THE GENE STRUCTURES OF SPONTANEOUS MUTATIONS AFFECTING A *CAENORHABDITIS ELEGANS* MYOSIN HEAVY CHAIN GENE

DAVID EIDE AND PHILIP ANDERSON

Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT

We have isolated spontaneous mutations affecting the *unc-54* major myosin heavy chain gene of *Caenorhabditis elegans* (variety Bristol). Spontaneous *unc-54* mutants occur in *C. elegans* populations at a frequency of approximately 3 $\times$ 10^{-7}. We have studied the gene structure of 65 independent *unc-54* mutations using filter-transfer hybridization techniques. Most *unc-54* mutations (50 of 65) exhibit no abnormalities detected with these techniques; these mutations are small lesions affecting less than 100 base pairs. Approximately 17% of the mutations (11 of 65) are simple deletions, ranging in size from less than 100 base pairs to greater than 17 kilobases. One isolate contains two separate deletions, each of which affects *unc-54*. Two mutants contain tandem genetic duplications that include a portion of *unc-54* and extend 8–10 kilobases beyond the 5' terminus of the mRNA. Conspicuously absent from our collection of spontaneous *unc-54* mutations are any resulting from insertion of transposable genetic elements. Such mutants, if they occur, must arise at a frequency of less than 5 $\times$ 10^{-9}.

T RANSPOSABLE genetic elements are responsible for a variety of genetic phenomena in many organisms. An understanding of their biological significance, however, is only beginning to emerge. Spontaneous mutations (those that arise naturally in the absence of any known mutagenic treatment) are perhaps the clearest examples of genetic events caused by transposons. Transposon insertion is often the most frequent type of spontaneous mutation. Mutants of this type were first described by McCLINTOCK (1949; reviewed by FEDEROFF 1983) for maize and have since been described in bacteria, yeast and Drosophila (reviewed by KLECKNER 1979; ROEDER and FINK 1983; RUBIN 1983). Retroviral proviruses are related to transposable elements (reviewed by VARMUS 1983) and cause similar mutations in mammalian cells (JENKINS *et al.* 1981; COPELAND, HUTCHINSON and JENKINS 1983; SCHNIEKE, HARBERS and JAENISCH 1983; KUFF *et al.* 1983).

In this report we describe the structures of spontaneous mutations in the nematode *Caenorhabditis elegans.* The *C. elegans* genome contains multiple copies of a dispersed repeated sequence, designated Tc*1*, that is almost certain to be a transposable element (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH

1983; EMMONS and YESNER 1984). We would like to measure the contribution of Tc*1* (and any other transposable elements) to spontaneous mutagenesis in *C. elegans*. E. PARK and H. R. HORVITZ (personal communication) have recently described genetic techniques that select directly for spontaneous mutations affecting the *unc-54* myosin heavy chain gene. *unc-54* encodes one of two myosin heavy chain isozymes expressed in body wall muscle cells (EPSTEIN, WATERSTON and BRENNER 1974; MACKENZIE, SCHACHAT and EPSTEIN 1978). We have used these techniques to isolate 65 independent *unc-54* mutants, and we present here an analysis of their gene structures.

MATERIALS AND METHODS

Genetic techniques: All *C. elegans* strains in this study were derived from the wild-type Bristol (N2) stock. The conditions for culturing *C. elegans* have been described (BRENNER 1974; SULSTON and BRENNER 1974). The strain *unc-105(n490)* was obtained from E. PARK. To measure spontaneous mutation frequencies and to isolate independent *unc-54* mutants, cultures of *unc-105(n490)* were grown from small inocula and screened periodically for spontaneous revertants having improved motility. Cultures were screened until each of them contained 1.9×10^5 animals (one 10-cm Petri dish), at which time they were discarded. Multiple revertants appearing in any single culture were assumed to represent the same mutational event; only one isolate was retained. Only revertant homozygotes can be detected in these experiments. Mutation frequencies were estimated by Poisson analysis of the distribution of each revertant class among total independent cultures. The proportion of total cultures not containing a given revertant class (P_0 class) was used to calculate the frequencies. Each estimate is based on 626 independent cultures; P_0 for *unc-54*, *unc-105* and *unc-22* was measured to be 0.946 (=592 of 626), 0.932 (=584 of 626) and 0.888 (=556 of 626), respectively. These statistical methods estimate for each revertant class the mutation frequency (mutations per animal) that accounts for the observed distribution of cultures that contain and do not contain revertants. This method corrects for multiple mutations within a single culture, is less sensitive to jackpots and is not affected by the differential growth rate of *n490* and its revertants. Of the 65 *unc-54* mutants isolated in this study, 44 were obtained following growth of *unc-105(n490)* at 20°. An additional 21 mutants were isolated among the F_2 progeny of *unc-105(n490)* populations that had been subjected to a heat shock treatment (4–6 hr at 37°). Approximately 50% of the animals survived this treatment. We measure the frequency of *unc-54* mutations following heat shock to be 4×10^{-7} (570 independent cultures; 1×10^5 animals per culture; $P_0 = 0.936$). This frequency is not significantly different from the frequency following continual growth at 20°, and we consider these mutants simply to be additional spontaneous alleles.

Reversion analysis of the tandem duplication alleles *unc-54(r104)* and *unc-54(r109)* was performed following removal of *unc-105(n490)* from our original isolates. This was done by backcrossing the primary isolates with wild-type males; the *unc-54* alleles were then segregated in the absence of *unc-105(n490)*. Cultures of *unc-54(r104)* or *unc-54(r109)* were periodically examined for the appearance of spontaneous wild-type revertants.

DNA isolation and Southern hybridization: Nematode cultures were harvested as described by SULSTON and BRENNER (1974). DNA was extracted essentially as described by MARMUR (1961), following disruption of the animals with Proteinase K. Restriction endonucleases were purchased from New England Biolabs. Filter-transfer hybridizations were performed according to the method of SOUTHERN (1975), with only minor modifications. DNAs were fractionated on 0.8% agarose gels containing a mixture of high- and low-gelling temperature agaroses (2:1 Sigma types V:VII) in 1 × TBE (MANIATIS, FRITSCH and SAMBROOK 1982). Radiolabeled probe DNAs for these experiments were prepared by nick translation (RIGBY *et al.* 1977). Recombinant phages SG23/1 and SG25/3 (see Figure 1) represent 25 kb of genomic DNA that include the *unc-54* gene and extensive flanking sequences (MACLEOD, KARN and BRENNER 1981). Individual restriction fragments were purified from digests of a wild-type *unc-54* clone according to the method of WEISLANDER (1979).

Cloning procedure: The *unc-54* mutant allele *r102* was cloned as follows: DNA isolated from

TABLE 1

Spontaneous mutation frequencies for three C. elegans genes

Gene	Spontaneous mutation frequency
unc-54	3×10^{-7}
unc-22	6×10^{-7}
unc-105	4×10^{-7}

The frequency of each mutant type was estimated by Poisson analysis of the distribution of mutants among a large collection of independent cultures (see MATERIALS AND METHODS).

r102 was partially digested with *MboI*. The digestion products were size fractionated on NaCl gradients (SLIGHTOM, BLECHL and SMITHIES 1980), and 12- to 20-kb fragments were collected. These fragments were ligated to λ1059 DNA that had been digested with *Bam*HI, and the products were packaged into virions as described by HOHN (1979). Hybrid phages carrying *unc-54* sequences were identified by plaque hybridization (BENTON and DAVIS 1977) using a gel-purified *unc-54* wild-type restriction fragment as probe. One phage, designated DE-9, was ultimately retained.

Analysis of DE-9: Heteroduplexes between DE-9 and the wild-type *unc-54* clone SG25/3 were formed by the method of DAVIS, SIMON and DAVIDSON (1971). Spreading and electron microscopy followed INMAN and SCHNOS (1970). Polyacrylamide gel electrophoresis of restriction digests has been described by MANIATIS, JEFFREY and KLEID (1975).

RESULTS

A selection for spontaneous unc-54 *mutants*: Spontaneous mutations are rare events. Their identification requires an efficient method of selection. E. PARK and H. R. HORVITZ (personal communication) have recently described genetic techniques that select directly for mutations affecting several *C. elegans* genes, including the *unc-54* myosin heavy chain gene. This selection is based on properties of the mutant strain *unc-105(n490)*. The properties of *unc-105(n490)*, together with an extensive genetic analysis of the *unc-105* gene, will be presented in detail by E. PARK and H. R. HORVITZ (unpublished data). We summarize here several of their observations important for our work: *unc-105(n490)* animals are extremely paralyzed. This appears to be due, at least in part, to a contractile defect of body wall muscle cells that leads to sustained contraction ("hypercontraction") of these cells. Body wall muscle of *unc-105(n490)* is abnormal. When viewed with polarized light optics, birefringent striations, although quite strong, are diffuse and irregular, having no apparent periodicity. Occasional patches of increased birefringence are found in these cells. Pharyngeal muscle seems unaffected, but pharyngeal pumping is often irregular. *unc-105(n490)* animals are short, attaining less than one-half normal body length. Revertants of *unc-105(n490)* (both full and partial revertants) usually contain mutations in the *unc-54*, *unc-22* or *unc-105* genes that suppress the paralysis caused by *unc-105(n490)*. The intragenic revertants are null alleles of *unc-105*. The selection for revertants is sufficiently strong that spontaneous mutations affecting these genes can be detected.

We have used these genetic techniques to isolate a large collection of spontaneous *unc-54*, *unc-22* and *unc-105* mutants. Table 1 presents the spontaneous

mutation frequency for each of these genes. *unc-54* mutants were of special interest to us, because *unc-54* clones (MACLEOD, KARN and BRENNER 1981) allow us to study the gene structure cf each of these mutants. We have isolated 65 independent *unc-54* mutants. Each isolate has been tested and fails to complement *unc-54(e1092)*, a recessive null allele of *unc-54* (MACLEOD *et al.* 1977). Our methods of isolation ensure that each isolate represents an independent mutational event (see MATERIALS AND METHODS).

The structure of spontaneous unc-54 *mutations*

"*Point*" *mutations*: The wild-type *unc-54* gene extends more than 7.2 kb and is interrupted by eight short introns (KARN, BRENNER and BARNETT 1983). We have analyzed each spontaneous *unc-54* mutation using filter-transfer hybridization techniques (SOUTHERN 1975). The radiolabeled DNA probe for these experiments includes all coding sequences plus 9 kb of material extending from both the 5' and 3' end of *unc-54*. Total genomic DNAs were digested with several different restriction enzymes. The enzymes chosen generate restriction fragments whose sizes allow us to detect all deletions or insertions that affect >100 base pairs. Most spontaneous *unc-54* mutations (50 of 65) exhibit no detectable abnormalities in these experiments (data not shown). These mutations, therefore, are point mutations or other lesions too small for detection. One mutation (*r274*) exhibits a restriction map abnormality, but the data are most consistent with *r274* being a point mutation or other small lesion. *r274* eliminates a single *Bam*HI restriction site (see Figure 1). Data with other enzymes, however, indicate that *r274* is not a detectable deletion (*e.g.*, *Bgl*II; see Figure 1). The position of *r274* relative to the *unc-54* restriction map is diagrammed in Figure 2.

Deletions: Eleven mutations are simple deletions that range in size from less than 100 base pairs to greater than 17 kb. Representative Southern blot experiments for these 11 mutations are included in Figure 1. In each case, one or more *unc-54* restriction fragments are absent and a novel junction fragment is present. Total genomic DNA was digested with several different restriction enzymes, and the fragments affected by the deletion were determined. Figure 2 diagrams the position and size of each deletion on the *unc-54* restriction map.

One-mutant strain [*unc-54(r102)*] contains two separate deletions. One of these, estimated to be 2 kb, extends across the 5' end of *unc-54*; the other, estimated to be 80 base pairs, is located within the *Bgl*II 3.868-kb fragment. A representative Southern blot experiment for *r102* is included in Figure 1, and the map positions of the two deletions are shown in Figure 2.

The structure of *r102* shown in Figure 2 has been confirmed by restriction mapping and heteroduplex analysis of a bacteriophage lambda clone that contains the *r102* mutant allele of *unc-54*. The identification of this clone, designated DE-9, is described in MATERIALS AND METHODS. Figure 3A shows a heteroduplex formed between DE-9 and SG25/3, a clone carrying a wild-type copy of *unc-54*. Figure 3B diagrams the heteroduplex structure pictured in Figure 3A. A single deletion/insertion loop is visible within the sequences
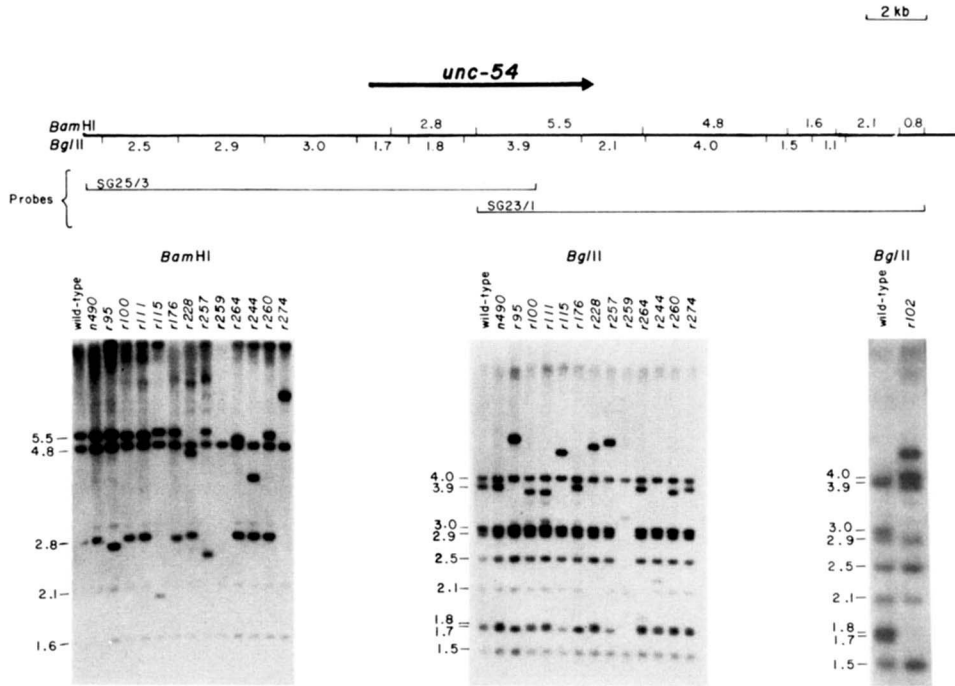
FIGURE 1.—Southern blot analysis of spontaneous *unc-54* mutations. Thirteen mutants exhibiting restriction map abnormalities are included in this survey. Total genomic DNAs were digested with restriction endonucleases and separated on 0.8% agarose gels. Radiolabeled probe DNA was a 1:1 mixture of phages SG25/3 and SG23/1. We have revised slightly the restriction map of MacLeod, Karn and Brenner (1981) to more accurately reflect the sizes of certain unsequenced restriction fragments. The transcribed portion of *unc-54* is indicated above its restriction map.

shared by these phages. This loop corresponds in size and in position to the larger (2 kb) deletion shown in Figure 2. The smaller deletion is not detected with heteroduplex techniques. Its location and size is indicated by analysis of the *Hin*dIII digestion products of DE-9. Figure 4 shows that the *Hin*dIII 294-base pair fragment present in SG25/3 contains an internal deletion of 83 ± 5 base pairs in DE-9. We have detected no other rearrangements in *r102*.

Tandem duplications: Two spontaneous *unc-54* mutations contain tandem chromosomal duplications that have one breakpoint within *unc-54* and extend several kilobases beyond the 5′ end of the gene. This conclusion is based on three lines of evidence:

1. *Southern blot phenotype*: Figure 5 presents total genomic Southern blots of *r104*, *r109* and control strains. When digested with restriction enzymes that cut the *unc-54* region frequently (*e.g.*, *Bam*HI and *Bgl*II), mutants *r104* and *r109* display a pattern of hybridization that includes all wild-type restriction fragments plus one additional band. The novel bands (indicated by arrows in Figure 5) are the fragments formed by juxtaposition of two tandemly repeated units. When *r104* and *r109* are cut with *Sal*I, however, the 21-kb fragment that includes *unc-54* is substantially larger than normal. Both endpoints of the tandem duplications are located within the large *Sal*I fragment.
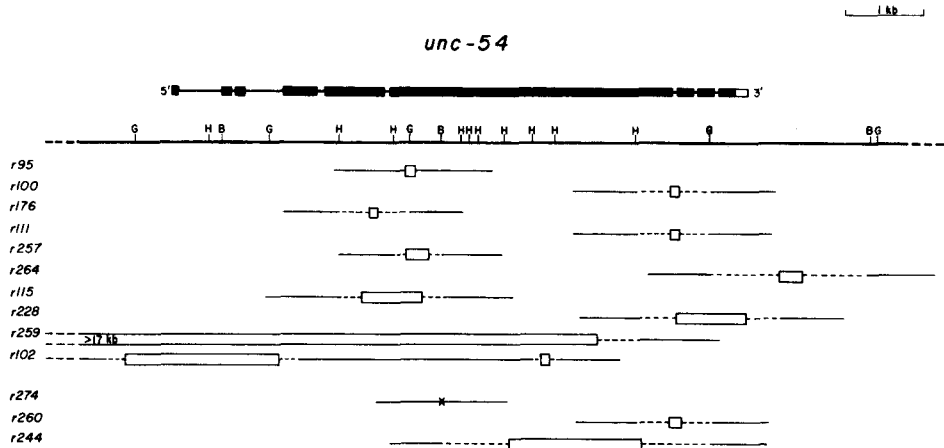
FIGURE 2.—Deletions affecting *unc-54*. The size and position of each deletion are diagrammed. A dotted line indicates the limits of precision for mapping data; each deletion resides somewhere within the dotted region. The length of each deletion box reflects the amount of material deleted. The *unc-54* gene spans more than 7.2 kb. The organization of *unc-54*-coding sequences is indicated above its restriction map. Boxed regions are exons; the untranslated 3′ trailer sequence is unfilled. The position of neither the 5′ nor the 3′ end of *unc-54* mRNA has been precisely defined. The limits of *unc-54* shown here are the AUG translational initiation codon (5′) and the AAUAAA polyadenylation signal (3′). The precise termini are thought to be nearby (KARN, BRENNER and BARNETT 1983). Mutations *r274*, *r260* and *r244* were isolated following heat shock treatment of *unc-105(n490)* (see MATERIALS AND METHODS). *r274* eliminates a single *Bam*HI restriction site. B = *Bam*HI; G = *Bgl*II; H = *Hind*III.

2. *Genetic instability*: *r104* and *r109* revert spontaneously to *unc-54⁺* (wild type) at a high frequency (between 10^{-5} and 10^{-6}; see *Materials and Methods*). Revertant strains exhibit a fully wild-type Southern blot pattern (see Figure 5).

3. *Junction fragment homologies*: We have shown that the novel fragments just described are caused by juxtaposition of sequences that are normally widely separated on the *unc-54* restriction map. Figure 6 shows an example of this analysis. *r104*, *r109* and control DNAs were digested with *Bgl*II, separated by electrophoresis and hybridized with six different probes from the *unc-54* region. The *r104 Bgl*II novel fragment, estimated to be slightly larger than 1.1 kb, hybridizes both to the *Hind*III 870-base pair and to the *Bgl*II 2.9-kb probes. It does not hybridize to other probes from the region. This demonstrates that the *r104* novel fragment contains sequence homology to both of these probes and localizes the *r104* duplication endpoints to these regions of the restriction map. Similarly, the *r109 Bgl*II novel fragment, estimated to be 2.0 kb, hybridizes both to the *Hind*III 706-base pair and to the *Bgl*II 2.5-kb probes. The results of several analyses of this type are summarized in Figure 7. Both *r104* and *r109* contain tandem duplications that include a portion of *unc-54* and extend several kilobases beyond the 5′ end of the gene. We estimate that *r104* and *r109* contain tandem duplications of 8 and 10 kb, respectively.
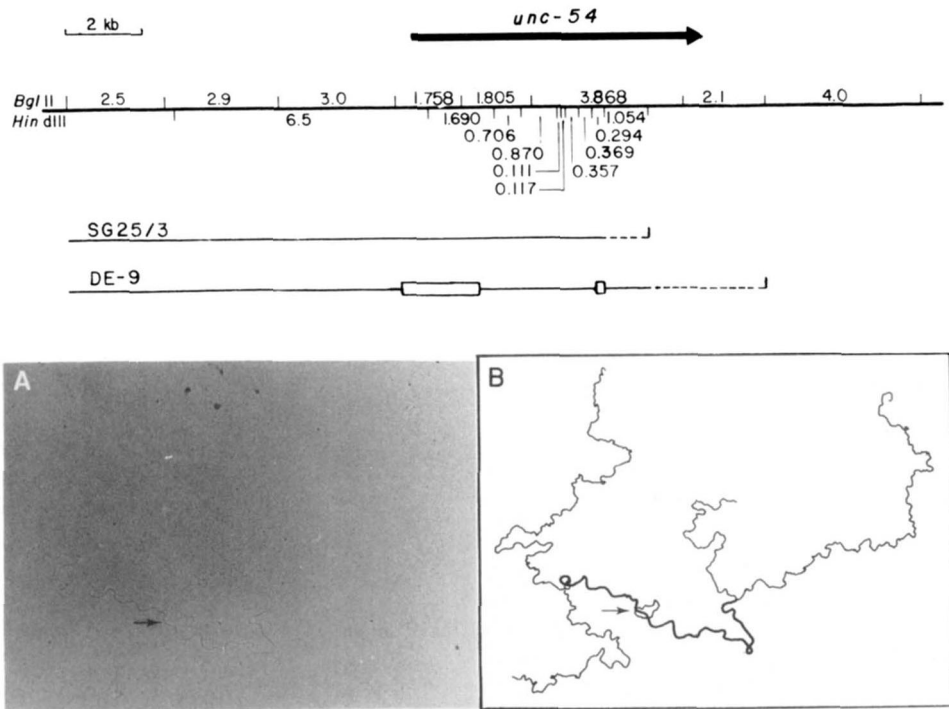
FIGURE 3.—Heteroduplex analysis of *unc-54(r102)*. The recombinant lambda phage DE-9 carries the *r102* mutant allele of *unc-54*. The phage SG25/3 carries a similar region from the wild-type gene. DE-9 and SG25/3 are each derivatives of λ1059, but the inserts are in opposite orientation. The region carried by each phage is diagrammed relative to the *unc-54* restriction map. Dotted lines indicate regions of uncertainty concerning the precise length of an insert. The transcribed portion of *unc-54* is indicated above its restriction map. A, A heteroduplex molecule formed between single strands of DE-9 and SG25/3. B, A diagram of this molecule indicating double-stranded (bold) and single-stranded (light) regions. An arrow indicates the single-stranded deletion loop of *r102*.

DISCUSSION

Our failure to obtain insertions among a collection of 65 independent mutants is remarkable. Spontaneous mutations in other organisms are frequently caused by insertion. Approximately half of spontaneous *gal* mutations and 70% of spontaneous *rps* mutations of *E. coli* are insertions of IS sequences (SAEDLER and STARLINGER 1967; JASKUNAS, LINDAHL and NOMURA 1975). In yeast, 10% of spontaneous *his4* mutations and 2% of spontaneous *lys2* mutations are due to insertion of the transposable element Ty1 (cited in ROEDER and FINK 1983; EIBEL and PHILIPPSEN 1984). In *Drosophila melanogaster*, approximately half of the spontaneous mutations affecting white (*w*), Antennapedia (*Ant*) and rosy (*ry*) and all spontaneous mutations affecting the bithorax complex are due to insertion of transposable elements (ZACHAR and BINGHAM 1982; SCOTT *et al.* 1983; BENDER *et al.* 1983). We have screened 2×10^8 nematodes during the course of these experiments. Insertions have not been detected and, therefore,
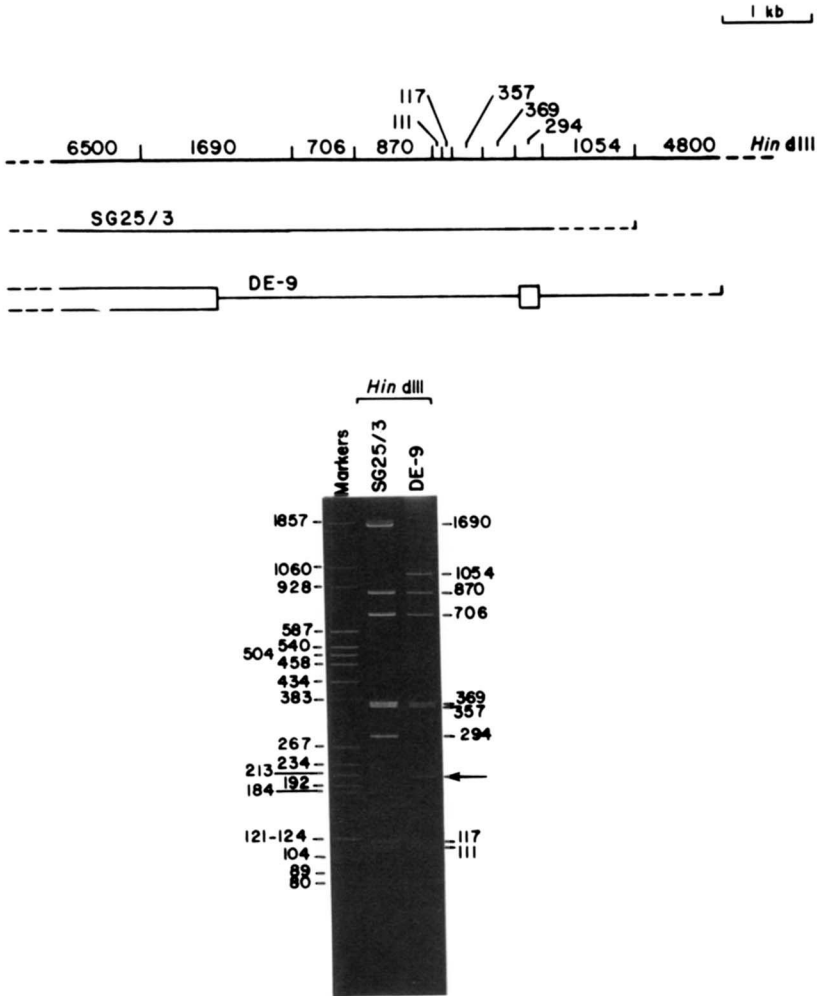
FIGURE 4.—Restriction analysis of *unc-54(r102)*. The *Hin*dIII digestion products of DE-9 and SG25/3 were separated by electrophoresis on a 3.5% polyacrylamide gel. The region carried by each phage is diagrammed relative to the *unc-54 Hin*dIII restriction map. Boxed regions of DE-9 indicate the material deleted by *r102*. Dotted lines indicate regions of uncertainty concerning the precise length of an insert. *r102* contains a small (approximately 80-base pair) deletion within the *Hin*dIII 294-base pair fragment. The deletion-containing fragment is indicated by an arrow.

must arise at a frequency of less than 5×10^{-9}. Included in this analysis are 21 mutants that were isolated following heat shock treatment of nematode populations (*see* MATERIALS AND METHODS).

All mutants described in this study were derived from the Bristol (N2) variety of *C. elegans*. Bristol strains contain in their genome approximately 25 copies of a dispersed repeated sequence, designated Tc*1*, that is almost certain to be a transposon (EMMONS *et al.* 1983; LIAO, ROSENZWEIG and HIRSH 1983). Why does Tc*1* not transpose? Perhaps all copies of Tc*1* in Bristol strains are
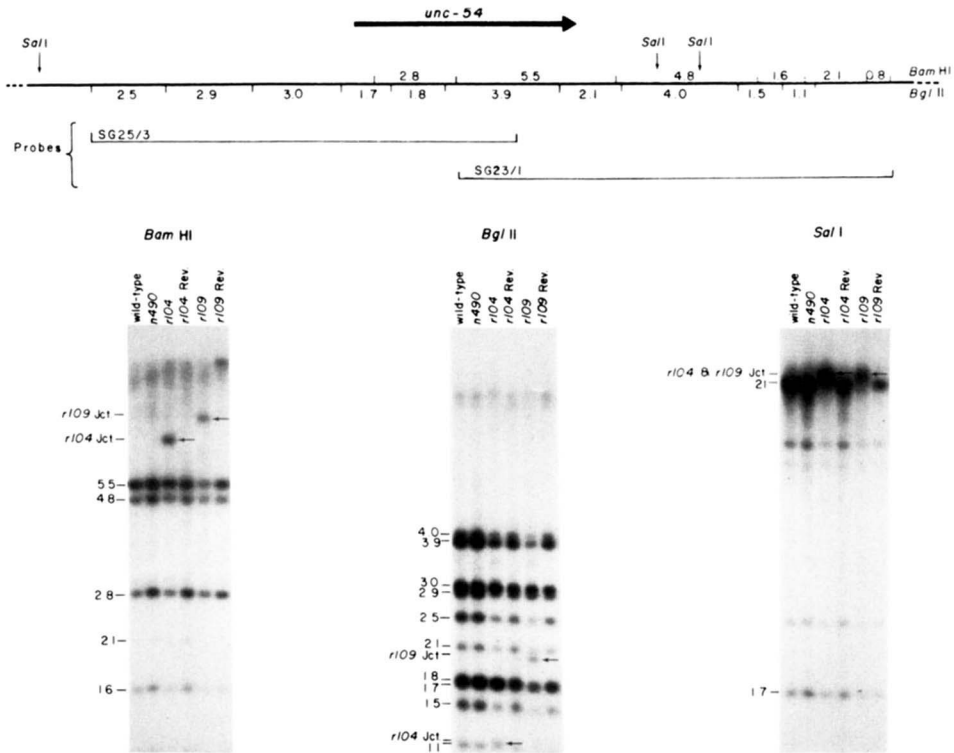
FIGURE 5.—Southern blot analysis of the tandem duplication alleles *r104* and *r109*. Total genomic DNAs were digested with restriction endonucleases and separated on 0.8% agarose gels. Radiolabeled probe DNA was a 1:1 mixture of phages SG25/3 and SG23/1. The novel restriction fragments formed by juxtaposition of two tandemly repeated units are indicated by an arrow. *r104* Rev and *r109* Rev are spontaneous wild-type revertants of *r104* and *r109*, respectively. The transcribed portion of *unc-54* is indicated above its restriction map. Three SalI sites within this region generate fragments of 21 and 1.7 kb.

defective for transposition. A situation similar to this has been described in Drosophila. In many strains, defective *P* factors substantially outnumber intact *P* factors (O'HARE and RUBIN 1983; reviewed by ENGELS 1983). We have recently analyzed a collection of spontaneous *unc-54* mutations isolated in the genetic background of the Bergerac variety of *C. elegans*. Surprisingly, we find that Tc*1* actively transposes in this strain, and most spontaneous *unc-54* mutations are due to Tc*1* insertion.

Two of the *unc-54* mutations described here are noteworthy. They contain tandem genetic duplications that partially include *unc-54* (see Figure 7). These mutants are unexpected because tandem duplications of the type diagrammed in Figure 7 should not cause *unc-54* defects (see, for example, ANDERSON and ROTH 1977). Each of these mutants contains an intact copy of *unc-54* as judged by Southern blots. Why are these structures nonfunctional? Three explanations seem plausible. First, our techniques for analysis of *r104* and *r109* detect only those lesions that affect more than 100 base pairs. Perhaps the *unc-54* gene copy that appears to be wild type by Southern blots actually contains an un-
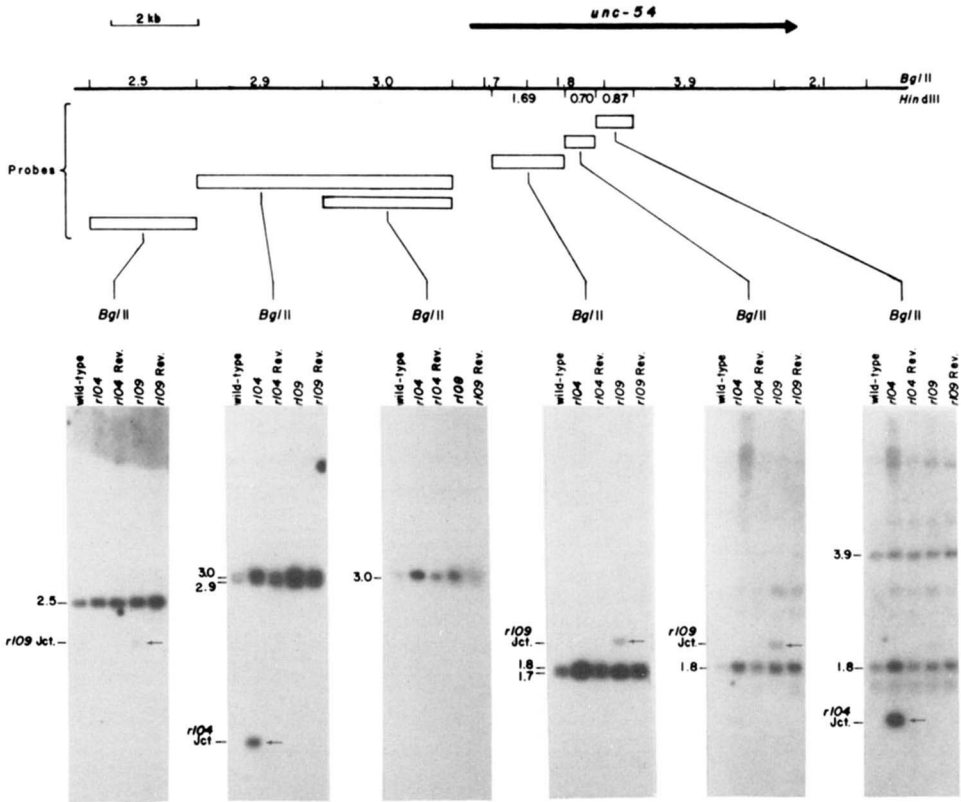
FIGURE 6.—Mapping duplication endpoints in *r104* and *r109*. Total genomic DNAs were digested with *Bgl*II and separated on 0.8% agarose gels. Restriction fragment probes were radiolabeled after being gel purified from the *Bgl*II or *Hind*III digestion products of SG25/3. The novel junction fragments produced by *r104* and *r109* are indicated by arrows. *r104* Rev and *r109* Rev are spontaneous wild-type revertants of *r104* and *r109*. The transcribed portion of *unc-54* is indicated above its restriction map.

detected mutation. If true, then the mutation must reside in the portion of *unc-54* that is duplicated, because both *r104* and *r109* revert to wild type by loss of the duplication. Undetected mutations would have to be excised with the duplication. A second explanation involves stable position effects (STURTEVANT 1925). The arrangement of sequences surrounding *r104* and *r109* are abnormal; sequences are juxtaposed that are not normally contiguous. This configuration might influence the expression of the nearby *unc-54* gene. A third explanation involves transcriptional interference between two closely spaced promoters whose transcripts overlap. Examples of such interference have been encountered in both bacterial and mammalian genes (HAUSLER and SOMERVILLE 1979; ADHYA and GOTTESMAN 1982; HOROWITZ and PLATT 1982; CULLEN, LOMEDICO and JU 1984). Perhaps, use of the "upstream" promoter of *r104* and *r109* precludes use of the "downstream" promoter; only the downstream promoter could express *unc-54*.
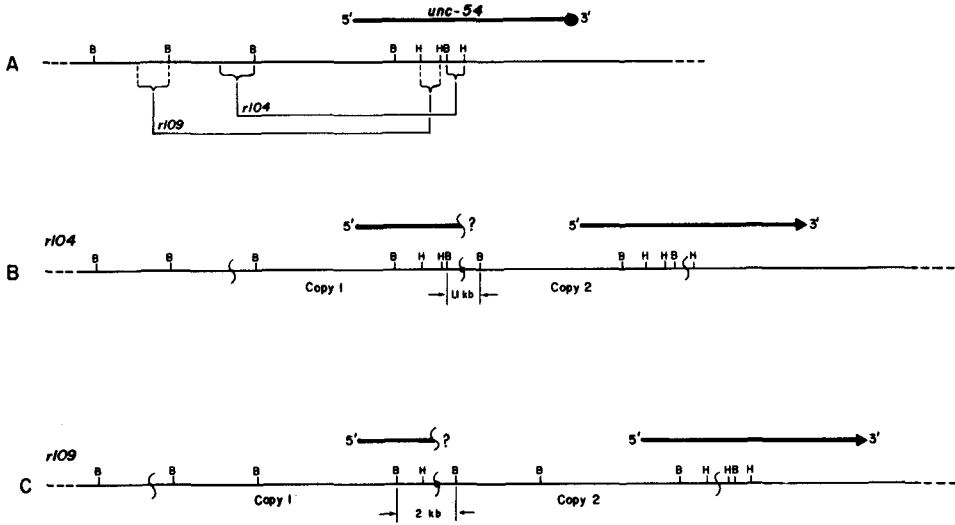
FIGURE 7.—*unc-54* sequences that are tandemly duplicated in *r104* and *r109*. A, An abbreviated restriction map of *unc-54* is diagrammed; only restriction sites near the duplication endpoints are shown. The transcribed portion of *unc-54* is indicated above its restriction map. The regions duplicated by *r104* and *r109* are indicated. B, The structure of *r104*. An 8-kb region extending across the 5' end of *unc-54* is duplicated in tandem. Regions of *unc-54(r104)* that are potentially transcribed are indicated above its restriction map. C, The structure of *r109*. A 10-kb region extending across the 5' end of *unc-54* is duplicated in tandem. Regions of *unc-54(r109)* that are potentially transcribed are indicated above its restriction map. B = *Bgl*II; H = *Hind*III.

LITERATURE CITED

ADHYA, S. and M. GOTTESMAN, 1982 Promoter occlusion: transcription through a promoter may inhibit its activity. Cell **29**: 939–944.

ANDERSON, R. P. and J. R. ROTH, 1977 Tandem genetic duplications in phage and bacteria. Annu. Rev. Microbiol. **31**: 473–505.

BENDER, W., M. AKAM, F. KARCH, P. A. BEACHY, M. PEIFER, P. SPIERER, E. B. LEWIS and D. S. HOGNESS, 1983 Molecular genetics of the *bithorax* complex in *Drosophila melanogaster*. Science **221**: 23–29.

BENTON, W. D. and R. W. DAVIS, 1977 Screening λ*gt* recombinant clones by hybridization to single plaques *in-situ*. Science **196**: 180–182.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77**: 71–94.

COPELAND, N. G., K. W. HUTCHINSON and N. A. JENKINS, 1983 Excision of the DBA ecotropic provirus in dilute coat-color revertants of mice occurs by homologous recombination involving viral LTRs. Cell **33**: 379–387.

CULLEN, B. R., P. T. LOMEDICO and G. JU, 1984 Transcriptional interference in avian retroviruses: implications for the promoter insertion model of leukaemogenesis. Nature **307**: 241–245.

DAVIS, R. W., M. SIMON and N. DAVIDSON, 1971 Electron microscope methods for mapping regions of base sequence homology in nucleic acids. Methods Enzymol. **21:** 413–428.

EIBEL, H. and P. PHILIPPSEN, 1984 Preferential integration of yeast transposable element Ty into a promoter region. Nature **307:** 386–388.

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., L. YESNER, K. RUAN and D. KATZENBERG, 1983 Evidence for a transposon in *Caenorhabditis elegans*. Cell **32:** 55–65.

ENGELS, W. R., 1983 The P family of transposable elements in Drosophila. Annu. Rev. Genet. **17:** 315–344.

EPSTEIN, H. F., R. H. WATERSTON and S. BRENNER, 1974 A mutant affecting the heavy chain of myosin in *Caenorhabditis elegans*. J. Mol. Biol. **90:** 291–300.

FEDEROFF, N. V., 1983 Controlling elements in maize. pp. 1–63. In: *Mobile Genetic Elements*, Edited by J. A. SHAPIRO. Academic Press, New York.

HAUSLER, B. and R. SOMERVILLE, 1979 Interaction *in vivo* between strong closely spaced constitutive promoters. J. Mol. Biol. **127:** 353–356.

HOHN, B., 1979 *In-vitro* packaging of lambda and cosmid DNA. Methods Enzymol. **68:** 299–309.

HOROWITZ, H. and T. PLATT, 1982 Regulation of transcription from tandem and convergent promoters. Nucleic Acids Res. **10:** 5447–5465.

INMAN, R. B. and M. SCHNOS, 1970 Partial denaturation of thymine- and 5-bromouracil-containing DNA in alkali. J. Mol. Biol. **49:** 93–98.

JASKUNAS, S. R., L. LINDAHL and M. NOMURA, 1975 Isolation of polar insertion mutants and the direction of transcription of ribosomal protein genes in *E. coli*. Nature **256:** 183–187.

JENKINS, N. A., N. G. COPELAND, B. A. TAYLOR and B. K. LEE, 1981 Dilute (*d*) coat color mutation of DBA/2J mice is associated with the site of integration of an ecotropic MuLV genome. Nature **293:** 370–374.

KARN, J., S. BRENNER and L. BARNETT, 1983 Protein structural domains in the *Caenorhabditis elegans unc-54* myosin heavy chain gene are not separated by introns. Proc. Natl. Acad. Sci. USA **80:** 4253–4257.

KLECKNER, N., 1979 Transposable elements in prokaryotes. Annu. Rev. Genet. **15:** 341–404.

KUFF, E. L., A. FEENSTRA, K. LUEDERS, L. SMITH, R. HAWLEY, N. HOZUMI and M. SCHULMAN, 1983 Intracisternal A-particle genes as movable elements in the mouse genome. Proc. Natl. Acad. Sci. USA **80:** 1992–1996.

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.

MACKENZIE, J. M., F. SCHACHAT and H. F. EPSTEIN, 1978 Immunocytochemical localization of two myosins within the same muscle cells in *Caenorhabditis elegans*. Cell **15:** 413–419.

MACLEOD, A. R., J. KARN and S. BRENNER, 1981 Molecular analysis of the *unc-54* myosin heavy chain gene of *Caenorhabditis elegans*. Nature **291:** 386–390.

MACLEOD, A. R, R. H. WATERSTON, R. M. FISHPOOL and S. BRENNER, 1977 Identification of the structural gene for a myosin heavy chain in *Caenorhabditis elegans*. J. Mol. Biol. **114:** 133–140.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

MANIATIS, T., A. JEFFREY and D. G. KLEID, 1975 Nucleotide sequence of the rightward operator of phage lambda. Proc. Natl. Acad. Sci USA **72:** 1184–1188.

MARMUR, J., 1961 A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3:** 208–218.

McCLINTOCK, B., 1949 Mutable loci in maize. Carnegie Inst. Wash. Publ. **48:** 142–154.

O'HARE, K. and G. M. RUBIN, 1983 Structures of P transposable elements of *Drosophila melanogaster* and their sites of insertion and excision. Cell **34:** 25–35.

RIGBY, P. W. J., M. DIEKMANN, C. RHODES and P. BERG, 1977 Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J. Mol. Biol. **113:** 237–251.

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.

RUBIN, G. M., 1983 Dispersed repetitive DNAs in *Drosophila*. pp. 329–361. In: *Mobile Genetic Elements*, Edited by J. A. SHAPIRO. Academic Press, New York.

SAEDLER, H. and P. STARLINGER, 1967 0° mutations in the galactose operon in *E. coli*. Mol. Gen. Genet. **100:** 178–189.

SCHNIEKE, A., K. HARBERS and R. JAENISCH, 1983 Embryonic lethal mutation in mice induced by retrovirus insertion into the α-1(I) collagen gene. Nature **304:** 315–320.

SCOTT, M. P., A. J. WEINER, T. I. HAZELRIGG, B. A. POLISKY, V. PIROTTA, F. SCALENGHE and T. C. KAUFMAN, 1983 The molecular organization of the *Antennapedia* locus of Drosophila. Cell *35:* 763–776.

SLIGHTOM, J. L., A. E. BLECHL and O. SMITHIES, 1980 Human fetal G_γ- and A_γ-globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes. Cell **21:** 627–638.

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

STURTEVANT, A. H., 1925 The effects of unequal crossing over at the *Bar* locus in Drosophila. Genetics **10:** 117–147.

SULSTON, J. E. and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans*. Genetics **77:** 95–104.

VARMUS, H. E., 1983 Retroviruses. pp. 411–503. In: *Mobile Genetic Elements*, Edited by J. A. SHAPIRO. Academic Press, New York.

WEISLANDER, L., 1979 A simple method to recover intact high molecular weight RNA and DNA after electrophoretic separation in low gelling temperature agarose gels. Anal. Biochem. **98:** 305–309.

ZACHAR, A. and P. M. BINGHAM, 1982 Regulation of *white* locus expression: the structure of mutant alleles at the *white* locus of *Drosophila melanogaster*. Cell **30:** 529–541.

Communicating editor: R. K. HERMAN