unc-54*, the gene for the major myosin heavy chain of the body wall musculature (23–25), can dominantly suppress this twitching and that at least two of these mutations are near the ATP-binding site of the myosin heavy chain add additional interest to understanding the function of the *unc-22* gene product (11, 12). The large size of the target for Tc*1* insertion suggests that *unc-22* encodes a very large protein (unpublished data). Blot hybridization analysis also indicates that the mRNA is unusually large (unpublished results). Identification of the *unc-22* gene product by using the DNA clones now available will likely identify a component of *C. elegans* muscle that would have been difficult to discover in any other way.

We thank S. Emmons for the plasmid pCe2001, E. Ferguson for the nematode strain MT1000, T. Rogalski for a strain containing *sDf19*, and J. Sulston for providing cosmid clones for the *unc-22* region. We also thank J. Kiff and L. Schrieffer for their assistance in constructing the restriction map of the *unc-22* region, G. R. Francis and R. Barstead for their constructive criticism of the manuscript, and K. Webb for typing the manuscript. This research was supported by U.S. Public Health Service Grant GM23883 and a Muscular Dystrophy Association Jerry Lewis Neuromuscular Research Center grant. G.M.B. was supported in part by a postdoctoral fellowship from the Muscular Dystrophy Association.

1. Shapiro, J. A., ed. (1983) *Mobile Genetic Elements* (Academic, New York).
2. Bingham, P. M., Levis, R. & Rubin, G. M. (1981) *Cell* **25**, 693–704.
3. Searles, L. L., Jokerst, R. S., Bingham, P. M., Voelker, R. A. & Greenleaf, A. L. (1982) *Cell* **31**, 585–592.
4. Modolell, J., Bender, W. & Meselson, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1678–1682.
5. Mattox, W. W. & Davidson, N. (1984) *Mol. Cell. Biol.* **4**, 1343–1353.
6. Fedoroff, N., Furtek, D. & Nelson, O. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3825–3829.
7. Moerman, D. G. & Waterston, R. H. (1984) *Genetics* **108**, 859–877.
8. Brenner, S. (1974) *Genetics* **77**, 71–94.
9. Waterston, R. H., Thomson, J. N. & Brenner, S. (1980) *Dev. Biol.* **77**, 271–302.
10. Moerman, D. G. (1980) Dissertation (Simon Fraser Univ., Burnaby, BC).
11. Moerman, D. G., Plurad, S., Waterston, R. H. & Baillie, D. L. (1982) *Cell* **29**, 773–781.
12. Dibb, N. J., Brown, D. M., Karn, J., Moerman, D. G., Bolten, S. L. & Waterston, R. H. (1985) *J. Mol. Biol.* **83**, 543–551.
13. Moerman, D. G. & Baillie, D. L. (1979) *Genetics* **91**, 95–103.
14. Rogalski, T. M., Moerman, D. G. & Baillie, D. L. (1982) *Genetics* **102**, 725–736.
15. Emmons, S. W., Klass, M. R. & Hirsh, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1333–1337.
16. Emmons, S. W., Yesner, L., Ruan, K. & Katzenberg, D. (1983) *Cell* **32**, 55–65.
17. Liao, L. W., Rosenzweig, B. & Hirsh, D. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3585–3589.
18. Rosenzweig, B., Liao, L. W. & Hirsh, D. (1983) *Nucleic Acids Res.* **2**, 4201–4209.
19. Emmons, S. W. & Yesner, L. (1984) *Cell* **36**, 599–605.
20. Eide, D. & Anderson, P. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 1756–1760.
21. Ruan, K. & Emmons, S. W. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 4018–4022.
22. Rose, A. M. & Snutch, T. P. (1984) *Nature (London)* **311**, 485–486.
23. Epstein, H. F., Waterston, R. H. & Brenner, S. (1974) *J. Mol. Biol.* **90**, 291–300.
24. MacLeod, A. R., Waterston, R. H. & Brenner, S. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5336–5340.
25. MacLeod, A. R., Waterston, R. H., Fishpool, R. M. & Brenner, S. (1977) *J. Mol. Biol.* **114**, 133–140.
26. Nigon, V. (1942) *Ann. Sci. Natl. Zool.* **11**, 1–132.
27. Sulston, J. E. & Brenner, S. (1974) *Genetics* **77**, 95–104.
28. Karn, J., Brenner, S., Barnett, L. & Cesareni, G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5172–5176.
29. Bolivar, R., Rodriguez, R. L., Greene, P. J., Betlach, M. C., Heyneker, H. L., Boyer, H. W., Crosa, J. H. & Falkow, S. (1977) *Gene* **2**, 95–113.
30. Wills, N., Gesteland, R. F., Karn, J., Barnett, C., Bolten, S. & Waterston, R. H. (1983) *Cell* **33**, 575–583.
31. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
32. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
33. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
34. Goodman, H. M., Olson, M. V. & Hall, B. D. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5453–5457.
35. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
36. Riddle, D. L. & Swanson, M. M. (1982) in *Genetic Maps*, ed. O'Brien, S. J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 2, pp. 244–258.
37. Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961–3965.
38. Rogalski, T. M. & Baillie, D. L. (1985) *Mol. Gen. Genet.* **201**, 409–414.
39. Ferguson, E. L. & Horvitz, H. R. (1985) *Genetics* **110**, 17–72.
40. Greenwald, I. (1985) *Cell* **43**, 583–590.