SPONTANEOUS UNSTABLE *UNC-22 IV* MUTATIONS IN *C. ELEGANS* VAR. BERGERAC

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ABSTRACT

This paper describes a mutator system in the nematode *Caenorhabditis elegans* var. Bergerac for the gene *unc-22*. Of nine *C. elegans* and two *C. briggsae* strains tested only the Bergerac BO strain yielded mutant animals at a high frequency and the *unc-22 IV* gene is a preferred mutational target. The forward spontaneous mutation frequency at the *unc-22* locus in Bergerac BO is about 1×10^{-4}, and most of these spontaneous *unc-22* mutations revert at frequencies between 2×10^{-3} and 2×10^{-4}. Both the forward mutation frequency and the reversion frequency are sensitive to genetic background. Spontaneous *unc-22* mutations derived in a Bergerac background and placed in a primarily Bristol background revert at frequencies of $<10^{-6}$. When reintroduced into a Bergerac/Bristol hybrid background the mutations once again become unstable.

The mutator activity could not be localized to a discrete site in the Bergerac genome. Nor did mutator activity require the Bergerac *unc-22* gene as a target since the Bristol *unc-22* homolog placed in a Bergerac background also showed high mutation frequency. Intragenic mapping of two spontaneous *unc-22* alleles, *st136* and *st137*, place both mutations in the central region of the known *unc-22* map. However, these mutations probably recombine with one another, suggesting that the unstable mutations can occur in more than one site in *unc-22*. Examination of the phenotypic effect of these mutations on muscle structure indicates that they are less severe in their effect than a known amber allele. We suggest that this mutator system is polygenic and dispersed over the nematode genome and could represent activity of the transposable element *Tc1*.

MUTATOR systems as defined in prokaryotes and the eukaryotes, yeast, Drosophila and maize, have been powerful tools in the application of genetics to a variety of problems. As described by GREEN (1976) mutator systems have generally occurred in one of three discrete classes: mutator genes, extrachromosomal mutators and mutable genes. Mutator genes act to induce stable mutations in other genes, and the mutator gene itself is a discrete mappable locus. GREEN (1976) has hypothesized that these mutators may operate through DNA repair or replication processes. Recently, a mutator gene has been found to be a T4 DNA polymerase mutant, *tsm19*, which increases reversion frequency of certain *rII* mutations by 50- to 100-fold (REHA-KRANTZ and BESSMAN 1981; REHA-KRANTZ and LIESNER 1984). Extrachromosomal mu-

tators are similar to mutator genes except that they cannot be localized to any position on a genetic map.

Mutable genes, on the other hand, are characterized as having one or more alleles that are mutationally unstable, and events associated with these genes are usually intragenic. Mutable genes were first described in 1914 by EMERSON (cited in FEDOROFF 1983) in maize. Later DEMEREC (1926) described a mutable gene, miniature-3 alpha (*mt-3α*) in *Drosophila virilis*. With hindsight one can see similarities between the observations of these early workers and the observations of MCCLINTOCK (1951, 1956) on the controlling elements of maize. More recently, mutator genes with mutations caused by the insertion of transposable elements have been described for several organisms including *E. coli*, *Saccharomyces* and *Drosophila melanogaster* (see SHAPIRO 1983 for reviews). The transposable element systems of these different organisms share many attributes, including high spontaneous forward mutation frequency, genetic instability of alleles and sensitivity of mutation frequency to genetic background.

In this paper we describe genetic evidence for a mutator system in the Bergerac strain of the nematode *C. elegans*, a strain initially isolated and described by V. NIGON (1949). This mutator system was identified in studies of the *unc-22* gene (BRENNER 1974). Mutations in this gene affect muscle structure disrupting thick and thin filament organization (WATERSTON, THOMSON and BRENNER 1980; MOERMAN 1980). Animals homozygous for an *unc-22* mutation are slow moving or even paralyzed and have an easily detectable twitch along their body wall. Even heterozygous animals can be induced to twitch after immersion in a solution of levamisole (BRENNER 1974) or 1% nicotine alkaloid (MOERMAN and BAILLIE 1979).

In *C. elegans* var. Bristol (BRENNER 1974), the most commonly used strain for genetic studies, the spontaneous forward mutation frequency at the *unc-22* locus is about 7×10^{-7} (R. P. ANDERSON, personal communication). In mutagenesis experiments using the Bergerac strain, we recovered incidentally an inordinate number of *unc-22* mutations. Further analysis of the parental strain revealed that *unc-22* mutations occur spontaneously at frequencies two to three orders of magnitude higher in the Bergerac strain than in the Bristol strain. Furthermore, these *unc-22* mutations are unstable. We describe genetic studies of these spontaneous mutations and their revertants which explore the process by which they are generated, maintained and altered. A comparison of the Bergerac mutator system, the first to be described in *C. elegans*, with mutators in other organisms suggests that the Bergerac mutator is a transposable element system.

MATERIALS AND METHODS

General procedures: The methods for stock maintenance, linkage studies, complementation testing and ethyl methanesulfonate (EMS) mutagenesis of *C. elegans* have been described by BRENNER (1974). Marker replacement crosses between the Bristol and Bergerac strains and tests of chromosomal and maternal influences on mutability are detailed in the appropriate RESULTS section. All experiments were performed at 20° unless otherwise noted.

Strains and genetic nomenclature: The *C. elegans* var. Bristol N2 (wild type) strain was originally isolated in England and was described by BRENNER (1974). This Bristol N2 strain is the parent of

all of the Bristol mutants used in this study. The *C. elegans* var. Bergerac strain was originally isolated in France and was described by NIGON (1949). Three laboratory strains of *C. elegans* var. Bergerac were used in this study. These strains were provided by J. BRUN to a number of laboratories between 1977 and 1980. In 1977 Brun provided the laboratory of D. HIRSH with Bergerac LY (LIAO, ROSENZWEIG and HIRSH 1983), which they sent to the Caenorhabditis Genetics Center (CGC) in 1980. This CGC strain was subsequently named Bergerac BO. The strain of BO used in this laboratory has been designated RW7000. A second strain, Bergerac FR, was sent by Brun directly to the CGC, and stock center notes indicate that Nigon isolated Bergerac FR in 1944. A third strain (now called BL1) was provided by Brun's laboratory to T. OTSUKA who was at that time working at the Medical Research Council Laboratory in Cambridge, England. We obtained Berg BO and Berg FR from the CGC, and BL1 from W. SHARROCK. We also obtained four wild-type strains of *C. elegans* from the CGC that were isolated from soil samples in or near Pasadena, California. These included GA-5, isolated by C. JOHNSON, PA-1 and PA-2, isolated by E. HEDGCOCK in 1973, and DH424, isolated by D. HIRSH (see LIAO, ROSENZWEIG and HIRSH 1983). A ninth wild-type strain of *C. elegans*, HA-8, was isolated by R. RUSSELL in Hawaii and sent to us by R. P. ANDERSON.

We also examined two strains of *Caenorhabditis briggsae* in this study. *C. brig* Z was sent to the CGC by B. ZUCKERMAN and *C. brig* G16 was isolated in India by A. FODOR. We obtained both strains from the CGC.

The genetic nomenclature used in this report conforms to the system of HORVITZ *et al.* (1979).

Determining forward mutation frequency and reversion frequency at the unc-22 *locus*: To determine the spontaneous mutation frequency for *unc-22*, single animals were placed on small (60 mm) or large (100 mm) Petri dishes and left until the plates were crowded. When crowded, but not starved, the plates were washed off with a few milliliters of a 1% nicotine alkaloid solution (Sigma) onto a clean (no agar) 35- or 60-mm Petri dish. The animals were left in this solution for several minutes (usually 5 min) and then examined to see if there were any twitching animals among the paralyzed animals. Wild-type worms become rigidly paralyzed in a 1% solution of nicotine, whereas worms homozygous or heterozygous for an *unc-22* mutation twitch or vibrate (MOERMAN and BAILLIE 1979). As few as one heterozygote *unc-22* animal could be detected in this assay, although often more than one animal was found. Plates yielding one or more "twitchers" were scored as positive and those containing only wild-type animals were scored as negative. Any twitcher found was picked and progeny tested to ensure that it segregated *unc-22* mutants.

For the reversion studies 60- or 100-mm Petri dishes seeded with single homozygous *unc-22* mutants were examined after one to two generations or more for wild-type animals. Wild-type revertants are relatively easys to see in a twitcher background because the revertants are generally larger and move faster than the Unc mutants.

All estimates of frequencies are based on previous counts which show that a 60-mm Petri plate can support 5×10^3 to 1×10^4 animals and that a 100-mm Petri plate can support about 1×10^5 animals (our unpublished data; also see GREENWALD and HORVITZ 1982; WATERSTON, SMITH and MOERMAN 1982). The frequencies of mutation or reversion were then estimated from the fractions of plates yielding no events using the Poisson distribution.

Intragenic mapping: To map spontaneous *unc-22* mutations intragenically we modified the earlier protocol of MOERMAN and BAILLIE (1979). The spontaneous *unc-22* mutation to be mapped (*x*) was placed in *cis* with *daf-14(m77)* and *dpy-4(e1166)*. The *daf-14* locus is about 0.8–1.2 map units left of *unc-22* and *dpy-4* is about 4.5 map units right of *unc-22* (RIDDLE 1980; RIDDLE and SWANSON 1982). This multiply marked chromosome was then placed *in trans* with *unc-43(e408) unc-22(y)* to make the heteroallelic strain *daf-14 unc-22(x) dpy-4/unc-43 unc-22(y)*. The *unc-43* to *unc-22* distance is 1.1 map units (ROGALSKI, MOERMAN and BAILLIE 1982). Individual heteroallelic animals were placed on separate 100-mm plates, and the plates were periodically examined for nontwitching animals. Any non-*unc-22* animals found were progeny tested to determine the genotype of the parental chromosomes.

This mapping study was done at 25° to make use of the *daf-14* mutation, a temperature-sensitive dauer constitutive (SWANSON and RIDDLE 1981). The principal of this genetic construct was to reduce viability of the homoallelic animals relative to the heteroallelic animals. Dauer animals will not contribute progeny to the next generation, nor will *unc-43 unc-22* doubles con-

tribute significantly because they grow very slowly and have small broods (D. G. MOERMAN, unpublished observations).

Microscopy: Polarized light microscopy procedures were similar to those used by WATERSTON, THOMSON and BRENNER 1980). Phalloidin staining of F-actin in whole animals and fluorescent microscopy procedures were as described by WATERSTON, LANE and HIRSH (1984).

RESULTS

Frequency and stability of spontaneous unc-22 *mutations in* C. elegans *var. Bergerac*: In *C. elegans* var. Bristol (strain N2) spontaneous forward mutations and reversions of mutations in most genes including *unc-22* are rare events, occurring at frequencies of $<10^{-6}$ (See Table 1). However, in screening *C. elegans* var. Bergerac (strain BO) animals for movement in a 1% nicotine solution, spontaneous mutants occur at a frequency of 1×10^{-4} (Table 1). Phenotypically, these spontaneous mutants resemble other *unc-22* alleles in being paralyzed and in having a prominent twitch with disorganized body wall muscle as determined by polarized light microscopy. This combination of twitch and abnormal body wall muscle structure has been found associated only with *unc-22* mutants, but we tested four of the spontaneous mutants for complementation of known *unc-22* alleles. All four fail to complement *unc-22(s12)* and *unc-22(s32)*, and we presume other isolates are also alleles of the *unc-22* locus.

Although we have not made an exhaustive survey in the course of these studies, we have looked for other mutant phenotypes. Mutants with Dpy, Bli or Unc phenotypes (involving more than 100 genes) could be readily recognized in simple screening of Bergerac animals, but to date we have found only three mutations in genes other than *unc-22*. One of these has a recessive Dumpy phenotype, another is a leaky recessive Roller and still another has a Blister phenotype. We have not obtained any revertants from the Dumpy or the Roller strains in $>10^5$ animals tested. The Blister mutant has not been tested for stability.

Of approximately 200 spontaneous *unc-22* mutants isolated in the BO strain, we tested 33 and found that 32 mutations are unstable and revert to a wild-type phenotype. The reversion frequency of all of these unstable alleles is similar, being between 2×10^{-3} and 2×10^{-4}. For the *unc-22(st136)* allele, for example, we found two wild-type revertants among 1229 progeny (see Table 2). In contrast, a mutagen-induced allele in the Bristol strain N2, *unc-22(s12)*, has not been observed to revert spontaneously; even after mutagenesis only extragenic suppressors have been recovered (Table 2; MOERMAN *et al.* 1982).

To determine whether a reverted allele differed from the initial BO wild-type *unc-22* allele in its ability to mutate once again, we looked for new *unc-22* mutants in a revertant strain. We found that the revertant allele was still capable of being mutated and, furthermore, that the new mutant allele from the revertant could itself still revert. Altogether we observed four sequential events; wild → twitcher → wild → twitcher → wild. The induction and reversion frequency for each event were similar to those we have already described.

We examined seven additional *C. elegans* strains and two *C. briggsae* strains

TABLE 1

A comparison of several C. elegans *and* C. briggsae *strains for the spontaneous induction of* unc-22 *mutations*

Strain	*unc-22* mutants[a]	Animals screened[b]	Induction frequency
N2	0	1.0×10^6	$\leq 10^{-6}$[c]
BO	10	9×10^4[d]	1×10^{-4}
DH424	0	1.3×10^6	$\leq 10^{-6}$
FR	0	1.2×10^6	$\leq 10^{-6}$
BL1	2	1.0×10^6	$\sim 10^{-6}$
HA-8	0	8×10^5	$< 10^{-5}$
GA-5	0	1.0×10^6	$\leq 10^{-6}$
PA-1	0	1.0×10^6	$\leq 10^{-6}$
PA-2[e]	0	$2\text{–}5 \times 10^5$	$\leq 10^{-5}$
C. brig. Z	0	1.0×10^6	$\leq 10^{-6}$
C. brig. G16[e]	0	$1\text{–}5 \times 10^5$	$< 10^{-5}$

[a] Mutants recovered resembled the *unc-22* reference allele in phenotype, but not all were demonstrated by complementation tests to be *unc-22* alleles.

[b] Except for the BO strain, estimates for the number of animals screened are based on a capacity of 10^5 animals per saturated 100-mm Petri dish.

[c] The estimate obtained by R. P. ANDERSON (personal communication) is 7×10^{-7}.

[d] Of 18 separate plates screened with an average of 5×10^3 animals each ($= 9 \times 10^4$ animals), ten plates yielded mutant animals. Using the Poisson distribution to correct for the possibility of multiple independent events in some of the plates, we estimated an overall frequency to be 1×10^{-4}.

[e] Animals from these two strains tend to burrow into the agar which makes estimates of the number of animals screened less reliable.

TABLE 2

A comparison of reversion frequencies for several unc-22 *mutant strains*

Strain	Genotype	Chromosomal background	Treatment	Revertants		Animals screened[b]	Reversion frequency[c]
				Intragenic	Extragenic[a]		
BC200	*unc-22(s12)*	Bristol	EMS[d]	0	6	8×10^5	$< 2 \times 10^{-6}$[e]
BL1	*unc-22*(st180)	Bergerac	None	1	0	1×10^6	5×10^{-7}
RW7002	*unc-22(st136)*	Bergerac	None	2	0	1229	0.8×10^{-3}
RW7008	*unc-22(st137)*	Mixed	None	4	0	1359	1.4×10^{-3}
RW7012	*unc-22(st136)*	Primarily Bristol	None	0	0	2×10^7	5×10^{-8}
RW7018	*unc-22(st137)*	Primarily Bristol	None	1	0	3×10^6	1.5×10^{-7}
RW7018	*unc-22(st137)*	Primarily Bristol	EMS	3	1	4×10^6	4×10^{-7}

[a] Extragenic suppressors were determined to be alleles of *unc-54* (see MOERMAN *et al.* 1982).

[b] Except for the BO strain, estimates for the number of animals screened are based on a capacity of 10^5 animals per saturated 100-mm Petri dish.

[c] Reversion frequency is calculated for intragenic events only.

[d] Animals in these experiments were treated with 0.05 M EMS for 4 h (BRENNER 1974).

[e] Data from MOERMAN *et al* (1982).

to determine whether any of these strains generate spontaneous *unc-22* mutations (Table 1). All of these strains show a frequency of spontaneous *unc-22* mutations of $<10^{-5}$. The BO strain, then, is unique in its ability to generate spontaneous *unc-22* alleles at a frequency of 10^{-4}.

Attempts to locate an unc-22 *mutator*: Given the strain (BO) and the relative gene (*unc-22*) specificity of the mutator, it was possible that a single locus in the BO strain was responsible for the high frequency of spontaneous *unc-22* mutations. This might be an unlinked site or the *unc-22* gene itself. In a search for a discrete mutator, we did a series of crosses between N2 and BO using, where possible, markers lying in the single central cluster of each chromosome to test for linkage of the site to known markers. Male animals carrying markers for each of the linkage groups in the N2 background, including LG IV, where *unc-22* is located, were mated to BO hermaphrodites. In the F_2 generation animals homozygous for these N2 markers were reisolated to establish 20 independent lines for each marker. The unmarked chromosomes were allowed to distribute randomly.

To score the presence or absence of the mutator activity in each of the lines established, we had to determine whether the *unc-22* mutation rate was characteristic of the N2 (7×10^{-7}) or BO (1×10^{-4}) strains. As it was impractical to determine accurately the mutation rate for each independent line, we chose to take advantage of the large disparity of *unc-22* mutation rates in the two backgrounds. In the absence of the Bergerac mutator activity, the probability of recovering an *unc-22* mutant spontaneously in 10^5 animals is about 0.06. In the presence of the Bergerac mutator, however, the probability of recovering one or more spontaneous *unc-22* mutants in 10^5 animals is >0.99. This is true even if the original parent of the line established was heterozygous for a recessive mutator at a single site, since the propagation of *C. elegans* under these conditions is exclusively by self-fertilization. This results after the several generations required to produce approximately 10^5 animals in a population in which >40% of the animals are progeny of animals homozygous for the mutator.

Following this reasoning each of the F_2 lines was propagated for three to five generations to produce approximately 10^5 animals (the number accommodated by a single large plate), and then the line was examined for the presence of *unc-22* mutants. If mutants were recovered the line was scored as positive for the mutator, and if no mutants were recovered, the line was scored as negative. With random assortment of the mutator in establishing the line, 0.75 of the lines should on average contain the mutator. Absolute linkage of the mutator site to the marker will yield lines with no mutator and only 0.06 of the lines will yield *unc-22* mutants on average. Intermediate values of linkage will, of course, give fractions between 0.06 and 0.75, depending on the linkage value.

Between 50 and 75% of the lines derived for most markers were positive for the ability to generate *unc-22* mutations (Table 3). However, *dyp-9 IV* and *dpy-10 II* each produced fewer positive lines than other markers used for these linkage groups, but the double mutant, DpyUnc, in each case grew poorly and

TABLE 3

Linkage tests of the unc-22 *mutator*

Linkage group	Marker	Spontaneous twitchers	No twitchers	% positive
X	*lon-2(e678)*	14	5	70
I	*dpy-5(e61)*	13	7	65
II	*dpy-10(e128)*	4	16	20
II	*unc-4(e120)*	7	5	58
III	*dpy-17(e164)*	11	9	55
III	*dpy-1(e1)*	13	5	71
IV	*dpy-13(e184)*	12	8	60
IV	*dpy-9(e12)*	5	14	26
V	*dpy-11(e224)*	15	5	75
IV	*dpy-13*(+)[Bergerac]	15	1	94

This screen is based on looking for heterozygous or homozygous *unc-22* mutants using 1% nicotine alkaloid. A no-decision was determined after screening $>10^5$ chromosomes.

was difficult to detect. We repeated the linkage tests using *unc-4* for LG II, which is a map unit to the right of *dpy-10*, and *dpy-13* for LG IV. These markers yielded a percentage of positives similar to other markers. In no case was there an indication of close linkage of the mutator activity to a marker mutant.

These chromosome replacement studies included replacement of the Bergerac *unc-22* gene by its Bristol homolog using a *dpy-13 IV* marker (Table 3). The *dpy-13* and *unc-22* genes are about 8 map units apart and recombination between *dpy-13* and *unc-22*(+) Bristol in establishing the lines should have occurred in only 15% of the cases; yet, 12 of 20 lines were positive (Table 3). Similar results were obtained using *unc-43(e408)* as the Bristol chromosome marker which is only 1.1 map units from *unc-22* (data not shown). Therefore, most of the mutational events had to occur in a Bristol *unc-22* gene showing that the BO *unc-22* gene is not the site of the mutator activity.

Sequential replacement of unstable unc-22 *alleles by wild type* unc-22 *alleles does not eliminate mutator activity*: If the mutator activity could occupy only a single site (apparently unlinked to the markers used above) or a very few sites in any one strain and resides at the *unc-22* locus in unstable alleles, it would be feasible to eliminate or at least substantially reduce mutator activity simply by replacing an unstable *unc-22* mutant allele with a wild-type allele. To test this, we crossed the twitcher strain RW7002, containing the *unc-22(st136)* unstable allele, with N2 males and selected +/+ animals among the F_2 progeny using a 1% solution of nicotine to establish independent lines. After expansion of the line to 10^5 animals two of 12 such lines yielded unstable *unc-22* alleles. One of the mutant strains, carrying the allele *unc-22(st137)*, was crossed as before, and 20 +/+ F_2 animals were used to establish lines. Of 15 fertile lines recovered, 14 yielded *unc-22* mutants in the 10^5 animals examined. The replacement of the unstable allele in two successive rounds did not reduce the mutator activity to Bristol levels.

Stabilizing the spontaneous unc-22 *mutations by altering their genetic background*: The *unc-22* spontaneous mutations in the BO background are unstable, reverting to wild type at a frequency between 2×10^{-3} and 2×10^{-4} (Table 2). Reversion also occurred for mutations recovered in the mixed Bergerac/Bristol backgrounds constructed in the mapping studies, suggesting that reversion might also depend on a polygenic mutator. It is reasonable to assume that this reversion activity represents a second aspect of the mutator system that generates the spontaneous mutations. As reversion is an easier process to monitor and is of similar or even higher event frequency than forward mutations, we used it to examine the relationship of overall genetic background to mutator activity.

To replace the Bergerac genome by the Bristol genome, we undertook an extensive series of the following cross: selfing scheme.

P_0 N2 ♂ × *st136/st136* ♀
F_1 +/*st136* ♀
F_2 *st136/st136* ♀

After one and two cycles of this scheme, two of two lines established yielded revertants in 10^5 animals. After a third cycle only one of two lines yielded revertants in 10^5 animals, indicating a significant decline in mutator activity as at least one revertant would be expected in 10^5 animals if the frequency were 10^{-3} ($P > 0.999$). After a fourth cycle no revertants were recovered in eight lines after expansion to 10^5 animals. Similar crosses were done using *st137* from strain RW7008, and after five cycles no revertants were found in 3×10^5 animals.

Further cross, selfing cycles were repeated for both *st136* and *st137* for a total of eight cycles, and single *st136* and *st137* homozygotes were picked to establish the strains RW7012 (containing *st136*) and RW7018 (containing *st137*). After this many mating cycles the contribution of the Bergerac genome to chromosomes unlinked to *unc-22* is expected to be one in 526 for RW7012 and less than this for RW7018. (The difference results from the fact that *st137* was derived in a mixed Bergerac/Bristol background.) A region of less than 10 map units to either side of *unc-22* is expected to be Bergerac in origin in RW7012 ($P > 0.95$).

We have examined strains RW7012 and RW7018 in order to obtain an accurate estimate of the reversion frequency of *st136* and *st137* in a predominately Bristol genetic background. For *st136*, we have not found any revertants after screening more than 2×10^7 animals (Table 2). We have isolated spontaneous revertants of *st137* in RW7018 at a frequency of about 1.5×10^{-7} (Table 2). These reversion frequencies are significantly lower than reversion frequencies observed for *st136* in RW7002, a Bergerac strain, or for *st137* in RW7008, a Bristol/Bergerac hybrid strain (Table 2).

To ensure that this reduced reversion frequency resulted from the expected reduction in the Bergerac genome and was not due to an inadvertantly induced change at the *unc-22* site in these new strains we crossed *st136*/+ ♂ × BO ♀ and *st137*/+ ♂ × BO ♀ to reintroduce Bergerac mutator activity, and reisolated the *st136* or *st137* homozygotes from the hybrid. This was done with the *st136*

strains after four and five of the cycles and with *st137* after six cycles. We derived 13 *st136* lines from *st136* outcrossed to N2 through four cycles which were then backcrossed to BO. Of these 13, 12 lines reverted, with revertants recovered after screening from 10^3 to 10^5 animals per line. Of nine *st136* lines from the five cycles of crosses with N2 and the BO backcross, eight reverted. Again, revertants were recovered after screening as few as 10^3 to as many as 10^5 animals. The 14 lines established from *st137* backcrossed into BO all reverted, again in 10^3 to 10^5 animals. Similar experiments done with *st136* and *st137* recovered from RW7012 and RW7018 show that these alleles retain their ability to revert in the presence of the Bergerac genome.

Maternal influence on mutator activity: The crosses done to construct the Bristol/Bergerac hybrids described before employed Bristol males and Bergerac or Bergerac/Bristol hybrid hermaphrodites as Bergerac males are of low fertility. This has meant that the oocyte cytoplasm has always been of Bergerac or hybrid origin. Cytoplasmic factors as well as nuclear factors can be important in determining mutator activity as in hybrid dysgenesis of *D. melanogaster* (ENGELS 1979a). To determine whether the Bristol cytoplasm contains factors that might inhibit mutator activity, we used the alternative mating protocol:

P_0 N2 ♂ × *st136/st136* ♀ (RW7002)
F_1 *st136*/+ ♂ × N2 ♀
F_2 *st136*/+ ♀
F_3 *st136/st136* ♀ Bristol/Bergerac hybrid.

This mating protocol introduces the hybrid genome into Bristol oocytes. If Bristol cytoplasm contains factors capable of inhibiting the mutator, then a single passage through this mating cycle might stabilize the *unc-22* mutation. In contrast we found that *st136* remained unstable in lines derived after even two and three cycles of the mating series. For example, after two cycles two of two lines established yielded revertants in 10^5 animals. These results appear to rule out a strong inhibitory activity of Bristol cytoplasm compared to Bergerac but does not rule out the possibility that mutator activity might be increased by passage through Bristol cytoplasm.

Intragenic location of st136 *and* st137: Because of the unusual specificity of the mutator for *unc-22*, it seemed plausible that a single site within the *unc-22* sequence presented a preferred target. The stability of *st136* and *st137* in the Bristol background made intragenic mapping feasible to determine whether these mutations are located at or near the same site. Table 4 shows the data from our mapping experiments. The *st136* and *st137* mutations are left of *s12*, the most distal allele of the *unc-22* gene, and both are left of *s14*. They are also both right of *s7*, and *st136* at least is right of *sDf19*, a small rearrangement which has a break point within the *unc-22* gene (ROGALSKI 1983). This places the two spontaneous mutations in the central region of the gene (see Figure 1).

To test whether *st136* and *st137* are at the same site in the central region we set up a heteroallelic strain of genotype *daf-14 unc-22(st136) dpy-4/+ unc-22(st137)* + and, after several generations, examined the plates for wild-type animals. We reasoned that, if these two mutations were at the same site, they

TABLE 4

Intragenic recombination data for st136 *and* st137

Alleles tested[a]	Total exceptions[b]	Plates screened[c]	Genotype of exceptional chromosome[d] *unc-43 dpy-4*[e] / *unc-43 unc-22*	*unc-43 dpy-4* / *daf-14 unc-22 dpy-4*	*daf-14* / *unc-43 unc-22*	Left/right
st136, s7	14	20	0	1	8	*s7-st136*
st136, s14	20	20	0	11	0	*st136-s14*
st136, s12	16	18	0	11	0	*st136-s12*
st136, sDf19[f]	7	17	ND	ND	ND	*sDf19-st136*
st137, s7	17	19	0	0	13	*s7-st137*
st137, s14	14[g]	14	0	9	0	*st137-s14*
st137, s12	18	19	0	9	0	*st137-s12*
st136, st137[h]	8	17	–	–	–	ND

ND = Not done.

[a] Allele at left was *in cis* with *daf-14* and *dpy-4*, whereas allele at right was *in cis* with *unc-43*.

[b] Only one exceptional event was scored for any single plate.

[c] Petri plates, 100 mm, capable of supporting 5×10^4 to 1×10^5 progeny.

[d] Only those events that were recovered over a normal parental chromosome are included. Therefore, the total of these columns may be less than the total exceptions obtained. We found no evidence of increased gene conversion nor of spontaneous excision of *st136* or *st137*.

[e] No exceptionals of the genotype *e408 e1166/e408 unc-22*(y) were isolated. We expected these animals to occur with the same frequency as Dpy recombinants. Worms of the former genotype move slowly, whereas Dpy recombinants move well. Perhaps because movement is an important cue in this recombinational analysis, we simply overlooked the slow individuals.

[f] *sDf19* is likely to be a small deficiency ending in *unc-22* and extending to the left (ROGALSKI 1983). We did not use flanking markers for *st136* but infer the left/right position from the fact that *st136/sDf19* recombined.

[g] One unusual Dpy "recombinant" from each of these heteroalleles was obtained. In each instance the exceptional animal isolated had a Dpy phenotype, but in the F_1 these animals produced Dumpy and larval lethal progeny. Other than the possibility that these exceptionals may have undergone a chromosomal rearrangement, or acquired some other mutation, we have no explanation for this observation.

[h] In this cross the genotype of parental worm was *daf-14 unc-22(st136) dpy-4/unc-22(st137)*. Of eight exceptionals one was +/+; three were +/*unc-22*; one was +++/*daf-14 unc-22 dpy-4*; one was *dpy-4/unc-22 dpy-4*; one was *unc-22/daf-14 dpy-4*; and one was ++/*unc-22 dpy-4*.

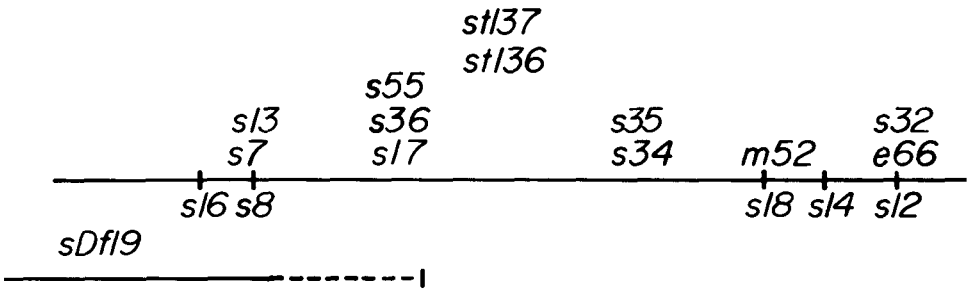

FIGURE 1.—Intragenic map of the *unc-22* gene (modified from MOERMAN and BAILLIE 1979; MOERMAN 1980; ROGALSKI 1983). Mutations shown below the line are all positioned left/right of each other. Mutations shown above *s8*, *s18* and *s12* were not separable from these alleles. The cluster of mutations in the central region have only been placed right of *s7* or *s8* and left of *s18* or *s14*.

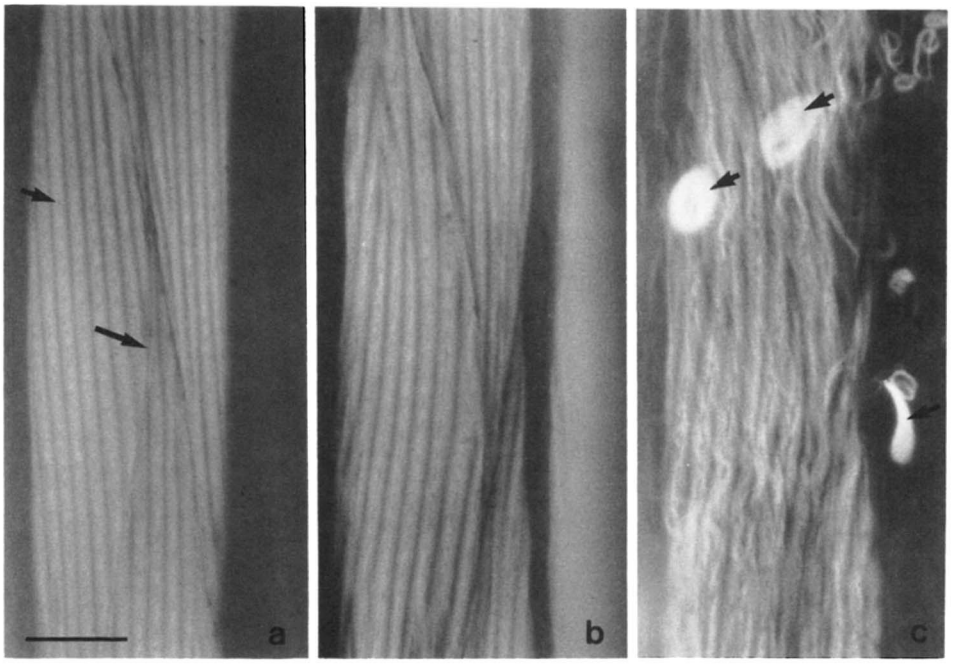

FIGURE 2.—Phalloidin-rhodamine-stained adult body wall muscle cells. a, N2 (wild type). Small arrow indicates a dense body, the nematode analog of the Z-line in vertebrate muscle. Large arrow indicates the boundary between two muscle cells. b, *unc-22(st136)* in RW7012. c, *unc-22(s32)*. Arrows point to aggregates of actin filaments. Bar = 10 μm. ×1435.

could neither recombine nor undergo gene conversion, and, therefore, any wild-type animals would be exceedingly rare [reversion in these stabilized strains occurs at a frequency of $<10^{-6}$ (Table 2)]. Nonetheless, nontwitching animals occurred at a frequency of approximately 5×10^{-6}. Of eight exceptions isolated, none had an array of markers that would allow us to unequivocally left/right position *st136* and *st137* (see legend to Table 4). Unfortunately, because there were no flanking markers on the *st137* chromosome, we could not distinguish whether the observed events resulted from recombination, gene conversion or reversion. However, the observed frequency of wild types was ten times the spontaneous reversion frequency of either parent. Therefore, it seems unlikely that *st136* and *st137* are at identical sites.

A comparison of the phenotypes of st136, st137 *and* s32: Although all EMS-induced alleles of *unc-22* show a prominent twitch, they vary considerably in their effects on muscle organization. Some alleles, like *e105*, have near-normal muscle structure, whereas others, like *s12*, have muscle that is mildly disorganized and still others, like the amber allele, *s32*, have severely disorganized muscle (WATERSTON, THOMSON and BRENNER 1980; MOERMAN 1980; also our unpublished results). To allow us to examine the structural integrity of the myofilament lattice, N2, *st136*, and *s32* animals were stained with the fungal toxin phalloidin labeled with rhodamine. This toxin binds specifically to F-actin (BARAK *et al.* 1980) and when labeled with a fluorophor it allows thin filaments in muscle to be visualized using fluorescence microscopy (Figure 2).

In wild type, F-actin is localized in discrete, sharply defined bands within the myofilament lattice near the hypodermis (Figure 2a). In *s32* muscle (Figure 2c) the thin filaments are irregularly arrayed and much of the actin has aggregated into clumps outside of the sarcomere. Thin filaments can also be seen at abnormal positions throughout the cell. In contrast, in *st136* muscle cells (Figure 2b) actin appears to be localized primarily to the sarcomere. There is some disorganization in the thin filaments, particularly at the ends of a cell, but overall the sarcomere organization in *st136* resembles an N2 muscle cell more closely than does *s32*. The body wall muscle in *st137* animals is structurally similar to that described for *st136* animals. This suggests that *st136* and *st137* do not result in null levels of *unc-22* gene product.

DISCUSSION

This paper describes a mutator system that acts in the Bergerac BO strain of *C. elegans* on the *unc-22* locus. In *C. elegans* var. Bristol spontaneous *unc-22* mutations occur at a frequency of 7×10^{-7} (R. P. ANDERSON, personal communication) and *unc-22* mutations, in general, are stable, reverting at a frequency of $<10^{-6}$. The Bergerac BO strain, however, yields spontaneous *unc-22* mutations at a frequency of 10^{-4} and these mutations are generally unstable, reverting to a wild-type phenotype at frequencies between 2×10^{-3} and 2×10^{-4}. The spontaneous *unc-22* mutations in the BO strain are phenotypically similar to previously described *unc-22* alleles (BRENNER 1974; MOERMAN and BAILLIE 1979; MOERMAN 1980; WATERSTON, THOMSON and BRENNER 1980). A sample of these new mutations when tested were found to map on LG IV near *unc-22* (D. G. MOERMAN and R. H. WATERSTON, unpublished data) and failed to complement the mild *unc-22* allele, *s12*, and an *unc-22* amber allele, *s32*.

A remarkable feature of this mutator system is its relative gene and strain specificity. In this study we found no other genes in the Bergerac BO strain that served as mutational targets at frequencies similar to those seen with *unc-22*. We did find rare spontaneous Dumpy, Blister or Roller mutants, but these mutations are comparatively stable (no revertants in 10^5 to 10^6 animals tested for *Dpy* and *Rol* mutants; the Blister mutant was not tested). Other workers, in particular, the laboratory of J. L. BRUN, have isolated several spontaneous Dumpy mutants from the Bergerac strain of *C. elegans* (see for example, DION and BRUN 1971). More recently, S. EMMONS and his coworkers have described the isolation of Dumpy and Uncoordinated mutants from the Bergerac BO strain after heat shock (S. W. EMMONS, personal communication). To our knowledge all of the mutations described by these two groups have not been observed to revert at frequencies comparable to that observed for *unc-22* in this study. Nonetheless, these mutations or some fraction of them might result from the same mutator activity we observe affecting *unc-22* at high frequency.

We examined nine strains of *C. elegans* and two strains of *C. briggsae* and found only the Bergerac BO strain produced spontaneous *unc-22* mutations at frequencies of 10^{-4}. The BL1 strain, which is of Bergerac origin, did produce spontaneous twitcher mutations and a revertant event (Table 1), but the frequency of 10^{-6} is well below that observed for the BO strain.

Our attempts to define a single site responsible for the mutator activity in the BO strain did not reveal a single site linked to one of the markers used. It remains possible, however, that linkage of a single mutator site to one of the markers might have been missed, since the difficulty of the assay of the mutator activity has limited our sample size in the mapping experiments. The mapping experiments would also seem to rule out multiple, independent mutators, any one of which could produce *unc-22* mutations at frequencies of 10^{-4}. Even as few as two such sites would yield fewer events of the null class than we observed.

An alternative explanation for the BO mutator consistent with our mapping results, and also with the experiments sequentially eliminating mutant *unc-22* alleles, is that the inducer is polygenic, at scattered sites, and the effects of the multiple mutators are additive. The strains established in the mapping studies would thus contain a reduced number of mutator sites, on average leading to reduced mutation rate. From the number of plates (10^5/plate) failing to yield mutants, we could estimate using the Poisson distribution that the mutation rate in these hybrids is about 1.2×10^{-5}. However, the fluctuation in the number of mutator elements (or Bergerac genome) between lines established complicates the interpretation of the null class.

The hypothesis that the mutator is polygenic and at scattered sites could also explain the necessity for multiple crosses with the Bristol strain to stabilize spontaneous BO *unc-22* alleles, of course assuming the same mutator activity is responsible for induction and reversion events. Similarly, reintroduction of Bergerac genome into strains with stabilized *unc-22* mutations yields high reversion frequencies in almost 100% of the lines established from F_2 animals. This sensitivity to strain background of both induction and reversion suggests it is the number of copies of a polygenic mutator that controls activity.

Mutator systems in other organisms involve a variety of different mechanisms. The *hi* and *mu* loci of *D. melanogaster* (cited by GREEN 1976) confer high general mutation rates, but the relative specificity of the Bergerac mutator for *unc-22* as a target rules out a general mutator in the present case. In other cases, specific loci have been found to be mutable. The W^{+u} allele mutates frequently to recessive *w* alleles and is sensitive to genetic background (GETHMANN 1971). This phenomenon is allele specific, and derived mutants are stable. The direct replacement of the Bergerac *unc-22* gene with its Bristol homolog does not prevent the spontaneous occurrence of *unc-22* mutations, and virtually all of the spontaneous *unc-22* alleles are unstable. The Bergerac mutator system is, therefore, not allele specific. Mutation to more severe phenotypes and reversion to wild type occur at high frequencies in Bar strains of *D. melanogaster* due to unequal crossing over (STURTEVANT 1925; BRIDGES 1936); however, the mutation and reversion frequencies are not dramatically senstive to strain background and a graded series of alleles are recovered. Furthermore, the Bar phenotype is known to involve duplication of an entire gene; the *unc-22* mutations observed here are intragenic, probably hypomorphic, and the frequency of generation and the stability of these mutations are sensitive to genetic background. A fourth possibility is that a flip/flop inversion mechanism similar to that of phase variation in Salmonella (ZIEG *et*

al. 1977) may be controlling the expression of the *unc-22* locus. If the *unc-22* gene is *on* in one direction and the BO strain turns *unc-22 off* by inverting a piece of DNA in, or adjacent to the gene, then the *off* state, exhibited by *st136* and *st137*, should have DNA in the same orientation. A prediction from this model is that *st136* and *st137* should fail to recombine, but we find that they are probably separable intragenic sites. This observation makes a simple flip/flop inversion model as an explanation for our results unlikely.

Transposable elements have been described in maize (McCLINTOCK 1951), bacteria (BUKHARI, SHAPIRO and ADHYA 1977 and references therein), the yeast Saccharomyces (CAMERON, LOH AND DAVIS 1979; ROEDER *et al* 1980) and *D. melanogaster* (GREEN 1967, 1977; ENGELS 1979b; see reviews by GREEN 1980 and ENGELS 1983). The Bergerac mutator system is similar to aspects of these classic systems; it is likely to be polygenic and dispersed; it is active in only one naturally occurring genetic background; it shows site preference, and the mutant alleles are highly unstable.

The preference we observe for a specific locus in this system has analogies in other transposable element systems. In prokaryotes, IS*4* has an almost exclusive preference for the *galT* gene and transposon Tn*7* has a particular "attachment site" in the *E. coli* chromosome (PFEIFER, HUBERMANN and KUBAI-MARONI 1977; LICHTENSTEIN and BRENNER 1982). In lambda, IS*2*-kan insertions lie at a single site (SAINT-GIRONS *et al.* 1981). Among eukaryote transposition systems a particularly intriguing analogy can be drawn between the effects of the Bergerac mutator system on the *unc-22* gene and the *P-M* hybrid dysgenesis effects on the singed (*sn*) bristle locus of *D. melanogaster*. The singed locus with a forward mutation frequency of 6×10^{-4} in *P-M* crosses of strain MR-h12 (GREEN 1977; BREGLIANO and KIDWELL 1983) and 1×10^{-2} in *P-M* crosses of π_2 (ENGELS 1979b) is the most active site for *P* insertion in the *P-M* system. Like spontaneous *unc-22* mutations in Bergerac, *P*-induced singed alleles are unstable, with typical excision frequencies in the range of 10^{-2} to 10^{-3} (GREEN 1977; ENGELS 1979b). A further similarity between the two loci is that, just as with spontaneous *unc-22* mutations, stability of singed mutations is controlled by the genetic background (ENGELS 1979a).

Our intragenic mapping studies suggest that there is not an absolute site specificity within the *unc-22* gene. Studies in *E. coli* demonstrate that Tn*10* and Tn*5* have site preferences within a target region (KLECKNER *et al.* 1979; MILLER, CALOS and GALAS 1980; BERG, SCHMANDT and LOWE 1983). This is at least partially related to AT content of the target site (MILLER, CALOS and GALAS 1980). In Drosophila, O'HARE and RUBIN (1983) have found that three of four sequenced *P* element-induced mutations in the white locus are at the same site. These are all in a GC-rich coding region of the gene. However, at the Notch locus in Drosophila KIDD, LOCKETT and YOUNG (1983) found seven transposable element events, all of which are at different locations in the complex. The four dominant Notch mutations are each in a different exon, whereas the three recessive mutations, all due to *copia*-like insertions, are at different locations in the large intron (KIDD, LOCKETT and YOUNG 1983). This latter result offers a possible explanation for our observation that the muscle structure in *st136* and *st137* animals is not as severely disorganized as it is in

the *s32* null animals. An insertional event into an intron, like that of the *copia*-induced Notch allele facet-glossy (fa^g) may produce mild alleles rather than null alleles.

Reversion of the *unc-22* alleles to a wild-type phenotype suggests that the putative excision events may be quite precise. In *E. coli*, both transposons Tn*10* and Tn*5* can undergo precise excision but this is rare and independent of transposase functions (FOSTER *et al.* 1981; EGNER and BERG 1981). The mechanism involved is thought to be similar to spontaneous deletion formation (ALBERTINI *et al.* 1982). In Drosophila the eye color mutant white-crimson (w^c), an allele of the white locus, mutates to white-ivory (w^i) at a frequency greater than 1×10^{-3} (GREEN 1967). Recently, COLLINS and RUBIN (1982) demonstrated that w^c is the result of an insertion of a 10-kb foldback (FB) transposable element, and by sequencing two revertants of w^c they determined that FB can excise precisely (COLLINS and RUBIN 1983). Whether FB excises using a transposase or via a mechanism similar to spontaneous deletion formation is unknown, but it is independent of strain background (GREEN 1967; and cited in COLLINS and RUBIN 1983).

The *P* elements in Drosophila can excise precisely, or imprecisely (RUBIN, KIDWELL and BINGHAM 1982; O'HARE and RUBIN 1983; VOELKER *et al.* 1984; ENGELS 1983), and do require element-encoded functions to excise (SPRADLING and RUBIN 1982; RUBIN and SPRADLING 1982; ENGELS 1983). The stability of spontaneous *unc-22* mutations is sensitive to genetic background, just as *P* element stability is, and, therefore, *unc-22* reversion may also require element-encoded functions. Whether a reversion event represents a precise or imprecise event may partially depend on the DNA context of the element, that is, whether it lies in an exon or intron. At the white locus in Drosophila only precise excision events were recorded among wild-type revertants, but the element studied is in an exon (O'HARE and RUBIN 1983). At the RNA polymerase II locus (*RpII*), where *P* is believed to be in an intron, imprecise events were observed (VOELKER *et al.* 1984). Until the molecular location of *st136* and *st137* within the *unc-22* gene is known we cannot say whether excision accompanying reversion is precise or imprecise.

The only transposable element sequence identified in the nematode to date is *Tc1* (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983; ROSENZWEIG, LIAO and HIRSH 1983) which is present in about 30 copies in the Bristol (N2) strain and several hundred copies in the Bergerac (BO) strain (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983). The element is 1.6 kb in length and contains a 54-base pair perfect inverted repeat at its ends and a large open reading frame (ROSENZWEIG, LIAO and HIRSH 1983). There is evidence for somatic excision (EMMONS *et al.* 1983; EMMONS and YESNER 1984), and recent experiments document germ line transposition of *Tc1* (D. G. MOERMAN, G. M. BENIAN and R. H. WATERSTON, unpublished results). Somatic excision of *Tc1* from *unc-22* during development offers an alternative explanation to the intron hypothesis for the weaker phenotypes of the unstable *unc-22* alleles.

Among the strains we examined for their ability to generate spontaneous *unc-22* mutations, Bergerac BO, Bergerac FR, BL1 and DH424 are high-copy number strains for *Tc1*, and N2, GA-5, PA-1 and PA-2 are low-copy number

strains for this element (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983). The Hawaiian strain, HA-8, has not been examined for *Tc1*. We thought it possible that, if *Tc1* was part of this mutator system, we might see a clear correlation between a strain's *Tc1* copy number and its ability to generate spontaneous *unc-22* mutations. We did not find a correlation but, instead, found the BO strain to be unique in its ability to generate spontaneous *unc-22* mutations at frequencies of 10^{-4}.

Quantitative differences may exist even among the high-copy number strains since the number of copies in the so-called "high-copy number strains" has not been precisely determined and the relationship between copy number and activity is unknown. There may also be qualitative differences in the *Tc1* elements of BO *vs.* the other strains we examined. In maize "dissociator" activity is controlled by "activator" elements (MCCLINTOCK 1947, 1948). In maize, these appear to be present in only one or at most a few copies per genome, but strains with multiple copies have been constructed. Copy number of *Ac* apears to influence the time and frequency of *Ds* transposition (MCCLINTOCK 1951). In *P-M* hybrid dysgenesis of Drosophila (ENGELS 1983; O'HARE and RUBIN 1983) both active *P* factors and related inactive *P* elements are recovered. Our results with hybrids between Bergerac and Bristol suggest only that frequencies of spontaneous mutation and reversion are sensitive to multiple sites. We do not know yet whether more than one element type is involved or whether different states of a single element are involved.

No unique molelcular probe for the *unc-22* gene is available so we cannot as yet examine the gene directly to determine whether spontaneous mutations are insertional events of *Tc1* or some other element. The strains we have developed in this study offer two avenues of approach toward recovering *unc-22*-specific sequences. *Tc1* elements closely linked to *unc-22*, present in Bergerac but absent in Bristol, are present in the backcrossed strain RW7012. (D. G. MOERMAN, G. M. BENIAN and R. H. WATERSTON, unpublished results). We could map these closely linked sites and possibly identify a polymorphism within 1 map unit (approximately 200 kb of DNA) of *unc-22*. We could then use chromosome "walking" techniques (BENDER, SPIERER and HOGNESS 1983) to reach the *unc-22* site. Alternatively, if *Tc1* is the cause of these spontaneous *unc-22* mutations we may identify a polymorphism at the *unc-22* site that correlates specifically with the mutated state of the locus. This variation on the transposon-tagging gene retrieval strategy described by BINGHAM, LEVIS and RUBIN (1981) for Drosophila would allow us to recover *unc-22* unique sequences. Once such sequences are available we could explore the molecular basis of the spontaneous *unc-22* mutations.

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