Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5325–5329, August 1987
Genetics

Isolation and characterization of a nematode transposable element from *Panagrellus redivivus*

(*unc-22* gene/mobile DNA element/hybrid dysgenesis)

CHRISTOPHER D. LINK, JULIE GRAF-WHITSEL, AND W. B. WOOD

Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309

Contributed by W. B. Wood, December 31, 1986

ABSTRACT **We have isolated a transposable element, designated PAT-1, from the free-living nematode *Panagrellus redivivus*. *P. redivivus* strain C15 was found to have a high spontaneous mutation frequency compared to the standard *Caenorhabditis elegans* laboratory strain N2. To characterize the genetic lesions occurring in spontaneous C15 mutants, we molecularly cloned the homolog of the *C. elegans unc-22* gene from wild-type *P. redivivus* and two strains carrying spontaneous mutations in this gene. One of these mutations resulted from the insertion of a 4.8-kilobase segment of repetitive DNA. This repetitive element (PAT-1) varies in copy number (10–50 copies) and location in different *P. redivivus* strains and is absent from *C. elegans*. The element could be useful as a transformation vector for *C. elegans*. Our approach is a general one that could be used to isolate additional nematode transposons from other species.**

The nematode *Caenorhabditis elegans* has been intensively studied as a model developmental system, and a few of its developmentally important genes have been molecularly cloned (1, 2). Characterization of these genes has been hampered by the lack of an efficient DNA transformation system. In *Drosophila melanogaster*, P elements have proven to be extremely useful transformation vectors for stable reintroduction of cloned genes (3, 4). Attempts to use Tc*1*, the only demonstrated transposon of *C. elegans* (2, 5–7), as a transformation vector have been unsuccessful (J. Shaw, personal communication). We have, therefore, sought a method for isolating additional nematode transposons, both to learn more about the types of transposons that exist in nematodes and to generate potential transformation vectors for *C. elegans*.

Compared to *D. melanogaster*, *C. elegans* appears to be surprisingly deficient in active transposable elements. Eide and Anderson (8) found that none of 65 spontaneous mutations in the *unc-54* gene of *C. elegans* strain N2 resulted from the insertion of a transposable element. Similarly, none of 28 spontaneous loss-of-function *unc-92* mutations (isolated as revertants of dominant *unc-92* alleles) was found to result from transposon insertion (9). [*unc-92* has since been renamed *act-1,2,3* (10).] In contrast, the majority of the spontaneous mutations examined for the white (11), rosy (12), notch (13), and antennapedia (14) loci of *Drosophila* have resulted from the insertion of mobile DNA elements. Attempts to identify *Drosophila*-like hybrid dysgenesis by mating different geographical isolates of *C. elegans* have also been unsuccessful (6). However, arguments that dysgenic elements are "selfish DNA" sequences that propagate in a population by colonizing virgin genomes via sex (15) would predict such elements to be uncommon in primarily self-fertilizing species such as *C. elegans*. We, therefore, chose to look for transposable elements in *Panagrellus redivivus*, a gonochoristic (male–female) free-living nematode similar to *C. elegans* (16).

C. elegans strains have been described that have high spontaneous mutation frequencies in the *unc-54* and *unc-22* genes, relative to the standard N2 strain (7, 17). In these strains, the enhanced mutation rate results from an increased frequency of gene inactivation by Tc*1* insertion (2, 7). These observations suggest that, in nematodes, high spontaneous mutation frequencies may be generally indicative of increased transposon activity. We discovered that *P. redivivus* strain C15, which contains no detectable Tc*1*-homologous sequences, also has a high spontaneous mutation frequency relative to the *C. elegans* N2 strain. We undertook to determine the molecular basis of these *P. redivivus* mutations and have demonstrated that one such mutation resulted from the insertion of a transposable element.

MATERIALS AND METHODS

Maintenance of Strains. *P. redivivus* strains were routinely maintained at 20°C and handled essentially as described for *C. elegans* (18). The C15 strain (19) was obtained from M. R. Samoiloff (University of Manitoba); the PS strain from P. W. Sternberg (Massachusetts Institute of Technology); the SC strain from the *Caenorhabditis* Genetics Center (Division of Biological Sciences, University of Missouri); the W strain from J. Pasternak (University of Waterloo); and the G strain from E. Geraert (State University of Ghent, Belgium). The C15 strain is the product of nine sibling matings (19); the remaining strains are not inbred. *P. redivivus unc-22* ("twitcher") mutants were maintained by mating individual female siblings of twitcher males to wild-type males; half of these matings produced twitcher males.

Construction of Genomic Libraries. Genomic DNA from wild-type *P. redivivus* was prepared from mixed-stage populations of animals harvested from NG plates (18) with agarose substituted for agar. Genomic DNA containing *P. redivivus* twitcher mutations was prepared from populations in which only a fraction of the chromosomes were mutant, as follows: female siblings of twitcher males were placed on individual NG/agarose plates and mated to wild-type males. Progeny from plates that produced twitcher male offspring were pooled and allowed to produce F_2 larvae before harvesting. (Approximately one-sixth of the X chromosomes in this F_2 population should be mutant.) Animals were lysed by incubating in 0.1 M Tris·HCl (pH 8.5), 0.05 M EDTA, 0.2 M NaCl, 1% $NaDodSO_4$, and proteinase K at 200 μg/ml (obtained from Boehringer Mannheim) at 65°C for 1–2 hr. DNA was purified by phenol extraction, RNase treatment, and ethanol precipitation.

Genomic libraries were prepared using vectors EMBL-3 (20) or Charon 20 (21). EMBL-3 libraries were prepared by

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.

Abbreviation: ssDNA, single-stranded DNA.

ligation of size-fractionated [15–20 kilobases (kb)] genomic DNA fragments from an *Mbo* I partial digest to *Bam*HI-cleaved vector DNA. Charon 20 libraries were prepared by ligating fragments of genomic DNA fragments from a *Hin*dIII limit-digest with purified Charon 20 *Hin*dIII arms. Packaging extracts were obtained from Vector Cloning Systems (San Diego, CA).

RNA Preparation. *P. redivivus* RNA was prepared from well-fed, mixed-stage animals grown at 25°C on NG/agarose plates, harvested, washed, then immediately frozen at −70°C. RNA was purified by the guanidine chloride method (22) as described (23). *C. elegans* total RNA was fractionated on 1.2% glyoxal gels (24) and transferred by blotting to Zetabind membranes (AMF, Meriden, CT). Zetabind filters were handled and hybridized according to the manufacturer's instructions.

Molecular Techniques. Restriction digests, ligations, Southern transfers, nick-translations, and hybridizations were performed using standard techniques (24). Hybridizations were routinely done at 42°C, in hybridization buffer containing 50% (vol/vol) formamide, 0.1 M sodium phosphate buffer (pH 7.0), 0.75 M NaCl, 0.25 M Tris·HCl (pH 7.8), 5 mM EDTA, 1% $NaDodSO_4$, and sheared, denatured salmon sperm DNA at 100 μg/ml; reduced stringency hybridizations were done in the same buffer at 32°C in 35% (vol/vol) formamide.

To hybridize two single-stranded (ss) DNA preparations, 2–20 μg of each was incubated in 10 μl of 0.5 M NaCl for 3 hr at 65°C, then cooled to room temperature. For S1 digestions, 1.2 μl of 10× S1 nuclease buffer [0.3 M sodium acetate (pH 4.6), 10 mM zinc acetate, 50% (vol/vol) glycerol] and 1 μl of S1 nuclease (100 units/μl, Bethesda Research Laboratories) were added, and reactions were incubated for 30 min at 37°C before quenching by addition of 1 μl of 0.5 M EDTA.

RESULTS

High Spontaneous Mutation Frequency in *P. redivivus* Strain C15. Because of the very limited genetic work done on *P. redivivus*, we first required an effective way to measure mutation frequency in *P. redivivus*. For this purpose, we employed an approach developed for measuring the mutation frequency of the *C. elegans unc-22* gene (17, 25), making the assumption that *P. redivivus* would contain a homologous gene. *C. elegans unc-22* mutants are defective in muscle structure (26, 27) and have a distinctive "twitcher" phenotype (18). The *unc-22* gene was chosen because (*i*) the *C. elegans* "twitcher" phenotype results almost exclusively from lesions in the *unc-22* gene; (*ii*) when suspended in nicotine or levamisol solutions, *unc-22* mutant animals continue to twitch, allowing easy identification among large numbers of drug-paralyzed wild-type animals; (*iii*) the *C. elegans unc-22* gene is large (25), highly mutable (28), and a frequent target for Tc*1* insertion (2, 17), and (*iv*) the *C. elegans* gene has been molecularly cloned (2).

To determine if *P. redivivus* can mutate to a twitcher phenotype, strain C15 males and females were mutagenized with ethyl methanesulfonate and mated as described (19). Twitcher mutants were readily recovered by screening F_2 progeny in 1 mM levamisole. Unexpectedly, these mutants were predominantly male, suggesting that the mutation might be X chromosome linked. (The *C. elegans unc-22* gene is autosomal.) Wild-type sibling females of the male twitcher mutants were recovered. Approximately half of these females, when mated to wild-type males, produced 50% twitcher males and wild-type females, indicating that the mutations were recessive and X chromosome linked [*P. redivivus* females are X/X, males are X/O (29)]. Unlike *C. elegans unc-22* heterozygotes, which twitch when suspended in 1 mM levamisol, *P. redivivus* females heterozygous for twitcher mutations could not be readily identified under these conditions. The allelism of independent twitcher mutants could not be ascertained by complementation testing because twitcher males were almost completely infertile. However, since all twitcher mutants were found to be X chromosome linked and since the twitcher phenotype in *C. elegans* results almost exclusively from mutations in the *unc-22* gene, it seems highly likely that these mutations are allelic.

The spontaneous twitcher mutation frequency was estimated to be 2×10^{-4} per germ cell per generation, based on the identification of four independent twitcher mutants among the progeny of 13,000 F_1 females of the C15 strain. This frequency is at least two orders of magnitude greater than the frequency in *C. elegans* strain N2, but similar to the spontaneous *unc-22* mutation frequency in the *C. elegans* Bergerac strain, which has an elevated level of Tc*1* transposition (17). To determine if these *P. redivivus* spontaneous twitcher mutants had resulted from the insertion of a transposable element into the *P. redivivus unc-22* homologous gene, we undertook to isolate this gene from wild-type and twitcher *P. redivivus* strains.

Isolation of *P. redivivus unc-22* Homolog. Genomic libraries were prepared from C15 DNA and screened at reduced stringency with pGB 1.1, a plasmid containing part of the *C. elegans unc-22* gene (kindly provided by D. Moerman and G. Benian, Washington University Medical School, St. Louis) (2). Positive clones were physically characterized, and the flanking regions were cloned by "chromosome walking." Restriction maps of the cloned region, and relevant subclones, are shown in Fig. 1*A*.

To estimate the extent of homology between the cloned region and the *C. elegans unc-22* locus, DNAs from two phages containing sequences spanning most of the *C. elegans* locus [λ DM17 and DM18 (2), also provided by D. Moerman and G. Benian] were used to probe, at reduced stringency, Southern blots of restriction enzyme digests of the cloned *P. redivivus* sequences. Both phage DNAs hybridized to several

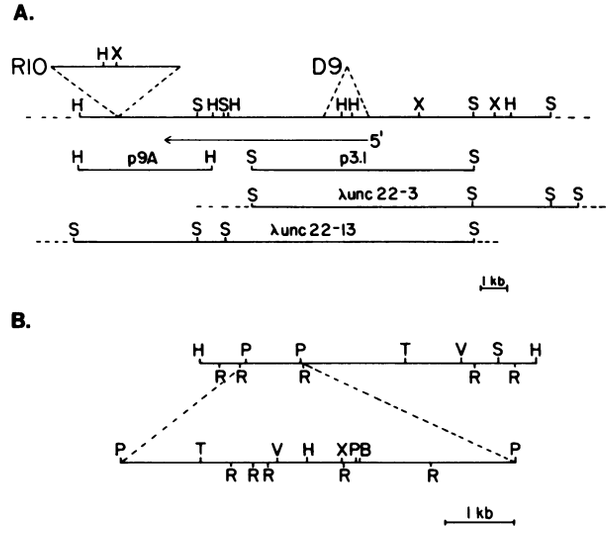

FIG. 1. (*A*) Restriction map of the *P. redivivus unc-22* region. The top line shows the map of the genomic region; relevant subclones are shown below. Also indicated are the sites of the R10 insertion and the D9 deletion (see text). Arrow indicates direction of transcription, assumed from homology to the *C. elegans unc-22* gene (G. Benian and D. Moerman, personal communication). (*B*) Detailed restriction map of the R10 mutant *unc-22* region. Top line shows map of the wild-type gene sequences contained in p9A; bottom line shows map of new sequences occurring in the R10 mutant. Precise endpoints of the insertion are not known; their approximate positions are indicated in Fig. 5. H, *Hin*dIII; X, *Xho* I; S, *Sal* I; R, *Eco*RI; P, *Pst* I; T, *Sst* I; V, *Eco*RV; B, *Bam*HI.

restriction fragments, indicating several regions of homology. When two of the *P. redivivus* restriction fragments were used to probe Southern blots of digested *C. elegans unc-22*-region DNA, they hybridized to fragments at roughly corresponding positions on the *C. elegans* map, consistent with colinearity of the *unc-22* locus in the two species (data not shown).

To determine if the cloned region, like the twitcher mutations, was X chromosome linked, we took advantage of a natural DNA polymorphism existing in this region between the C15 and PS strains of *P. redivivus*. When *Sal* I-digested C15 DNA is probed with p3.1, a band of 9.1 kb is seen, while similar probing of PS DNA reveals bands of 5.4 and 3.9 kb. To determine if this polymorphism is X chromosome linked, we relied on the fact that male progeny contain both maternal and paternal autosomes, but only the maternal X chromosome. As shown in Fig. 2, DNA from male progeny of a PS male × C15 female cross does not yield the 5.4 and 3.9 kb bands, indicating that the region is X chromosome linked.

Cloning Mutated *unc-22* Genes from *P. redivivus*. DNA from two spontaneous twitcher mutants, R10 (recovered from strain C15) and D9 (recovered among offspring of a C15 × PS mating), was prepared and screened by Southern blot using clones containing the *P. redivivus unc-22* region. Both mutations reproducibly showed new bands when probed with specific portions of the *unc-22* region, suggesting that DNA rearrangements were associated with the original twitcher mutations (Fig. 3*A*, lanes 2 and 4). The 9.0-kb *Hin*dIII fragment associated with the D9 mutation and the 6.5-kb *Hin*dIII fragment associated with the R10 mutation were cloned by screening genomic libraries constructed from R10 or D9 DNA with the appropriate *unc-22* subclones. These restriction fragments were then used to probe Southern blots of wild-type genomic *P. redivivus* DNA (Fig. 3*A*, lanes 5 and 7) and *unc-22* subclones (data not shown). These studies demonstrate that the D9 mutation is a 1.6-kb deletion that has eliminated two *Hin*dIII sites in the *unc-22* region, thus fusing neighboring 6.0- and 42-kb *Hin*dIII fragments into the 9.0-kb *Hin*dIII fragment associated with the D9 mutation (shown schematically in Fig. 1*A*). In contrast, the R10 6.5-kb *Hin*dIII fragment hybridizes to numerous fragments (Fig. 3*A*, lane 7) indicating that this rearrangement involved a repetitive sequence.

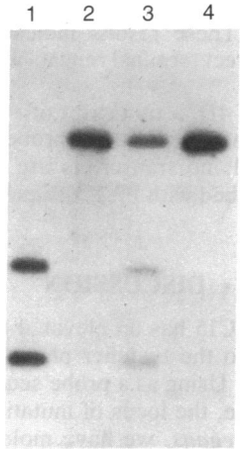

FIG. 2. Polymorphism mapping of the *P. redivivus unc-22* homologous region. PS males were mated to C15 females. Twenty virgin female progeny were batch mated to C15 males, and 20 male progeny were batch mated to C15 females. After one generation of growth, animals were harvested, and DNA was prepared. The figure shows a Southern blot of *Sal* I-digests of these DNAs hybridized with labeled p3.1. Lanes: 1, PS DNA; 2, C15 DNA; 3, DNA from the population produced by the female offspring of the initial cross (note the presence of both PS and C15-specific bands); 4, DNA from the population produced by the male offspring of the initial cross. The absence of the PS-specific bands in lane 4 indicates that the cloned region must reside on a chromosome not present in the male progeny of the initial cross, which can only be the X chromosome.

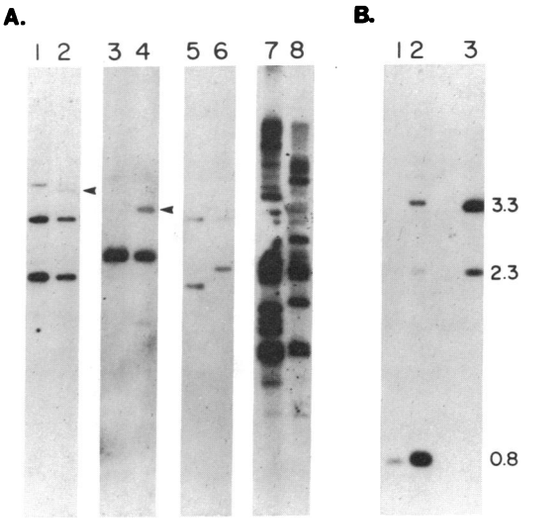

FIG. 3. (*A*) Genomic Southern blots of wild-type and mutant DNAs. Southern blot of *Hin*dIII digests of C15 (lane 1) and D9 population (lane 2) DNA hybridized with nick-translated λ *unc-22*-3 DNA (see Fig. 1*A*). Note the 9.0-kb band of less-than-unique sequence intensity in the D9 lane. (Higher-mobility band observed in both lanes is due to incomplete *Hin*dIII digestion). Southern blot of *Hin*dIII digests of C15 (lane 3) and R10 population (lane 4) DNA probed with nick-translated p9A DNA (see Fig. 1*A*). Note the 6.5-kb band in R10 digest. Southern blot of *Hin*dIII digests of C15 (lane 5) and PS (lane 6) DNA hybridized with the nick-translated 9.0-kb *Hin*dIII fragment from λ CH20/D9. (The band >4.2 kb in the PS digests results from a C15/PS DNA polymorphism.) Southern blot of *Hin*dIII digests of C15 (lane 7) and PS (lane 8) DNA hybridized with nick-translated 6.5-kb *Hin*dIII fragment from λ CH20/R10 (see text). (*B*) Southern blot of *Pst* I digests of C15 (lane 1), R10 population (lane 2), and pPAT-1 (lane 3) DNA hybridized with a nick-translated 0.8-kb *Pst* I fragment of p9A that spans the PAT-1 insertion site in the R10 mutation.

Restriction mapping of the R10 6.5-kb *Hin*dIII fragment and other R10 library clones that overlap the mutated region demonstrated that the original R10 mutation resulted from a 4.8-kb insertion of repetitive DNA (Fig. 1*B*), designated PAT-1 (*Pa*nagrellus *t*ransposon). Because no R10 library clones contained the entire PAT-1 insertion, the element was reconstructed by subcloning into pUC8 a 2.6-kb *Pst* I–*Hin*dIII fragment containing the left half of the R10 insertion and a 5.7-kb *Hin*dIII–*Sal* I fragment containing the right half of the insertion, thus generating pPAT-1 (left and right designations are arbitrary). To confirm the identity of pPAT-1 with the original mutation, the 0.8-kb *Pst* I fragment of p9A, which contains the insertion site, was used to probe *Pst* I digests of genomic C15, R10 population, and pPAT-1 DNA (Fig. 3*B*). In addition to a major 0.8-kb band, the R10 population digest contains the predicted minor bands of 3.3 and 2.3 kb. These bands are identical in size to the corresponding bands in the pPAT-1 digest.

Characterization of the PAT-1 Element. To determine the distribution of PAT-1 elements among *P. redivivus* strains, genomic DNA was prepared from five independent *P. redivivus* isolates. *Pst* I digests of these DNAs were blotted and probed with DNA fragments containing the entire PAT-1 element. As shown in Fig. 4*A*, the PAT-1 element varies from 10 to 50 copies per genome among these strains. This element is not detectable in *C. elegans*, even under reduced stringency conditions (data not shown).

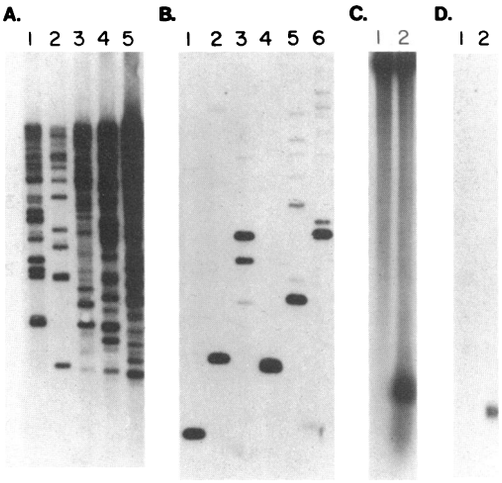

FIG. 4. (*A*) Southern blot of *Pst* I-digested *P. redivivus* genomic DNAs hybridized with two nick-translated *Pst* I fragments of pPAT-1, which contain the entire PAT-1 element. Note that each genomic copy of the element should produce two hybridizing *Pst* I fragments. Lanes: 1, strain C15; 2, strain PS; 3, strain SC; 4, strain G; 5, strain W. (*B*) Southern blot of restriction digests of C15 DNA probed with a nick-translated PAT-1 internal 0.7-kb *Bam*HI–*Hin*dIII fragment. Lanes: 1, *Bam*HI/*Hin*dIII; 2, *Bam*HI/*Eco*RV; 3, *Bam*HI/*Sst* I; 4, *Eco*RI; 5, *Bam*HI/*Sal* I; 6, *Sal* I. (*C*) Sizing terminal repeats. ssDNA was obtained from pEMBL-18 derived subclones by F_1 superinfection as described (30). Subclone p18-1 contains a 2.6-kb *Pst* I–*Hin*dIII fragment that includes the left half of the PAT-1 element. (The end of the PAT-1 element farthest downstream from the *unc-22* transcription unit is arbitrarily designated the "left" end). Subclone p18-14 contains a 5.6-kb *Bam*HI–*Hin*dIII fragment that includes the right half of the PAT-1 element. Subclone p18-18 contains a 5.6-kb *Hin*dIII–*Sal* I fragment that also includes the right half of the PAT-1 element. The fragments subcloned in p18-1 and p18-14 are in the same relative orientation as in the PAT-1 element, whereas the p18-18 fragment is in the opposite orientation. Subclones p18-1 and p18-14 will, therefore, yield ssDNA from the same strand upon superinfection, while p18-18 will yield DNA from the opposite strand. In the experiment shown, ssDNA from the left half of the PAT-1 element (subclone 18-1) was annealed to ssDNA from each strand of the right half (subclones 18–14 and 18–18; see Fig. 5), S1 digested, then run on a 2% agarose gel. The fractionated DNAs were then transferred to nitrocellulose and probed with a 1.15-kb *Pst* I–*Sst* I fragment that contains the left PAT-1 terminus. Lane 1 contains annealed and digested ssDNA from subclones p18-1 and p18-14, which yield same DNA of the same polarity strands (see Fig. 5). No S1-resistant fragments are observed. Lane 2 contains annealed and digested ssDNA from subclones p18-1 and p18-18, which yield DNA strands of opposite polarity. An S1-resistant fragment of ≈170 bp is observed. (The hybridizing fragments at the top of the gel result from double-stranded DNA contaminating the original ssDNA preparations.) (*D*) RNA gel blot of total RNA from *C. elegans* (lane 1) and *P. redivivus* strain C15 (lane 2) hybridized with nick-translated *Pst I* fragments containing all of the PAT-1 element. The band observed in lane 2 corresponds to a transcript of ≈900 bp.

The overall structure of the individual PAT-1 elements in the C15 strain appears to be similar to that of the cloned element as judged by hybridization studies using PAT-1 internal fragments as probes (Fig. 4*B*). This view is also supported by the *Pst* I digests shown in Fig. 4*A*, which indicate that there is a minimum size *Pst* I fragment of 2.1 kb, implying that the large majority of elements are not smaller than the cloned copy. The bulk of the PAT-1 elements in the C15 strain do appear to differ from the cloned copy, however, in that they contain two internal *Sal* I sites, while the cloned element contains none.

Cross-hybridization studies indicated homologous sequences in fragments containing the termini of the PAT-1 element, suggesting that this element contains terminal repeated sequences. To determine the orientation and approximate size of these repeats, we devised an S1 nuclease protection experiment using ssDNA from both termini (Fig. 5). DNA fragments from left and right halves of the PAT-1 element were inserted into pEMBL-18, which enabled us to produce PAT-1 ssDNA by F_1 superinfection (Fig. 4). ssDNA preparations from each half of the PAT-1 element were annealed and then digested with the single-strand-specific nuclease S1. The digest (including any S1-resistant duplexes resulting from the annealing of repeated sequences) was then subjected to gel electrophoresis, blotted to nitrocellulose, and probed with a labeled DNA fragment containing one of the PAT-1 termini. As shown in Fig. 4*C*, a fragment of ≈170 base pairs (bp) is observed in these experiments. This fragment is not produced when ssDNA is used, and it is orientation dependent: it is observed only when the ssDNAs represent opposing strands (Fig. 5). In addition, this fragment is not seen using a probe containing only the *unc-22* sequences present at the insertion site and no PAT-1 sequences (data not shown). These results indicate that the PAT-1 element contains direct terminal repeats with a minimum size of 170 bp.

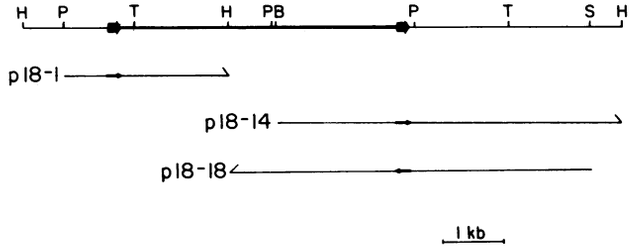

FIG. 5. Schematic diagram of PAT-1 DNA (thick line) and the ssDNA molecules derived from three subclones described in the text and Fig. 4*C*. Restriction sites are indicated as in Fig. 1. Heavy arrows represent repeated sequences (detected by cross-hybridization) found at or near the termini of the PAT-1 element. If the repeats are direct (as shown), then p18-1 ssDNA should anneal with p18-18 ssDNA in the repeat region to form S1-resistant duplexes but should not anneal with p18-14 ssDNA. If the repeats are inverted, then p18-1 ssDNA should anneal with p18-14 ssDNA but not with p18-18 ssDNA. As shown in Fig. 4*C*, p18-1 ssDNA anneals with only p18-18 ssDNA to form S1-resistant duplexes, confirming that the PAT-1 element contains direct repeats.

As shown in Fig. 4*D*, a transcript of ≈900 bp is detected when PAT-1 sequences are used to probe a blot of C15 total RNA. As expected, no transcripts are detected when *C. elegans* RNA is probed with PAT-1 sequences (Fig. 4*C*, lane 1).

DISCUSSION

P. redivivus strain C15 has an elevated frequency of spontaneous mutation to the twitcher phenotype relative to *C. elegans* strain N2.* Using as a probe sequences from the *C. elegans unc-22* gene, the locus of mutations to the twitcher phenotype in *C. elegans*, we have molecularly cloned homologous sequences from *P. redivivus* wild-type and twitcher

*Our attention was initially drawn to the C15 strain by a striking nonreciprocal effect on fertility in crosses between C15 and another *P. redivivus* isolate, PS. The brood size of C15 ♂ × PS ♀ crosses was <10% the brood size of PS ♂ × C15 ♀ crosses. Because of the difficulties inherent in working with genetically uncharacterized and noninbred strains, we have not attempted to determine if genetic factors are involved in the unidirectional brood-size reduction. However, we do note a correlation between PAT-1 copy number and nonreciprocal mating effects: the four strains with high PAT-1 copy number (20–50 copies) (C15, SC, W, and G) show similar effects when males are mated to females of the PS strain, which has the lowest PAT-1 copy number (≈10 copies).

mutant strains. We have shown that in *P. redivivus*, both the *unc-22* sequence and mutation to the twitcher phenotype are X chromosome linked and that at least one spontaneous twitcher mutant resulted from the insertion of a 4.8-kb transposable element, designated PAT-1, into the *unc-22* locus. The PAT-1 element varies in copy number among different *P. redivivus* isolates and is absent from *C. elegans*. Based on its size, long direct terminal repeats, and structural conservation, this element resembles retrotransposon elements such as Ty in yeast and *copia* in *Drosophila* (31) more closely than P in *Drosophila* or Tc*1* in *C. elegans*, although classification of the PAT-1 element will require further physical characterization. A retrotransposon-like element has been isolated from *Ascaris lumbricoides* (32).

The relative contribution of PAT-1 transposition to the spontaneous *unc-22* mutation frequency in *P. redivivus* cannot be determined from the limited number of mutants we have examined. Our finding that the second characterized spontaneous *unc-22* mutation resulted from a deletion could indicate that the *P. redivivus unc-22* region is inherently unstable, perhaps because of some repeated structure. Alternatively, the deletion may have resulted from an insertion–deletion event of the kind often associated with transposon activity (33). Also unexpected was the discovery that the *P. redivivus unc-22* gene is X chromosome linked, since the *C. elegans* gene is on autosome IV (18). Because *P. redivivus* has one fewer pair of chromosomes than *C. elegans* (5 vs. 6), and in *C. elegans* IV/X chromosome fusions are viable (34), it is tempting to speculate that *P. redivivus* has undergone a chromosome fusion relative to *C. elegans*. However, this simple interpretation is not supported by cytology, since in both species all chromosomes appear to be roughly equal in size (29, 35).

The PAT-1 element can now be tested as a transformation vector for *C. elegans*. In undertaking such tests, it is encouraging that the P elements derived from *D. melanogaster* are active in distantly related Hawaiian Drosophilid species (36). Perhaps the PAT-1 element could be introduced into *C. elegans* by germ-line microinjection (37, 38) and subsequently screened for progeny containing integrated PAT-1 sequences. Alternatively, by analogy to P element rosy vectors (39), a selectable *C. elegans* marker such as the *sup-7* gene (40) could be readily incorporated into the PAT-1 element, thus allowing direct identification of transformed progeny after coinjection of modified and intact PAT-1 elements.

In view of the observation that spontaneous twitcher mutants in both *P. redivivus* and *C. elegans* can result from transposon insertion into the *unc-22* gene, we believe *unc-22* homologues may be useful as general "transposon traps" for the isolation of transposable elements from rhabdatid nematode species.

We thank R. Pulak, L. Meheus, M. R. Samoiloff, P. W. Sternberg, J. Pasternak, and E. Geraert for help in obtaining *P. redivivus* strains. Other strains were obtained from the *Caenorhabditis* Genetics Center, which is supported by a contract between the National Institutes of Health and the Curators of the University of Missouri. We are grateful to R. Waterston, D. Moerman, and G. Benian for providing *C. elegans unc-22* clones; to P. Mains, C. Trent, and J. Shaw for helpful suggestions during the course of this work; to D. Moerman and R. Waterston for critical reading of the manuscript; and to J. Wilson for help in its preparation. We also thank Scott W. Emmons, Albert Einstein College of Medicine, and Allan Spradling, Carnegie Institution of Washington, for reviewing the manuscript and for their suggestions. This research was supported by a grant to W.B.W. from the National Institutes of Health (HD-11762) and a postdoctoral fellowship to C.D.L. from the National Institutes of Health (GM-08668).

1. Greenwald, I. (1985) *Cell* **43,** 583–590.
2. Moerman, D. E., Benian, G. M. & Waterston, R. H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2579–2583.
3. Spradling, A. C. & Rubin, G. M. (1982) *Science* **218,** 341–342.
4. Rubin, G. M. & Spradling, A. C. (1982) *Science* **218,** 342–348.
5. Emmons, S. W., Yesner, L., Ruan, K. & Katzenberg, D. (1983) *Cell* **32,** 55–65.
6. Liao, L. W., Rosenzweig, B. & Hirsh, D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3585–3589.
7. Eide, D. & Anderson, P. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1756–1760.
8. Eide, D. & Anderson, P. (1985) *Genetics* **109,** 67–79.
9. Landel, C. P., Krause, M., Waterston, R. H. & Hirsh, D. (1985) *J. Mol. Biol.* **180,** 497–513.
10. Albertson, D. G. (1985) *EMBO J.* **4,** 2493–2498.
11. Zachar, Z. & Bingham, P. M. (1982) *Cell* **30,** 529–541.
12. Cote, B., Bender, W., Curtis, D. & Chovnick, A. (1986) *Genetics* **112,** 769–783.
13. Kidd, S., Lockett, T. J. & Young, M. W. (1983) *Cell* **34,** 421–433.
14. Scott, M. P., Weiner, A. J., Hazelrigg, T. I., Polisky, B. A., Pirrotta, V., Scalenghe, F. & Kaufman, T. C. (1983) *Cell* **34,** 763–776.
15. Hickey, D. A. (1982) *Genetics* **101,** 519–531.
16. Sternberg, P. W. & Horvitz, H. R. (1981) *Dev. Biol.* **88,** 147–166.
17. Moerman, D. G. & Waterston, R. H. (1984) *Genetics* **108,** 859–877.
18. Brenner, S. (1974) *Genetics* **77,** 71–94.
19. Burke, D. J. & Samoiloff, M. R. (1980) *Can. J. Genet. Cytol.* **22,** 295–302.
20. Frischauf, A.-M., Lehrach, H., Poustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170,** 827–842.
21. DeWet, J. R., Daniels, D. L., Schoeder, J. L., Williams, B. G., Denniston-Thompson, K., Moore, D. D. & Blattner, F. R. (1980) *Virology* **33,** 401–410.
22. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
23. Cox, G. N., Kramer, J. M. & Hirsh, D. (1984) *Mol. Cell. Biol.* **4,** 2389–2395.
24. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
25. Moerman, D. G. & Baillie, D. L. (1979) *Genetics* **91,** 95–103.
26. Waterston, R. H., Thomson, J. N. & Brenner, S. (1980) *Dev. Biol.* **77,** 271–302.
27. Moerman, D. E. (1980) Dissertation (Simon Fraser Univ., Burnaby, BC).
28. Zengel, J. M. & Epstein, H. F. (1980) *Cell Motil.* **1,** 73–97.
29. Hechler, H. (1970) *J. Nematol.* **2,** 355–361.
30. Dente, L., Cesareni, E. & Cortese, R. (1983) *Nucleic Acids Res.* **11,** 1645–1655.
31. Finnegan, D. J. (1985) *Int. Rev. Cytol.* **93,** 281–326.
32. Aeby, P., Spicher, A., deChastonay, Y., Muller, F. & Tobler, H. (1986) *EMBO J.* **5,** 3353–3360.
33. Calos, M. P. & Miller, J. H. (1980) *Cell* **20,** 579–595.
34. Sigurdson, D. C., Herman, R. K., Horton, C. A., Kari, C. K. & Pratt, S. E. (1986) *Mol. Gen. Genet.* **202,** 212–218.
35. Albertson, D. G. & Thomson, J. N. (1982) *Chromosoma* **86,** 409–428.
36. Brennan, M. D., Rowan, R. G. & Dickinson, W. J. (1984) *Cell* **38,** 147–151.
37. Stinchcomb, D. T., Shaw, J. E., Carr, S. H. & Hirsh, D. J. (1985) *Mol. Cell. Biol.* **5,** 3484–3496.
38. Fire, A. (1986) *EMBO J.* **5,** 2673–2680.
39. Spradling, A. C. & Rubin, G. M. (1983) *Cell* **34,** 47–57.
40. Wills, N., Gesteland, R. F., Karn, J., Barnett, L., Bolten, S. & Waterston, R. H. (1983) *Cell* **33,** 575–583.