Mol Gen Genet (1982) 188:286–291

MGG

The Linkage Mapping of Cloned Restriction Fragment Length Differences in *Caenorabditis elegans*

A.M. Rose[1], D.L. Baillie[2], E.P.M. Candido[3], K.A. Beckenbach[2], and D. Nelson[2]

[1] Department of Medical Genetics, University of British Columbia, Vancouver, V6T 1W5, Canada,
[2] Department of Biological Sciences, Simon Fraser University, Burnaby, V5A 1S6, Canada
[3] Department of Biochemistry, University of British Columbia, Vancouver, V6T 1W5, Canada

Summary. The genomic DNA of two closely related strains of the nematode, *Caenorhabditis elegans, Bristol* (*N2*), and *Bergerac* (*Bo*), has different restriction endonuclease sites (Emmons et al. 1979). Since these two strains interbreed, it is possible to regard the restriction fragment length differences (RFLDs) as mutant variants. The *N2* and *Bo* pattern can be segregated and mapped using classical genetic techniques.

Utilizing a number of genetic markers existing in the *N2* strain, we have constructed hybrid populations homozygous for either *Bristol* or *Bergerac* over a given chromosomal region with random *Bristol-Bergerac* composition for the remainder of the genome. Genomic restriction digests from these hybrid populations were probed with random cloned fragments of *Bristol* DNA. In this way, fragments were mapped to genetically well characterized regions of the *C. elegans* genome. 27 probes which hybridize to a total of 310 Kb of DNA were found to exhibit six restriction fragment differences. Four of these differences have been mapped, providing probes for four different genomic regions. We have combined classical genetics and recombinant DNA technology to construct linkage maps of cloned DNA fragments using restriction fragment length differences. We are pursuing this approach in order to advance the knowledge of the genetic organization of *C. elegans* and to provide a means of cloning genes in an organism which provides an experimental model for the study of many biological systems. It is hoped that this approach will also provide a practical solution to some difficult problems in nematode strain identification. Furthermore, the characterization of the families of transposable elements responsible for generating many of the RFLDs will undoubtedly contribute to the understanding of the biological significance of these elements.

Introduction

In the free-living nematode, *Caenorhabditis elegans,* restriction fragment length differences (RFLDs) between two closely related strains, *Bristol* (*N2*) and *Bergerac* (*Bo*) have been described (Emmons et al. 1979). Since *Bristol – Bergerac* heterozygotes can be established, these RFLDs can be genetically mapped. From RFLDs the *Bristol – Bergerac* heterozygotes, hybrid populations homozygous for a single *Bristol* (or *Bergerac*) chromosome, but otherwise randomly heterozygous, can be established. DNA prepared from these hybrid populations can be used to map RFLDs by linkage to morphological mutants. In this way, we have applied the classical genetic tools of segregation and linkage to cloned pieces of DNA. Previously, specific cloned genes have been analysed by linkage to RFLDs (Petes and Botstein 1977; Kan and Dozy 1978; Olson et al. 1979; Hirsh, personal communication). More recently, the identification and mapping of restriction enzyme polymorphisms in humans has been proposed in order to extend the range of available genetic markers (Solomon and Bodmer 1979; Botstein et al. 1980). This approach does not require the isolation of a specific gene; rather, randomly identified RFLDs are used to establish a linkage map of cloned DNA fragments. In this paper, we describe our pursuit of this approach in an experimental organism. We have undertaken this study for a number of reasons: (1) to demonstrate the feasibility of generating an extensive linkage map of RFLDs in *C. elegans* (2) to facilitate the cloning of genes known to be of general biological importance, but for which no standard biochemical cloning methods exist; (3) to provide a model for the application of nuclear DNA polymorphisms to numerous taxonomic and evolutionary problems; and (4) to identify and characterize transposable elements in *C. elegans*, thus extending our knowledge generally about their biological significance. In this paper we describe an approach to generate a set of molecular probes for genetically well-characterized regions of the genome.

Offprint requests to: A.M. Rose

Materials and Methods

Preparation of DNA. C. elegans DNA was prepared by the method of Emmons et al. (1979) and banded on a cesium chloride gradient in the presence of EtBr (28 g of CsCl were added to 30 ml of DNA plus EtBr). The EtBr was extracted with NaCl and H_2O saturated isopropanol. The CsCl concentration was lowered by addition of 2 volumes of H_2O and DNA precipitated at −20 with EtOH (Davis et al. 1980) and stored in 10 mM Tris pH 7.5 and 1 mM EDTA. DNA prepared in this way is readily restrictable when nematodes are isolated from agar plate cultures.

Plasmid DNA was prepared from transformed *E. coli RR1* (*F^-, pro, leu, thi, lac*), *y, str^r, r_K^-, m_K^-, endo I^-, trpC9830*) after chloramphenicol amplification by the modified cleared lysate procedure of Guerry et al. (1979). Plasmid DNA was purified from CsCl density gradients.

0026-8925/82/0188/0286/$01.20

Construction of Recombinant Plasminds. High molecular weight genomic *Bristol* DNA and pBR322 were completely digested with *Eco* R1 and *Hind* III, ligated and transformed into *E. coli RR1. pCe s102* was prepared by digestion of genomic and pBR322 DNA with *Bam* H1.

Restriction Digests, Ligation and Transformation. Restriction digests and ligations were done under conditions recommended by the enzyme supplier. Restriction enzymes and T4 DNA ligase were purchased from Bethesda Research Labs. The size of restriction fragments was estimated by comparison with *Hind* III and *Eco* R1-*Hind* III digests of *λCI857* DNA. Bacteria were made competent for transformation and transformed by the method of Mandel and Higa (1979) with minor modifications. Transformed cells were plated on nutrient agar plates containing 20 μg/ml tetracycline or 50 μg/ml ampicillin.

DNA Hybridization Procedures. Following electrophoresis in horizontal agarose gels, DNA fragments were transferred to nitrocellulose filters (Schleicher and Schuel) by either the method of Southern (1975) or that of Smith and Summers (1980). Filters were pretreated at hybridization temperature in 5 × SSPE (1 × SSPE = 0.18 M NaCl; 0.010 M sodium phosphate; 1 mM EDTA pH 7.0). Hybridization was carried out in 5 × SSPE, 0.3% SDS at 62° C. Filters were washed extensively in 2 × SSPE, 0.2% SDS, at 45° C.

Small amounts of plasmid DNA were prepared according to the procedure of Davis et al. (1980) in order to estimate the size of the insert. Large amounts of plasmid DNA were prepared as described and the inserts purified from low melting point agarose (Langridge et al. 1980). ^{32}P-labelled probe DNA was prepared by nick translation as described by Maniatas et al. (1975). α^{32}P-dCTP was purchased from New England Nuclear.

Strains and Nomenclature. Caenorhabditis elegans var. *Bristol,* strain *N2,* and the mutant strains used for genetic mapping were obtained from S. Brenner and from the *Caenorhabditis elegans* genetic stock center. *N2* is the wild-type strain; all mutants were derived originally from *N2.* This paper conforms to the published guidelines for nomenclature (Horvitz et al. 1979). Gene designations used are *unc* (uncoordinated) and *dpy* (dumpy).

The naming of the plasmids used in this study was adapted from the *C. elegans* nomenclature. Thus, each plasmid from *C. elegans* (*pCe*) carries an "s" prefix to designate the laboratory in which it was generated (s = Simon Fraser University).

C. elegans Culture Conditions. For DNA preparation, *C. elegans* were grown on high peptone NGM plates: 3 g NaCl; 17 g Agar; 20 g Bacto-peptone (Difco) in 1 liter H_2O; autoclaved; and added: 2 ml cholesterol (5 mg/ml in EtOH), 1 ml 1 M $CaCl_2$; 1 ml 1 M $MgSO_4$; and 25 ml 1 M KPO_4; pH = 6.0. *C. elegans* crosses and other genetic manipulations were carried out as described by Brenner (1974).

Results

Our results demonstrate the feasibility of generating an extensive linkage map of RFLDs in *C. elegans.* In order to map these RFLDs, we have constructed hybrid populations

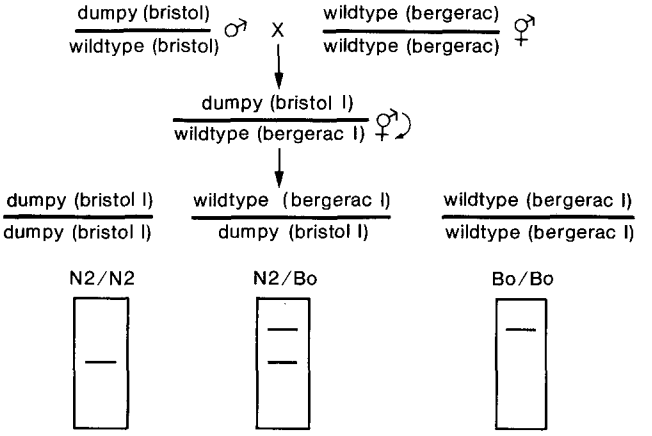

Fig. 1. The genetic protocol for generating strains used for mapping RFLDs. At the bottom of the figure is shown the expected hybridization pattern for a probe from chromosome I

of *Bristol* and *Bergerac.* It appears that the genomes of these two strains are very similar since genetic crosses between these two strains indicate that no large translocations exist between them, nor does any inversion exist for a region of chromosome *I* near *dpy-5* (data not shown). Thus, linkage of DNA differences to known *Bristol* markers can be demonstrated. We have done this by the following method. For each of chromosomes, *I, II, IV,* and *V,* a recessive mutation was chosen, for example, *dpy-5(e61)* on chromosome *I. Dpy-5* hermaphrodites were mated to wild-type *Bristol* males and the progeny, *dpy-5/+,* heterozygous males were mated to *Bergerac* hermaphrodites. Outcross progeny from this mating were allowed to self-cross individually and the expected phenotypic ratio of 3:1 wild types to dumpies was observed in the progeny. For our purposes, the dumpy marker represents a region of DNA around *dpy-5* and can be used to identify *Bristol* chromosome *I* DNA. Therefore, homozygous *dpy-5* chromosomes are homozygous for *Bristol* chromosome *I* DNA in the *dpy-5* region. A population of *dpy-5* homozygotes was then obtained as follows. For any *dpy-5* homozygote, there is a chance that a second chromosome may also be homozygous for *Bristol* DNA. However if a large number of Dpy individuals are selected, the probability of the population being randomly mixed for all other chromosomes increases. To be sure of a random mixture of all other chromosomes, we selected approximately 200 individual dumpies. A large number of progeny from each of these dumpies were grown on petri plates at 20° C. In order to control for selection against individuals in the population, all the progeny were pooled just prior to the preparation of the DNA. This DNA, referred to as *Bristol I* DNA, was then used for mapping RFLDs to the *dpy-5* region of chromosome *I.* In addition, phenotypically wild-type (non-Dpy) individuals, which did not segregate Dpy progeny, were selected individually and used to establish a supply of *Bergerac I* DNA. In a mapping experiment, a probe for a RFLDs that originated from DNA in the *dpy-5* region would show only the *Bristol* pattern with *Bristol I* DNA, and only the *Bergerac* pattern with *Bergerac I* DNA, but both patterns with DNA that was homozygous *Bristol* for any other region of the genome. Another supply of DNA, *Bristol IV,* for example, would be heterozygous *Bristol/Bergerac* for the *dpy-5* region of chromosome *I.* This mapping procedure is illustrated in Fig. 1.

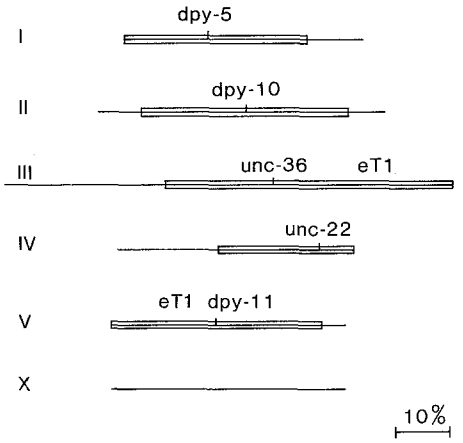

Fig. 2. Representation of the six chromosomes of *C. elegans* and the regions from which homozygous *Bristol* or *Bergerac* DNA has been prepared

Samples of DNA that could be used to map RFLDs to either the *dpy-5* region of chromosome *I*, *dpy-10*(e128) region of *II*, *unc-22*(*s18*) region of *IV*, or *dpy-11*(*e224*) region of *V* have been prepared by the method described above. In addition, a *Bristol* translocation, *eT1*, exists which eliminates recombination in the right half of *III* and the left half of *V* (Rosenbluth and Baillie 1981). This rearrangement has an uncoordinated (*Unc-36*) phenotype which was used to construct genetic strains that carry only *Bristol* (or *Bergerac*) DNA for the right half of *III* and left half of *V*. Thus, we have prepared samples of DNA from various genetic strains which are homozygous for either *Bristol* or *Bergerac* in several regions of the genome. These genomic regions along with the genetic markers used to make the DNA are illustrated in Fig. 2. Due to increasing recombination frequency distal from the genetic marker, we were not able to map RFLDs at the tips of the autosomes. The exception to this was the region covered by *eT1* (*unc-36* to the right tip of *III* and *dpy-11* to the left tip of *V*) which suppresses crossing over absolutely in this region (Rosenbluth and Baillie 1981).

In order to investigate differences in hybridization patterns between *Bristol* and *Bergerac*, we generated a random set of probes from Eco R1-Hind III digested genomic *Bristol* DNA inserted into pBR322. The recombinant clones thus obtained provided us with an average probe size of 1,500 base pairs which were hybridized to either Eco R1 or Hind III digested genomic DNA.

The results of these hybridizations are summarized in Table 1. Since we were initially concerned with detecting major hybridization band differences that could be used for RFLDs linkage experiments, the minor hybridization bands of *pCe s2* and *pCe s9* have been ignored. Our rationale has been that we are primarily interested only in bands which strongly hybridize the probes.

Six restriction fragment length differences have been detected in this study. Using 27 labelled probes and blot hybridization we have examined the 60 bands described in Table 1. Thus, 720 base pairs (bps) (12×60) have been sampled for nucleotide changes. These 60 bands represent approximately 310,000 base pairs of genomic DNA which were sampled for rearrangements. Therefore, our observed frequency for RFLDs occurrence (6/720) was one per 120 bps for nucleotide changes or one per 52,000 bps for rearrangements (6/310,000). Blot hybridizations used to screen for RFLDs are show in Figs. 3 (no RFLD) and 4 (RFLD).

Table 1. Probes used in this study. The probe size is given in base pairs, as is the total size of the major homologous genomic fragments

Probe	Size	# bands	Size	RFD	LG[a]
pCe s1	E/H 1,600	1	4,000	–	
pCe s2	E/H 2,000	9 major 3 minor	40,000	+	IV
pCe s3	E/E 1,400	5	17,500	+	III
pCe s4	H/H 1,800	1	3,000	–	
pCe s5	E/H 1,000	1 major 9 minor	2,200	–	
pCe s6	E/H 1,000	1	1,000	–	
pCe s7	E/H 1,500	1	3,500	–	
pCe s8	E/H 1,600	1	3,000	–	
pCe s9	E/H 1,200	1 major	15,000	+	NM[b]
pCe s10	H/H 400	1 major 4 minor	18,300	–	
pCe s11	E/E 1,600	1	2,000	–	
pCe s12	H/H 1,600	1	500	–	
pCe s13	E/E 600	1	15,000	–	
pCe s14	E/H 700	2	2,000	–	
pCe s15	E/H 1,000	1	2,000	–	
pCe s16	E/H 1,400	1	4,500	–	
pCe s17	E/H 800	12	44,300	+	NM[b]
pCe s18	E/H 1,600	1	5,000	+	I
pCe s19	E/H 1,500	1	2,000	–	
pCe s20	E/H 400	1	1,800	–	
pCe s21	E/H 1,200	1	4,300	–	
pCe s22	E/H 1,000	2	3,000	–	
pCe s23	E/H 2,200	1	1,500	–	
pCe s25	E/H 2,000	1	2,000	–	
pCe s27	E/H 1,600	2 major 1 minor	5,500	–	
pCe s30	E/H 5,000	2 major 1 minor	9,800	–	
pCe s102	B/S 2,200	1	7,000	+	IV
$n=27$	$x=1,478$	$n=60$	$\Sigma=309,700$	$n=6$	

[a] LG = Linkage Group
[b] NM = Not mapped; B/S = Bam HI Sal I digest

Four RFLDs have been mapped by hybridizing the probe to *Eco* R1 digested *Bristol* DNA from selected regions of the genome. The first of these to be mapped was *pCe s2*. This probe hybridized to 9 major bands detectable on *Eco* R1 digested *Bristol* DNA (Fig. 5). The smallest of these was reduced by a few hundred base pairs on *Eco* R1 digested *Bergerac* DNA. Despite the number of bands to which this probe hybridized, it has been possible to map the shift shown by this lowest band. When *pCe s2* was hybridized against *Eco* R1 cut *Bristol I* and *Bristol IV* DNA, the *Bristol* IV DNA showed only the *Bristol* pattern. The *Bristol* I DNA however, shows both the *Bristol* and *Bergerac* patterns indicating that *pCe s2* originated from chromosome *IV*. Since crossing over would have occurred in the strains from which this DNA was made, we would expect to see a faint *Bergerac* band if *pCe s2* were more than 5% from *unc-22*. Even on over exposed autoradiographs no band was seen (data not shown) and we conclude that *pCe s2* is tightly linked to *unc-22*.

In contrast, *pCe s102* which has also been mapped to

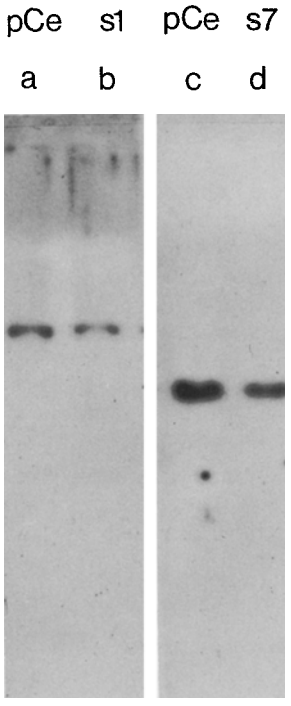

Fig. 3. Genomic blot hybridization patterns of two non-RFLD probes. *Eco* R1 digested *Bristol* DNA (lane a, c) and *Bergerac* DNA (lane b, d) was hybridized with probe *pCe s1* (lane a, b) or *pCe s7* (lane c, d)

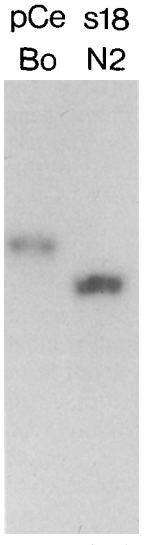

Fig. 4. Blot hybridization pattern for a RFLD probe. This probe, *pCe s18* hybridizes to a 5,500 bp fragment in *Bristol* (*N2*) and a 7,200 bp fragment in *Bergerac* (*Bo*)

chromosome *IV* shows a reduced *Bergerac* band in the *Bristol IV* lane (Fig. 6) indicating that *pCe s102* is on chromosome *IV*, but weakly linked to *unc-22*.

A third RFLD, that shown by *pCe s3*, was mapped to *Bristol eT1* DNA, but not to *Bristol V*, indicating that it is located close to or within the region affected by *eT1* on chromosome *III* (data not shown).

A fourth probe, *pCe s18*, has been mapped to the *dpy-5* region of chromosome *I* (Fig. 7). In this case, as for *pCe s2*, there is no indication of a *Bergerac* band in the *Bristol I* DNA, suggesting tight linkage to *dpy-5*. *pCe s18* has been used to screen *Bristol* Charon 4 and *Bergerac* Charon 4A libraries. From these screens, homologous phage containing *Bristol* and *Bergerac* DNA have been isolated. Examination of cloned DNA from the *pCe s18* region of chromosome I confirms that an insertion of approximately 1,700 bps of DNA exists in the *Bergerac* DNA that is absent in *Bristol*. When *pCe s18* is hybridized to the *Bergerac* genome, a minor band can be seen in the position expected if excision of the 1,700 bp insert had occurred (Fig. 7).

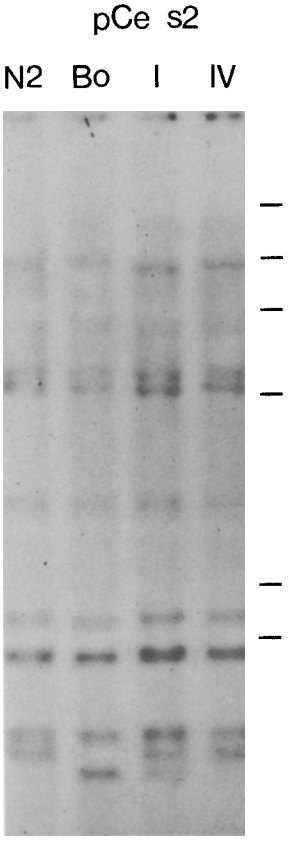

Fig. 5. Blot hybridization of *pCe s2*. The first two lanes show the *Bristol* (*N2*) and *Bergerac* (*Bo*) patterns. The bottom band is larger in *N2* than *Bo*. This difference in size is linked to a marker on chromosome IV (lane 4). The last two lanes show the hybridization patterns for *Bristol* I and *Bristol* IV strains. Both bands are present in the *Bristol* I strain (indicating heterozygousity for the probe), but only the *Bristol* band appears in *Bristol* IV. The horizontal lines at the right edge represent size markers from *Hind* III digested *λ CI857* DNA (23.3, 9.3, 6.5, 4.3, 2.2, and 1.9 Kb)

We have hybridized probes *pCe s3* and *pCe s18* to the genome of another *C. elegans* strain *FF*. In both cases the *FF* strain had homologous restriction fragments the same size as the *N2* strain, but different than *Bo*.

Discussion

Our study has shown that RFLDs in *C. elegans* are inherited as Mendelian co-dominant alleles, and thus can be used to establish a linkage map of cloned genomic fragments that overlaps the standard genetic linkage map. Furthermore, the frequency with which RFLDs between the *N2* and *Bo* strains were recovered (1/5 probes) and the high

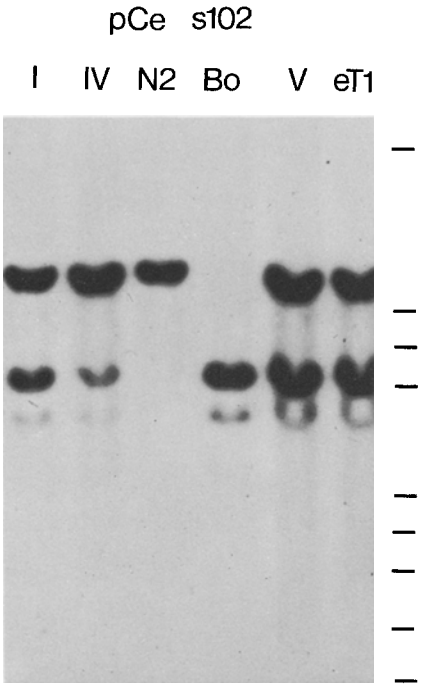

Fig. 6. Blot hybridization of *pCe s102*. This probe hybridizes to a larger fragment in *Bristol* (*N2*) than in *Bergerac* (*Bo*). When hybridized to 4 strains counstructed for mapping, only the *Bristol* IV strain showed a reduction in the amount of *Bo* fragment. Horizontal lines at the edge represent an *Eco* RI-*Hind* III digestion of *λ CI857* DNA (21.7, 5.2, 4.3, 3.5, 2.0, 1.6, 1.4, 0.9, 0.8, Kb)

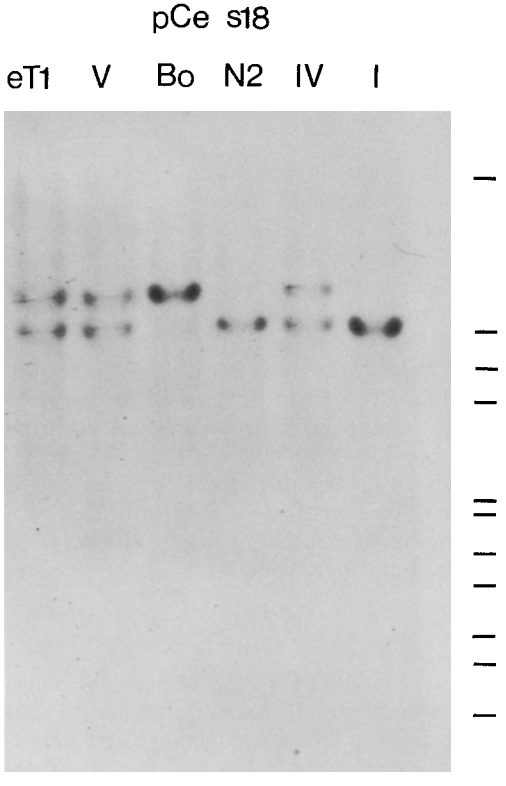

Fig. 7. Blot hybridization of *pCe s18* showing the linkage of this RFLD to *dpy-5* (*I*)

proportion of unique sequence probes (17/27) enhance the feasibility of generating an extensive linkage map of RFLDs for the *C. elegans* genome. We have developed markers for four separate chromosomal regions in our initial screen, and are encouraged that the generation of more probes will result in random coverage of the *C. elegans* genome. It will be possible to accummulate a bank of probes which would provide molecular markers for the entire genome. Our data indicate that RFLDs between *Bristol* and *Bergerac* occur at least every 50,000 bps. Consequently one of every two or three cloned Charon 4 fragments could be expected to carry a RFLD which can be used to place almost any cloned fragment on the genetic map.

The reliability of the mapping procedure depends upon the quality of the genetic strains used to make the DNA. We have taken great care in the construction and culturing of these strains to ensure that no chromosome is preferentially lost due to culture conditions. This was important because the *Bo* strain possesses a temperature sensitive and an uncoordinated phenotype which differ from *N2*. Also by growing individual progeny on uncrowded, high nutrient plates, we hope to have reduced selection against deleterious genes that could eliminate certain chromosomes. Finally, by pooling the progeny from large numbers of individuals, we hope to have minimized any selection that has occurred. As our catalogue of mapped probes increases it will be possible to confirm the heterozygosity of each DNA by hybridization. For example, the *Bristol* DNA from the *eTl, V,* and *IV* strains appears to be equally representative of both *Bristol* and *Bergerac* chromosome *I* DNA as shown by hybridization to *pCe s18* (Fig. 7). Within a lane the *Bristol* and *Bergerac* bands appear to be of equal intensity.

In the nematode cloned genes have been isolated by hybridization to biochemically characterized probes, for example, the cloning of the myosin heavy chain gene (MacLeod et al. 1981). However, one of *C. elegans* greatest strengths as an experimental organism is its potential usefulness for the genetic dissection of a wide range of biological pathways, particularly since some mutants exist in *C. elegans* (for example, in muscle and cell lineage) that have not been identified in other organisms (for reviews see Brenner 1974; Riddle 1978; Zuckerman 1980). At present there is no method for obtaining molecular probes for most of these characterized genes, whose products have not been biochemically identified. Our probes can be used to screen lambda libraries, as we have done with *pCe s18* in order to "walk" into the specific region of interest. In *C. elegans* the small genome size (8×10^7 bps of DNA) and the relative lack of repetitive DNA (83% unique sequence) (Sulston and Brenner 1974) make chromosome walking realistic. Our own experience is that the amount and distribution of repetitive DNA in *C. elegans* is such that walking between RFLDs is feasible.

At least one of the RFLDs described in this paper, *pCe s18*, is the result of a insertion of DNA into the *Bo* strain. A 1,700 bp transposon in *C. elegans*, Tc1, has been identified (Emmons, personal communication). Tcl inserts differentially into *N2* and *Bo*, and excises at a low level. Thus, it is most likely that an insert of Tcl has occurred in the *Bo* strain within the DNA homologous to *pCe s18*. Not all of our RFLDs however can be accounted for by Tcl insertion. We are characterizing the source of some others.

The high frequency of occurrence of RFLDs between

the *Bristol* and *Bergerac* strains suggests the feasibility of using nuclear RFLDs as a taxonomic tool. There are many cases in agriculture, fisheries, and forestry where strain identification is important. RFLDs (DNA polymorphisms) in mitochondrial DNA are being used to investigate evolutionary relationships; we (Curran, Rose and Baillie) are characterizing nuclear RFLDs in closely related parasitic nematodes as a solution to an extremely difficult taxonomic problem. This promising approach could eventually provide a reliable and relatively fast method for identifying the parasitic strain, and hence, for recommending an appropriate resistant host. The biological and economic contribution of this application would be extensive. In this regard, the feasibility of other applications of nuclear RFLDs characterization is being investigated.

The method described in this paper, that of randomly selecting cloned fragments of DNA and characterizing them using RFLDs provides a novel approach to many biological questions about genome organization and evolutionary relationships, not only in *C. elegans* but in a wide range of organisms including man. We have demonstrated in this paper the feasibility of such an approach in one experimental organism, and are in the process of extending the methodology to include economically relevant applications.

Acknowledgements. We thank R. Rosenbluth, T. Rogalski, B. Honda for construction of the following genetic strains; *Bristol* (and *Bergerac*) *eTI, IV*, and *V*; T. Snutch for construction and screening of the *Bristol* Charon 4 library; T. Snutch and S. Wood for construction of the *Bergerac* Charon 4A library; C. Salamanca and L. Hale for technical assistance. Some *C. elegans* strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is supported by contract number NO1-AG-9-2113 between NIH and the Curators of the University of Missouri. This work was supported by grants to DLB from NSERC, MDA (Canada) and B.C. Health. AMR is a MDA (Canada) postdoctoral fellow.

References

Brenner S (1974) The genetics of *Caenorhabditis elegans*. Genetics 77:71–94

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Butler MH, Wall SM, Luehrsen KR, Fox GE, Hecht RM (1981) Molecular relationships between closely related strains and species of nematodes. J Mol Evans 18:18–23

Davis RW, Botstein D, Roth JR (1980) A manual for genetic engineering: advanced bacterial genetics. Cold Spring Harbor Laboratory, New York

Emmons SW, Klass MR, Hirsh D (1979) Analysis of the constancy of DNA sequences during development and the evolution of the nematode *Caenorhabdits elegans*. Proc Natl Acad Sci USA 76:1333–1337

Guerry P, Leblanc DJ, Falkow S (1979) In: Wu R (ed) Methods in enzymology, vol 68, Recombinant DNA. Academic Press, New York, p 270

Horvitz HR, Brenner S, Hodgkins J, Herman RK (1979) A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Genetics 86:275–287

Kan YM, Dozy AM (1978) Polymorphism of DNA sequence adjacent to human β-globin structural gene: relationship to sickle mutation. Proc Natl Acad Sci USA 75:5631–5635

Langridge J, Langridge P, Berquist PL (1980) Extraction of DNA from agarose gels. Anal Biochem 103:264–271

MacLeod AR, Karn J, Brenner S (1981) Molecular analysis of the *unc-54* myosin heavy chain gene of *Caenorhabditis elegans*. Nature 291:386–390

Mandel M, Higa A (1979) In: Wu R (ed) Methods in enzymology, vol 68, Recombinant DNA. Academic Press, New York, p 252

Maniatas T, Jeffrey A, Kleid DG (1975) Nucleotide sequence of the rightward operator of phage. Proc Natl Acad Sci USA 72:1184–1188

Olson MV, Loughney K, Hall BD (1979) Identification of the yeast DNA sequences that correspond to specific tyrosine-inserting nonsense suppressor loci. J Mol Biol 132:387–410

Petes TK, Botstein D (1977) Simple Mendelian inheritance of the reiterated ribosomal DNA of yeast. Proc Natl Acad Sci USA 74:5091–5095

Smith GE, Summers MD (1980) The bidirectional transfer of DNA and RNA to nitrocellulase or DBM paper. Anal Biochem 109:123–129

Solomon E, Bodmer WF (1979) Evolution of sickle variant gene. Lancet I:923

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoesis. J Mol Biol 98:503–517

Sulston JE, Brenner S (1974) The DNA of *Caenorhabditis elegans*. Genetics 77:95–104

Riddle DL (1978) The genetics of development and behavior in *Caenorhabditis elegans*. J Nematol 10:1–16

Rosenbluth RE, Baillie DL (1981) The genetic analysis of a reciprocal translocation, *eT1* (III: V), in *Caenorhabditis elegans*. Genetics 99:415–428

Zuckerman BM (ed) (1980) Nematodes as biological models. Academic Press, New York

Communicated by M.M. Green

Received August 23 / September 2, 1982