Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3585–3589, June 1983
Biochemistry

Analysis of a transposable element in *Caenorhabditis elegans*

(nematode genome organization/repetitive DNA/inverted repeat/strain variation)

LOUISE W. LIAO, BRADLEY ROSENZWEIG, AND DAVID HIRSH*

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

Communicated by William B. Wood, March 9, 1983

ABSTRACT **A transposable element, designated Tc1, has been characterized in *Caenorhabditis elegans*. Tc1 is 1.7 kilobases long, has an inverted terminal repeat of <100 base pairs, and is repeated as a highly conserved element. The copy number and genomic positions of Tc1 are extremely variable among strains, implying that Tc1 is mobile. However, progeny of interstrain crosses did not show hybrid dysgenic traits that might be due to Tc1 transposition.**

Transposable elements are discrete genetic units capable of integrating into many sites in the genome (for review, see refs. 1 and 2). Since their discovery (3), several eukaryotic transposable elements have been described. The *copia*, P, and FB elements in *Drosophila* and the Ty elements in yeast all have terminal repeats and are members of dispersed repetitive families (4–7). Evidence for their transposition initially came from analyses of their distributions in the genome by the Southern blot technique (8), which showed that the copy numbers and sites of these elements are hypervariable among different strains of the same species and, to a lesser degree, among individuals of the same strain (4, 9–11). Direct evidence for transposition of Ty, *copia*, and P elements came from analyses of mutations caused by their insertions into nonhomologous genomic sites (6, 12). The P elements are exceptional for their remarkably high frequency of transposition during interbreeding of certain *Drosophila* strains (13). Transposition of P elements is the most likely basis of P–M hybrid dysgenesis, in which progeny of hybrid crosses exhibit sterility, high rates of mutation, and chromosomal aberrations (14).

In this paper, we report the characterization of a transposable element in the nematode *Caenorhabditis elegans* and compare it with other known eukaryotic transposable elements. Previous studies in this laboratory have shown that the two strains of *C. elegans*, Bristol and Bergerac, give occasional differences in restriction endonuclease cleavage patterns on Southern blots when probed with randomly selected cloned fragments (15). One such DNA polymorphism was found to be a 1.7-kilobase-pair (kb) difference adjacent to the actin gene cluster (16). Here, we demonstrate that the observed polymorphism near the actin genes is due to the presence of the transposable element "Tc1" at that site in the Bergerac strain. Another polymorphism between the Bristol and Bergerac strains has been characterized by Emmons *et al.* (17) and it also was found to be due to Tc1.

MATERIALS AND METHODS

Nematodes. All *C. elegans* var. Bristol worms used in this study are descendants of a single Bristol N2 hermaphrodite (18). All laboratory stocks are derived from a culture of Bristol N2 frozen in 1972 in separate vials. Stocks stored frozen since 1972 were obtained from this laboratory and the Medical Research Council laboratories in Cambridge, England, and a stock stored frozen since 1974 was obtained from the Caenorhabditis Genetics Center. Laboratory stocks that were passaged regularly for the past 3–8 years were obtained from D. Baillie, R. Edgar, D. Riddle, and S. Ward. These cultures were generally propagated for a few generations after being thawed, then allowed to survive starvation conditions as dauer larvae for a few weeks. Aliquots of the starved cultures were then transferred to fresh medium, repeating the continuous cycle of growth and starvation.

Two laboratory strains of *C. elegans* var. Bergerac were used. DNA analysis was done on the Bergerac LY strain from this laboratory, obtained from J. Brun in 1977 and originally isolated in France in 1949 (19). For the hybrid dysgenesis tests, we used the Bergerac FR strain, which the Caenorhabditis Genetics Center obtained independently from Brun in 1980. The Bergerac FR strain has male fertility levels and brood sizes comparable with Bristol N2, while the Bergerac LY strain has low male fertility and brood sizes. Bergerac stocks were propagated from single hermaphrodites thawed in recent months and maintained similarly to Bristol stocks.

Wild strains of *C. elegans* obtained from R. Russell included CL2a, GA-1, PA-2, and PaC-1. EPC-4, also called DH424, is a wild isolate that we collected. All wild strains were collected independently from soil samples in or near Pasadena, CA, and found to be fertile with the Bristol N2 strain.

DNA Analysis. Procedures for the isolation of *C. elegans* DNA from first-stage (L1) larvae, restriction endonuclease digestion, gel fractionation of DNA fragments, and hybridization to Southern blots have been described (15). Electron microscopy of renatured single-stranded molecules and heteroduplex molecules was by published procedures (20, 21). All spreads contained linearized pBR325 DNA, which can form stem-loop structures, as a size marker (22).

Phage and Plasmid Recombinants. Recombinant phage containing *C. elegans* DNA were isolated from an *Eco*RI partial digest library in λ Charon 10 or a *Sau*3A partial digest library in λ 1059 (16, 23). *Eco*RI fragments were subcloned into pBR325 by published methods (15). Isolation of recombinant DNA carrying *C. elegans* sequences containing actin gene *I*, actin gene *IV*, collagen gene *col-1*, and ribosomal DNA have been described (16, 24, 25).

Hybrid Dysgenesis Tests. For each mating, three males from a given stock were transferred to a plate with a single fourth-stage (L4) larval hermaphrodite; matings giving 45–50% male progeny were analyzed further. Five to 10 F_1 hermaphrodites per mating were transferred individually as larvae to fresh plates to propagate by self-fertilization. F_1 sterility was scored by counting both the number of eggs laid and the number of prog-

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.

Abbreviations: bp, base pairs; kb, kilobase pair(s).

* To whom reprint requests should be addressed.

eny hatched. Crosses were carried out at 20°C because the Bergerac FR, PA-2, and EPC-4 strains are not viable at or above 25°C. Stocks were maintained at 16°C.

RESULTS

Identification of a 1.7-kb Insert Near the Actin Gene Cluster. The DNA polymorphism observed between the Bristol and Bergerac strains when probed with actin genes was localized to a 3.5-kb *Eco*RI fragment in the Bristol strain and a 5.2-kb *Eco*RI fragment in the Bergerac strain (Fig. 1*A*). Restriction maps of the Bristol *Eco*RI fragment subcloned in pCe(Br)T1 and the Bergerac *Eco*RI fragment subcloned in pCe(Be)T1 showed that the DNA polymorphism results from an insert of 1.7 kb in the Bergerac strain (Fig. 1*B*). This 1.7-kb insert is called Tc1.

Tc1 Is a Member of a Dispersed Repetitive Family. The Bergerac recombinant plasmid pCe(Be)T1, containing the *Eco*RI fragment carrying the Tc1 element, was hybridized to Bristol and Bergerac DNAs digested with *Eco*RI (which does not cleave within the element). The Bristol strain gives 25–30 restriction fragments that hybridize to the probe (Fig. 2*A*, lane a). Under identical conditions, the Bergerac hybridization pattern appeared as a black streak but, when one-eighth the standard amount of Bergerac DNA was analyzed, a complex pattern of discrete bands was superimposed on a streak (lane b). By comparison, when the hybridization probe was the Bristol recombinant plasmid pCe(Br)T1 containing the *Eco*RI fragment that lacks the Tc1 element, only unique genomic restriction fragments hybridized (lanes c and d). Thus, the sequences surrounding Tc1 in the hybridization probe are present only once in the Bristol and Bergerac genomes whereas multiple copies of elements homologous to Tc1 are present in the Bristol and Bergerac genomes.

The Southern blot patterns include several hybridizing fragments that have unique sizes that are smaller than 3.4 kb. Thus, their repeat length cannot accommodate even two complete elements. Most hybridizing bands have the same intensity; the few of higher intensity probably represent equal-sized restriction fragments or two Tc1 copies on the same restriction fragment. Therefore, the Tc1 elements are not organized in repetitive arrays.

The apparent difference in copy number of Tc1-homologous sequences between the Bristol and Bergerac genomes can be interpreted in two ways. Either these strains have a single class of Tc1 elements and the Bergerac strain contains at least 10 times more copies of Tc1 than the Bristol strain or the Bristol DNA has sequences with only partial homology to the Bergerac-derived hybridization probe (explaining why similar hybridization intensities were obtained when a given amount of Bristol DNA was compared with one-eighth the amount of Bergerac DNA). To test these alternatives, DNA from a recombinant phage carrying Tc1-homologous DNA from the Bristol strain was hybridized to genomic digests of Bristol and Bergerac DNAs. As shown in Fig. 2*A* (lanes e–g), these Southern blot profiles are essentially identical to those obtained with the Bergerac Tc1 element as the probe. The only differences between patterns obtained with the Bristol-derived Tc1 probe and the Bergerac-derived Tc1 probe are due to hybridization by homology to single-copy sequences surrounding Tc1 in each of the probes, as indicated by controls (lanes c, d, and h). We conclude that the Bristol and Bergerac genomes have a single class of Tc1 elements, that Bristol has 25–30 Tc1 elements, and that Bergerac has several hundred copies of Tc1.

Tc1-Homologous Sequences Occur as Intact 1.7-kb Elements. The Bristol and Bergerac genomes were digested with several restriction endonucleases that do not cleave within the copy of Tc1 cloned in pCe(Be)T1. Southern analyses of those genomic digests probed with pCe(Be)T1 showed hybridization only to fragments at least 1.7 kb long. The results suggest that 1.7 kb is the size of the repeated DNA homologous to the probe and, moreover, that each member of the Tc1 family contains the entire 1.7-kb sequence.

To assess the degree to which the sequence is conserved among members of the Tc1 family, the genomic DNAs were digested with the restriction endonuclease *Hae* III. This enzyme cleaves the Tc1 element cloned in pCe(Be)T1 into five pieces; three are approximately 0.5 kb long and two are approximately 0.1 kb long. From sequence analyses, we know that

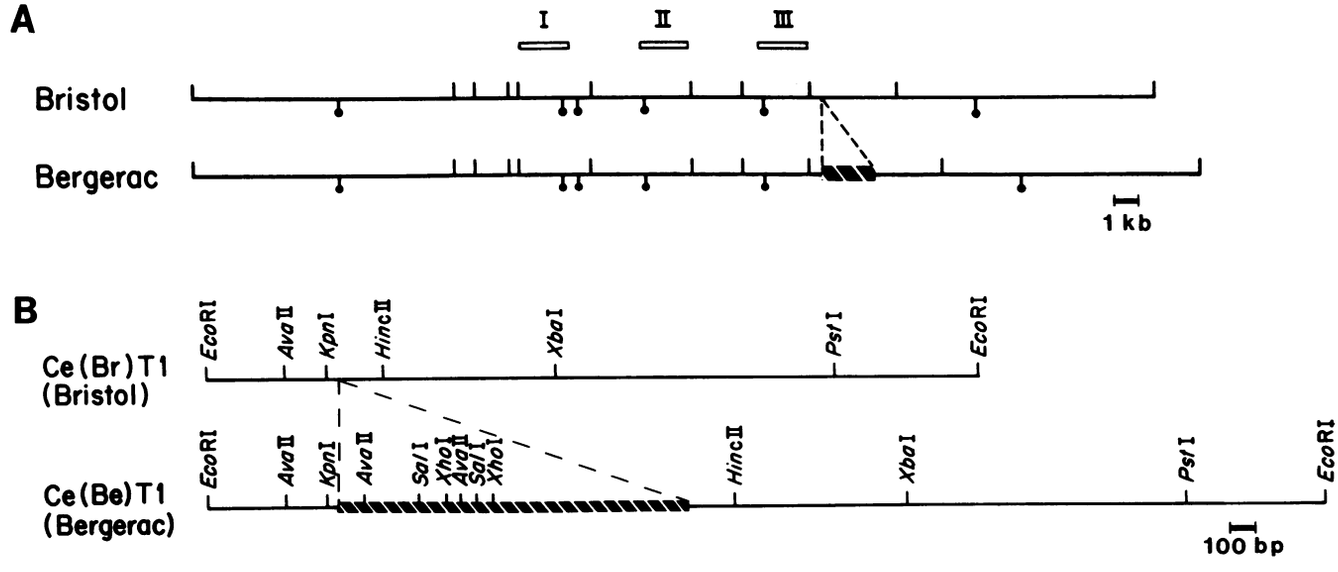

FIG. 1. Comparison of restriction endonuclease cleavage maps from *C. elegans* strains with and without Tc1 inserted near actin gene *III*. (*A*) The upper map was derived from the Bristol strain and the lower map is the corresponding region from the Bergerac strain. Boxes indicate positions of actin genes *I*, *II*, and *III*; actin gene *IV* is not in this region (16). Positions of *Eco*RI sites (⊥) and *Bam*HI sites (⊤) are indicated. (*B*) Detailed maps of the Bristol 3.5-kb *Eco*RI fragment in pCe(Br)T1 and of the Bergerac 5.2-kb *Eco*RI fragment in pCe(Be)T1. The Tc1 element is depicted as a thick bar.

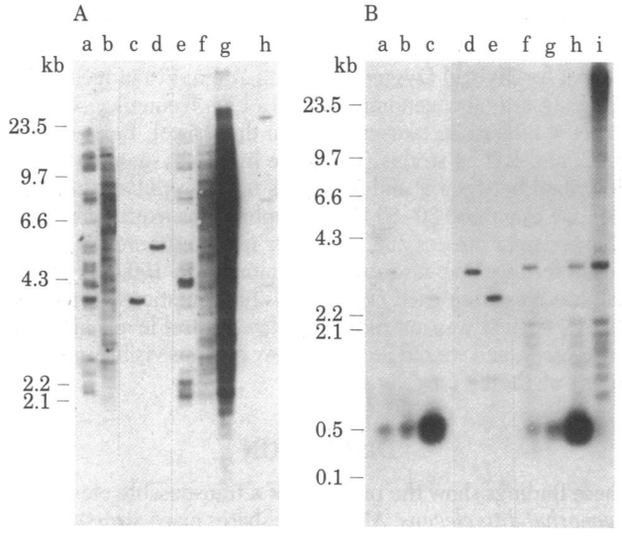

FIG. 2. Hybridization of Tc1 to Bristol and Bergerac DNAs. Genomic DNA digests were fractionated on a 0.7% agarose gel, transferred to nitrocellulose paper, and hybridized to DNA labeled to $>10^8$ cpm per μg with [α-^{32}P]TTP. (*A*) Hybridization to *Eco*RI geomic digests. The hybridization probe to lanes a and b was pCe(Be)T1, containing the Bergerac-derived Tc1 element and flanked by single-copy sequences. The probe to lanes c and d was pCe(Br)T1, which contains the corresponding single-copy sequences from Bristol and thereby serves as a control for hybridization not due to Tc1. The probe to lanes e–h was DNA from a recombinant phage containing a Bristol-derived Tc1 element flanked by single-copy sequences. Lane h is a control identifying hybridization bands due to single-copy sequences as well as Tc1 and phage sequences in the probe. Sources and amounts of *Eco*RI-digested DNA in each lane were as follows: a, Bristol, 3 μg; b, Bergerac, 0.4 μg; c, Bristol, 3 μg; d, Bergerac, 3 μg; e, Bristol, 3 μg; f, Bergerac, 0.4 μg; g, Bergerac, 3 μg; h, DNA from the recombinant phage containing a Bristol Tc1 element, 1 ng. Positions of *Hin*dIII-digested λ DNA size standards are indicated on the left. (*B*) Hybridization to *Hae* III genomic digests. The probe to lanes a–c was pCe(Be)T1, the probe to lanes d and e was pCe(Br)T1, and the probe to lanes f–i was DNA from a recombinant phage containing a Bristol-derived Tc1 element. Sources and amounts of *Hae* III-digested DNA in each lane were as follows: a, Bristol, 3 μg; b, Bergerac, 0.4 μg; c, Bergerac, 3 μg; d, Bristol, 3 μg; e, Bergerac, 3 μg; f, Bristol, 3 μg; g, Bergerac, 0.4 μg; h, Bergerac, 3 μg; i, DNA from the recombinant phage containing a partially digested Bristol-derived Tc1 element, 2 ng.

the cloned copy of Tc1 contains *Hae* III restriction sites 9 base pairs (bp) from its boundaries (unpublished data). If all members of the Tc1 family conserve the *Hae* III restriction sites, then a Tc1 probe would hybridize to the 0.5- and 0.1-kb restriction fragments of every genomic copy of Tc1. Because the cloned copy of Tc1 used as the hybridization probe also contains flanking sequences, the probe will also hybridize to DNA flanking the one genomic copy of Tc1 that was cloned. Under standard conditions, the probe would not hybridize to the *Hae* III restriction fragments that include only the 9-bp termini of the element and the nonhomologous sequences flanking other genomic copies of Tc1. The results (Fig. 2*B*) indicate that the *Hae* III restriction sites are indeed highly conserved. Southern blots probed with pCe(Be)T1 show hybridization to wide bands approximately 0.5 and 0.1 kb long (lanes a–c). In addition, pCe(Be)T1 hybridized to bands that represent the single-copy sequences surrounding Tc1 in pCe(Be)T1, as shown by the patterns obtained when pCe(Br)T1 is the probe (lanes d and e). In an identical manner, a probe containing a Bristol-derived Tc1 element hybridized to the 0.5- and 0.1-kb internal *Hae* III restriction fragments of Tc1 and to the single-copy sequences surrounding Tc1 in the Bristol recombinant phage DNA probe (lanes f–i). Only one other hybridizing fragment was apparent; however, variants of Tc1 that yield *Hae* III restriction fragments smaller than 0.5 kb long would not have been detected because small fragments were not retained well on Southern blots. The results indicate that one member of the Tc1 family has a *Hae* III restriction pattern that would generate a fragment more than 0.5 kb long. Thus, nearly all members of the Tc1 family have highly conserved DNA sequences.

Tc1 Has Inverted Terminal Repeats. A *Kpn* I/*Xho* I restriction fragment from pCe(Be)T1, containing only one end of Tc1, hybridized to itself as well as to a DNA restriction fragment from the other end of Tc1 but not to any fragment from the central region. By contrast, restriction fragments from the central region of Tc1 did not hybridize to any fragment of pCe(Be)T1 except themselves. This result implies that Tc1 is terminally repetitious. To determine whether Tc1 has an inverted or direct repeat, we examined single-stranded Tc1 DNA by electron microscopy. The 5.2-kb *Eco*RI fragment from pCe(Be)T1 was denatured and allowed to renature under conditions favoring intramolecular interactions. Electron microscopic analysis revealed stem-loop structures, indicating that Tc1 has inverted terminal repeats (Fig. 3*A*). The single-stranded loop is 1.6 ± 0.1 kb long and the double-stranded stem is <0.1 kb long. Analyses of heteroduplexes between the 5.2-kb *Eco*RI fragment from pCe(Be)T1 and the 3.5-kb *Eco*RI fragment from pCe(Br)T1 confirm the interpretation that the stem-loop structure is confined to the Tc1 element (Fig. 3*B*). Detailed restriction maps of the termini failed to indicate any direct repeat (data not shown). Thus, we conclude that the terminal redundancy is limited to an inverted repeat of <100 bp.

Tc1 Is a Mobile Element. The organization of Tc1 in several laboratory Bristol strains was compared by Southern blot hybridizations. The extent to which the laboratory strains had been passaged independently of each other ranged from two strains that had been kept frozen for 10 years and propagated only a few weeks to prepare DNA samples to one strain that had been passaged regularly for 8 years (approximately 500 generations). All Bristol N2 stocks had an identical genomic arrangement and copy number of the Tc1 element (Fig. 4).

Examination of hybridization patterns of five *C. elegans* strains isolated from soil near Pasadena, CA, indicated that Tc1 is mobile in evolutionary time. The GA-1, PaC-1, CL2a, and PA-2 strains differed from Bristol in the positions of 0, 1, 5, or 6 hybridizing bands, respectively, while maintaining practically the same copy number of Tc1 elements. The Southern blot profile of the wild strain EPC-4 diverges dramatically from those of Bristol and other wild Pasadena strains and resembles the profile of the Bergerac strain (Fig. 5*A*). In sharp contrast to the diversity among Bristol, Bergerac, PA-2, and EPC-4 when

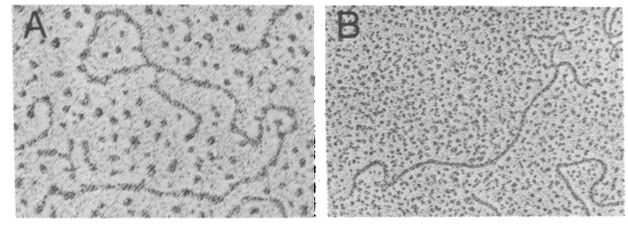

FIG. 3. Electron micrographs showing the stem-loop structure of Tc1. (*A*) *Eco*RI-digested pCe(Be)T1 was denatured and briefly renatured. Size measurements for the stem and loop of the Bergerac 5.2-kb *Eco*RI fragment and the pBR325 moiety represent mean ± SEM for at least 10 molecules of each DNA. (*B*) Heteroduplex molecule between the 3.5-kb *Eco*RI fragment in pCe(Br)T1 and the 5.2-kb *Eco*RI fragment in pCe(Be)T1. The lengths of the stem, loop, and arms are the same as in *A*.

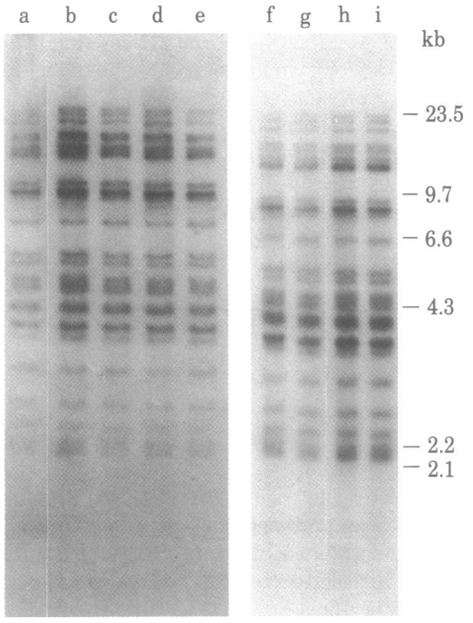

FIG. 4. Tc1 hybridization patterns to Bristol laboratory stocks. *Eco*RI digests of 3 μg of genomic DNA were hybridized with pCe(Be)T1 as described in Fig. 2. Sources of Bristol N2 stocks in each lane were as follows: a, Hirsh; b, Ward; c, Riddle; d, Caenorhabditis Genetics Center; e, Medical Research Council laboratories; f, Hirsh; g, Edgar; h, Baillie, S stock; i, Baillie, S♂ stock.

probed with Tc1, their DNA organization appeared essentially identical when other DNA fragments, such as collagen, actin, or ribosomal DNA, were used as hybridization probes. For example, the four strains show identical Southern blot profiles for

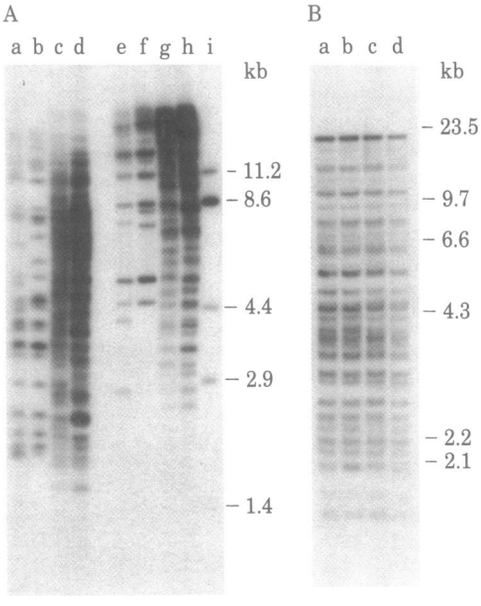

FIG. 5. Comparison of restriction fragments in various *C. elegans* strains homologous to Tc1 or collagen DNA. (*A*) pCe(Be)T1 was hybridized to DNA digested with *Eco*RI (lanes a–d) or *Bam*HI (lanes e–h) as described in Fig. 2. Sources and amounts of genomic DNA in each lane were as follows: a and e, Bristol, 3 μg; b and f, PA-2, 3 μg; c and g, Bergerac, 0.4 μg; d and h, EPC-4, 0.4 μg. Positions of DNA size markers (lane i) are indicated on the right. (*B*) A recombinant plasmid containing the collagen gene *col-1* was hybridized to 3 μg of *Eco*RI-digested DNA from various sources: lane a, Bristol; lane b, PA-2; lane c, Bergerac; lane d, EPC-4.

the collagen multigene family, in which >30 hybridizing bands are resolved (Fig. 5*B*).

Test for Hybrid Dysgenesis. In an attempt to induce Tc1 to integrate into new genomic sites at a high frequency, we constructed interstrain crosses between the Bristol, Bergerac FR, PA-2, and EPC-4 strains. Using the hybrid dysgenesis system described between P and M strains in *Drosophila* as a model (14), we examined 30–60 F_1 hermaphrodites from each of the 12 interstrain crosses and from four intrastrain crosses for reduced fertility. We also examined more than 100,000 F_2 hermaphrodites from each cross for visible mutations. We found that F_1 fertility was not reduced below control levels in any of the crosses, and we did not detect any obvious visible mutation in any of the F_2 progeny scored.

DISCUSSION

These findings show the presence of a transposable element in *Caenorhabditis elegans*. Although it shares many structural features with other eukaryotic transposable elements, the characteristics of Tc1 define a unique class. Tc1 is the smallest known eukaryotic transposable element, only 1.7 kb long, and has short inverted terminal repeats (<100 bp). All copies of Tc1 appear to be full length and have the same cleavage patterns with several restriction endonucleases, unlike the variable-length copies of the P element and FB element families in *Drosophila* (6, 7). The high degree of conservation at restriction enzyme sites reflects a highly conserved DNA sequence, consistent with the notion that Tc1 codes for products mediating its transposition.

In interstrain comparisons, the genomic arrangement of Tc1 has diverged much more rapidly than other multigene families. The Bristol N2, CL2a, GA-1, PA-2, and PaC-1 strains contain 25–30 copies of Tc1 per haploid genome and vary among themselves in a few positions, while the Bergerac and EPC-4 strains contain several hundred copies of Tc1 and also differ from one another in at least a few genomic locations. It appears that most of the Pasadena strains are closely related to the Bristol, England, isolate and that the EPC-4 strain from Pasadena is closely related to the Bergerac strain from France. However, although Bristol stocks were propagated in Pasadena laboratories a few years ago, the Bergerac strain was not; so it is doubtful that EPC is a recent lineage derived from a laboratory stock. Thus, it is curious that distinct relationships appear among geographic isolates. Perhaps mobility of the Tc1 family occurred more recently than strain separation such that an independent mechanism has allowed Tc1 to occupy a broader range of sites in some strains than others.

Analyses of Bristol stocks separated by up to 500 generations (≤8 years) gave no evidence for integration of Tc1 into new genomic sites. Similarly, the *copia* element in *Drosophila* moves at a very low frequency; a single case of a gain or loss of a *copia* element within a stock was noted in 50 years of laboratory propagation (11). By contrast, the yeast Ty1 element showed differences in Southern blot profiles between subcultures from a single colony passaged independently for 1 month (4). Movement of Ty elements apparently occurs more frequently by gene conversion than by transposition (26). Therefore, the mobility of Tc1, as assessed by Southern analyses, may be a consequence of homology-dependent mechanisms as well as of true transposition events. Proof of transposition awaits characterization of specific mutations caused by Tc1 insertions.

Using transposable elements as tools for mutagenesis and in gene-isolation studies requires either mobility at a high enough frequency to screen for mutants or a selection scheme for rare transposition events. The latter strategy is used in bacterial systems because many prokaryotic transposons carry antibiotic-re-

sistance genes. The only molecularly characterized systems in which transposable elements move at a high frequency *in vivo* are the P–M system in *Drosophila*, which causes hybrid dysgenesis, and the *Ac–Ds* controlling element system in maize (6, 14, 27, 28). We analyzed the progeny of interstrain crosses in *C. elegans* for hybrid dysgenesis but detected none. An alternative approach to introducing Tc1 into new genomic sites is to microinject exogenous Tc1 into *C. elegans* gonads, where the DNA may be incorporated into maturing gametes. In light of the success with introducing P elements into *Drosophila* embryos (29), microinjection of high numbers of the Tc1 sequence might lead to integration of Tc1 into new genomic sites.

We thank S. Emmons for communicating unpublished results and J. Karn for providing a recombinant λ1059 library. We are grateful to D. Baillie, R. Edgar, J. Hodgkin, D. Riddle, and S. Ward for providing Bristol N2 stocks and to R. Russell for providing the wild strains CL2a, GA-1, PA-2, and PaC-1. A Bristol N2 stock and the Bergerac FR stock were provided by the Caenorhabditis Genetics Center, which is supported by Contract N01-AG-9-2113 between the National Institutes of Health and the Curators of the University of Missouri. This research was supported by National Institutes of Health Grant GM26515 to D.H. and a postdoctoral fellowship from the American Cancer Society (PF-1720) to L.W.L.

1. Calos, M. P. & Miller, J. H. (1980) *Cell* **20,** 579–595.
2. Kleckner, N. (1981) *Annu. Rev. Genet.* **15,** 341–404.
3. McClintock, B. (1956) *Cold Spring Harbor Symp. Quant. Biol.* **21,** 197–216.
4. Cameron, J. R., Loh, E. Y. & Davis, R. W. (1979) *Cell* **16,** 739–751.
5. Finnegan, D. J., Rubin, G. M., Young, M. W. & Hogness, D. S. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 1053–1063.
6. Rubin, G. M., Kidwell, M. G. & Bingham, P. M. (1982) *Cell* **29,** 987–994.
7. Potter, S., Truett, M., Phillips, M. & Maher, A. (1980) *Cell* **20,** 639–647.
8. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
9. Eibel, H., Gafner, J., Stotz, A. & Philippsen, P. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 609–617.
10. Strobel, E., Dunsmuir, P. & Rubin, G. M. (1979) *Cell* **17,** 429–439.
11. Young, M. W. & Schwartz, H. E. (1980) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 629–640.
12. Roeder, G. S. & Fink, G. R. (1980) *Cell* **21,** 239–249.
13. Bingham, P. M., Kidwell, M. G. & Rubin, G. M. (1982) *Cell* **29,** 995–1004.
14. Kidwell, M. G., Kidwell, J. F. & Sved, J. A. (1977) *Genetics* **86,** 813–833.
15. Emmons, S. W., Klass, M. R. & Hirsh, D. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1333–1337.
16. Files, J. G., Carr, S. & Hirsh, D. (1983) *J. Mol. Biol.* **164,** 355–375.
17. Emmons, S. W., Yesner, L., Ruan, K. & Katzenberg, D. (1983) *Cell* **32,** 55–65.
18. Brenner, S. (1974) *Genetics* **77,** 71–94.
19. Nigon, V. (1949) *Ann. Sci. Nat. Zool. Biol. Anim.* **11,** 1–132.
20. Emmons, S. W., Rosenzweig, B. & Hirsh, D. (1980) *J. Mol. Biol.* **144,** 481–500.
21. Davis, R. W., Simon, M. & Davidson, N. (1971) *Methods Enzymol.* **21,** 413–428.
22. Prentki, P., Karch, F., Iida, S. & Meyer, J. (1981) *Gene* **14,** 289–299.
23. Karn, J., Brenner, S., Barnett, L. & Cesareni, G. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5172–5176.
24. Kramer, J. M., Cox, G. N. & Hirsh, D. (1982) *Cell* **30,** 599–606.
25. Files, J. G. & Hirsh, D. (1981) *J. Mol. Biol.* **149,** 223–240.
26. Roeder, G. S. & Fink, G. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5621–5625.
27. McClintock, B. (1956) *Brookhaven Symp. Biol.* **8,** 58–71.
28. Burr, B. & Burr, F. A. (1982) *Cell* **29,** 977–986.
29. Spradling, A. C. & Rubin, G. M. (1982) *Science* **218,** 341–347.