

Cloning within the *unc-43* to *unc-31* interval (linkage group IV) of the *Caenorhabditis elegans* genome using Tc1 linkage selection

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The region around the twitcher gene, *unc-22*, flanked by *unc-43* on the left and by *unc-31* on the right, has been intensively studied in our laboratory over the period of the last 8 years. In this paper we describe the identification and isolation of probes specific for several restriction fragment length differences (RFLDs) which lie within this region. Many RFLDs in *Caenorhabditis elegans* are caused by the insertion of a transposable element, Tc1. The method we used involved the isolation of Tc1-containing genomic fragments. These were recovered from a λ gt10 library of DNA from a specially constructed genetic strain containing the *unc-43* to *unc-31* interval from the BO strain and the rest of the genome from N2. Because the BO strain is rich in Tc1 insertion sites and the N2 strain has few, the majority of Tc1-bearing genomic fragments in the constructed strain were derived from the *unc-22* region. Of nine such Tc1-bearing genomic fragments isolated, six were found which mapped within the region of interest. The 350 kilobases of genomic sequences isolated as a result of these studies are being used to study the molecular organization of this region. The method described here for Tc1 linkage selection is one that is rapid, general, and may be targeted to any genetically characterized region of the *C. elegans* genome.

Key words: Tc1, *unc-22*, restriction fragment length differences, *Caenorhabditis elegans*, linkage selection.

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La région autour du gène de contraction (saccades), *unc-22*, flanquée sur la gauche par le *unc-43* et sur la droite par le *unc-31*, a été étudiée de façon intensive au cours des huit dernières années. Dans ce document, les auteurs décrivent la méthode d'identification et d'isolation de sondes spécifiques pour plusieurs fragments de restriction de différentes longueurs (RFLDs) qui sont situés dans cette région. Plusieurs RFLDs chez *C. elegans* sont causés par l'insertion d'un élément transposable, le Tc1. La méthode utilisée implique l'isolation de fragments génomiques contenant du Tc1. Ceux-ci furent recouverts d'un feuillet λ gt10 d'ADN chez une souche génétique spécialement développée qui contient l'intervalle *unc-43* à *unc-31* de la souche BO et le reste du génome de la souche N2. Puisque la souche BO est riche en sites d'insertion de Tc1 et que la souche N2 n'en a que peu, la majorité des fragments génomiques porteurs de Tc1 chez la souche développée fut dérivée de la région *unc-22*. Sur neuf tels fragments génomiques isolés porteurs de Tc1, six se sont avérés appartenir à la région d'intérêt. Les 350 kilobases de séquences génomiques isolées comme résultat de ces recherches sont utilisées aux fins d'une étude sur l'organisation moléculaire de cette région. La méthode décrite ici, soit celle de la sélection de linkages de Tc1, en est une rapide, générale qui peut être appliquée à toute région génétiquement caractérisée du génome de *C. elegans*.

Mots clés: Tc1, *unc-22*, fragments de restriction de différentes longueurs, *Caenorhabditis elegans*, sélection de linkage.

[Traduit par le journal]

Introduction

In this paper we describe the targeted isolation and partial characterization of cloned probes defining restriction fragment length differences (RFLDs) around the region flanking the *unc-22* gene in *Caenorhabditis elegans*. Previously we described a method for obtaining cloned molecular probes for genetically defined genes whose products had not been biochemically identified (Rose et al. 1982). That method involved the

two closely related *C. elegans* strains, var. *Bristol* (N2) and var. *Bergerac* (BO) (Emmons et al. 1979) and cloned probes for two regions of the genome were identified: pCe s18 which identifies a strain polymorphic site (*sP1*) in the *unc-15* region of linkage group (LG) I (Rose and Baillie 1980; Rose et al. 1982; Harris and Rose 1984) and pCe s102 (*sP2*) which maps into the highly polymorphic region surrounding the heat shock genes on LG IV (Snutch and Baillie 1983, 1984;

targeted cloning of genes. Our method makes use of a special class of RFLD caused by the insertion of the transposable element, Tc1 (Emmons et al. 1983). Unlike our previous approach of mapping randomly obtained RFLDs, here we describe a method for the directed cloning of a specific region of the genome.

The region we selected is the interval between *unc-43* and *unc-31* on LG IV. This region contains the twitcher gene *unc-22*. The organization of this gene (Moerman and Baillie 1979; Moerman 1980) and the region around the gene (Rogalski et al. 1982; Rogalski 1983; Rogalski and Baillie 1985; L. Donati and D. L. Baillie, unpublished results) has been the object of intense genetic analysis. Mutational analysis of this region has revealed a cluster of 16 essential genes to the left of *unc-22*, whereas in the same interval to the right of *unc-22* only 4 essential genes were identified (Rogalski and Baillie 1985). The isolation of cloned probes for the *unc-22* region of LG IV makes possible studies of the molecular organization of this region.

Materials and methods

Caenorhabditis elegans strains and genetic crosses

Wild-type and mutant strains were maintained and mated on nematode growth medium (NGM) streaked with *Escherichia coli* OP50 (Brenner 1974). Crosses were carried out on 10 × 35 mm culture plates. All experiments were performed at 20°C. The wild-type strain N2 and some mutant strains of *C. elegans* var. *Bristol* were originally obtained from the MRC stock collection in Cambridge, England. Others were received from the *Caenorhabditis* Genetics Center at the University of Missouri, Columbia. *Caenorhabditis elegans* var. *Bergerac* (BO) was obtained from D. Hirsh in Boulder, Colorado. The following mutant genes and alleles were used: *unc-22* (*h3*), *unc-22* (*s7*), *unc-31* (*e169*), and *unc-43* (*e266*).

Ethyl methanesulfonate mutagenesis

Individuals from the *Bergerac* strain, BO, were treated with 0.01 M ethyl methanesulfonate in M-9 buffer for 4 h. The F₂ descendents were screened for mutant phenotypes and a twitcher recovered. This allele (*h3*) failed to complement a known *unc-22* allele, *s7*.

Preparation of *C. elegans* DNA

DNA was isolated from the strains of *C. elegans* as previously described (Rose et al. 1982).

Construction of λ gt10 library

A λ gt10 vector, a gift from T. Huynh, was used to construct a library of *unc-22*(*h3*); N2/BO DNA in the following way. *unc-22* DNA (0.1 μ g) was digested to completion with *Eco*RI and ligated in the presence of 1 μ g of *Eco*RI-digested λ gt10 vector. The ligation mix was packaged according to the method described in Davis et al. (1980) using packaging extracts prepared by T. Snutch. Approximately 10⁶ phages/ μ g of DNA were recovered. Restriction digests were done under conditions recommended by the supplier (Bethesda Research Labs). T4 ligase was prepared from an *E. coli* lysogen

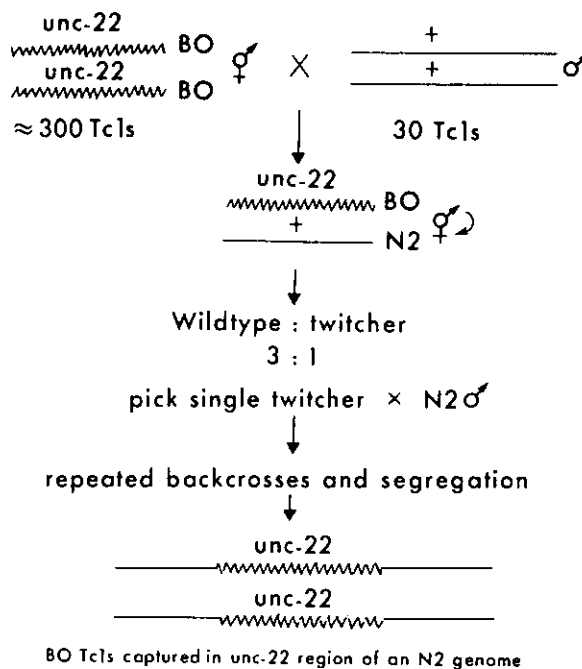


FIG. 1. The genetic protocol followed to generate the *unc-22* hybrid strain. BO DNA is shown by wavy lines and N2 DNA by straight lines.

Phage purification

The probes used to isolate Tc1-containing phages from the λ gt10 library were Tc1 (compliments of S. Emmons) and Tc1(Hin), a variant which differs by the presence of a *Hind*III site (Rose et al. 1985). A pUC13 vector (Viera and Messing 1982) containing Tc1(Hin) was nick translated (Davis et al. 1980) to a specific activity of 10⁷–10⁸ cpm/ μ g. Phages (10⁵) were screened for homology to the probe by the method of Benton and Davis (1977). Plaque-purified phages were amplified and 10⁷ phages were plated on media prepared in 25 × 35 cm lasagna trays. After 8 h of growth at 37°C the tray was flooded with 70 mL of λ diluant (10 mM Tris pH 7.5; 10 mM MgSO₄) at 4°C. The phages in λ diluant were left overnight at 4°C, collected, and purified by centrifugation in a CsCl gradient (Davis et al. 1980). Phage DNA was extracted by the formamide method described in Davis et al. (1980).

Subcloning

Subcloning was carried out as described by Snutch (1984). One microgram of λ gt10 carrying the fragment to be cloned and 0.1 μ g of plasmid vector pUC13 were mixed in 10 μ L of *Eco*RI restriction buffer and restricted with 2 units of *Eco*RI for 2 h. The reaction was stopped by heat inactivation at 70°C for 5 min. After cooling to room temperature, 0.1 μ g of DNA ligase was added. Ligation was performed at 16°C for 2 h followed by a sixfold dilution and stored at 4°C overnight. Fifty microlitres of ligation mix were used to transform competent *E. coli* strain JM83 by the method of Morrison (1979). Transformed cells were plated on XGal, IPTG, and ampicillin-containing L-broth plates, and white colonies were se-

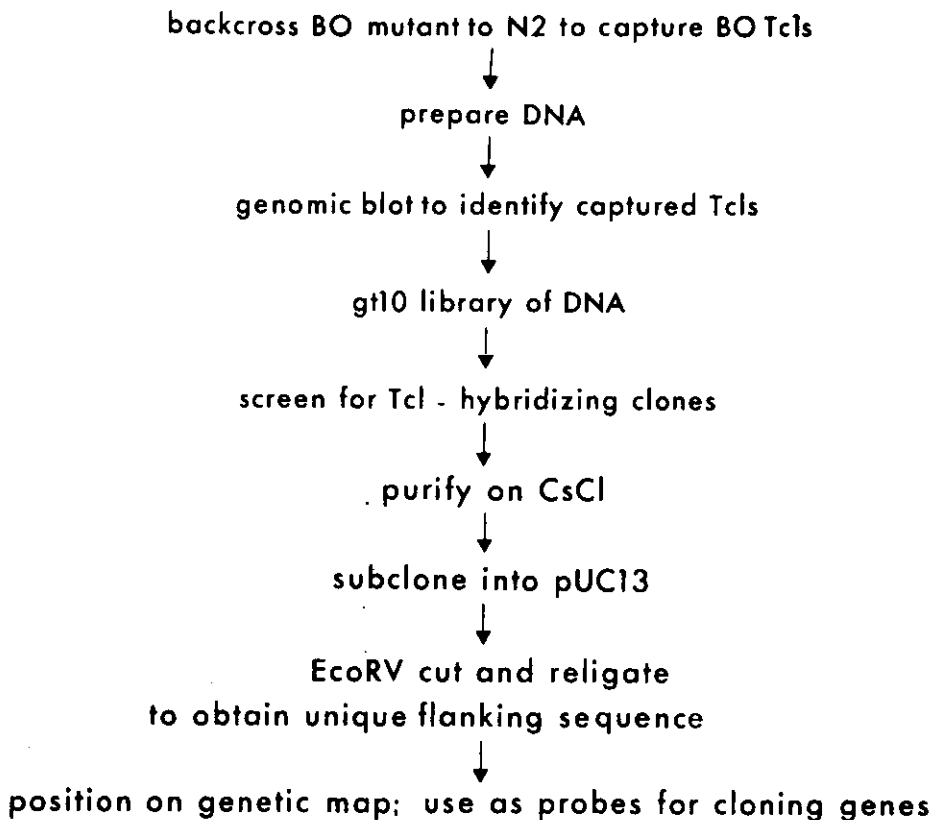


FIG. 2. Flow diagram of the cloning procedure.

from 10 mL overnight cultures by a method described in Davis et al. (1980).

Flanking sequences of the TcIs were isolated by digesting the subcloned fragment with *EcoRV*, which cuts in the terminal repeats of Tc1. The ends were then religated and transformed into JM83. Single colonies were isolated and screened for the absence of Tc1. These were then grown to maturity, and the DNA that was isolated was used to map the RFLDs.

Blot hybridization

Genomic DNA was digested with restriction endonucleases and electrophoresed in submerged horizontal agarose gels. DNA fragments were transferred to nitrocellulose filters (Schleicher and Schuel) by the method of Smith and Summers (1980). Prehybridizations were carried out in $5 \times$ SSPE (SSPE = 0.18 M NaCl, 1 mM Na₂EDTA, 10 mM phosphate buffer, pH 7.0) and 0.3% SDS at either 62 or 68°C for at least 1 h. Overnight hybridizations were done under the same conditions after adding 10^6 cpm of [³²P]labelled probe DNA per millilitre of hybridization solution. Filters were washed in three changes of $2 \times$ SSPE at 62°C and air dried prior to autoradiography. Hybridizations to phage or plasmid DNA were done for 1–4 h.

Construction of three-factor mapping strain DNA

RFLDs were initially mapped to LG IV. The LG IV mapping DNA was N2 for LG IV in a mixed N2/BO genome

To position the Tc1-induced RFLDs with the 2 map unit interval defined by *unc-43* and *unc-31*, we constructed N2–BO recombinant chromosomes in either the *unc-43* to *unc-22* or the *unc-22* to *unc-31* region. These recombinants were used to prepare DNA. This was done as follows. The *cis*-heterozygote, *unc-22 unc-31* (N2)/+ + (BO), was constructed and six *Unc-31* crossover individuals were isolated from the self-cross progeny by T. Rogalski. From each of the six *Unc-31* individuals, + (BO) *unc-31* (N2)/*unc-22 unc-31* (N2), a single individual homozygous for the crossover chromosome was identified. Homozygous recombinant individuals were grown separately for DNA. In a similar manner, nine *Unc-43* recombinants were isolated by L. Turner and grown. DNA from each recombinant was studied separately, or in some cases, equal amounts of DNA from individual isolates of one recombinant class were pooled for genomic blot hybridization experiments.

Results

Genetic strains that contained BO DNA in the *unc-22* region of linkage group IV and N2 DNA for the rest of the genome were constructed using a *Bergerac* mutant allele, *h3*. Individuals homozygous for *unc-22* (*h3*) were outcrossed to N2 males, and heterozygous hermaphrodite progeny resulting from this mating were

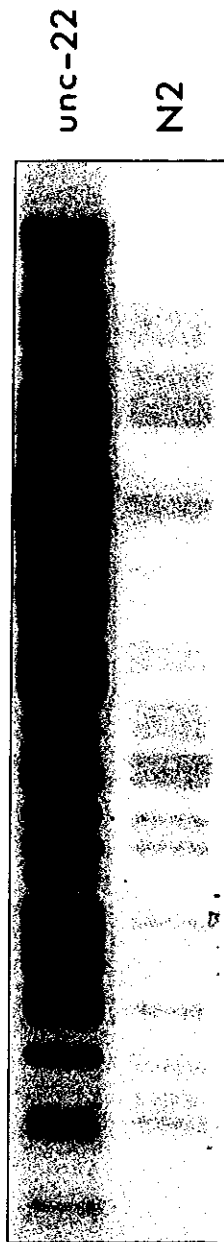


FIG. 3. Autoradiograph of TcI hybridization to N2 and *unc-22* genomic DNA digested with *EcoRI*. Two micrograms of DNA were loaded in each lane.

was isolated as the progenitor of the first backcross strain. This procedure was repeated six times, such that approximately 98% of the *h3* genome was replaced with N2 DNA except for the region immediately adjacent to the *unc-22* gene (Fig. 1). Because each backcross diluted the remaining BO portion of the genome by 50%, then after six backcrosses the BO portion is $(1/2)^6$, that

on 100-mm culture plates and the DNA was prepared. This DNA was referred to as "*unc-22*" DNA.

The flanking DNA from sites containing TcI insertions in the BO strain provided probes for RFLDs which were mapped within the *unc-22* region. A flow diagram of each of the steps to obtain the RFLD probes is shown in Fig. 2. A number of BO TcIs were present in the "*unc-22*" DNA as can be seen in Fig. 3. In N2, 30 or more bands can be identified, whereas in the *unc-22* strain considerably more (greater than 60) are present. As described in the Materials and methods, a λ gt10 library was prepared from the "*unc-22*" DNA. Using TcI(Hin) as the probe, the library was screened for TcI-hybridizing clones. After plaque purification of the identified phage, phage bands were collected from CsCl gradients. Thirteen phages were isolated in this way. Each of the phages were digested with *EcoRI* to identify the insert sizes. Nine different inserts were observed (four fragments were independently isolated twice) and are shown in Fig. 4. In this sample of TcI-containing phages the fragment sizes ranged from 1900 to 4300 base pairs (bp). *EcoRI*-digested phage inserts were subcloned into the plasmid vector pUC13. DNA from pUC13 subclones was prepared using 10 mL overnight cultures, and digested with *EcoRV* which cuts in the TcI terminal repeats (Rosenzweig et al. 1983) producing a 1600-bp fragment (Fig. 5). All the subclones but one, lane 4, gave a 1600-bp band as expected. Further analysis of this subclone is not described in this paper. The two largest inserts each had an additional *EcoRV* site in the flanking sequence. (The additional *EcoRV* fragments did not hybridize to TcI.) One (lane 8) gave a 1600-bp doublet and another (lane 9) also gave a 1600-bp doublet plus an 1100 bp fragment. (In addition both has pieces attached to the pUC13 vector.)

In order to clone flanking sequence after TcI elimination, a portion of the *EcoRV* digestion mix for each of eight subclones was diluted 10-fold to favor a ring-closure reaction and religated. Following transformation into *E. coli* JM83, individual white colonies were isolated. DNA was prepared from 10-mL overnight cultures and digested with *EcoRI*. If the TcI sequence had been removed, each subclone should now contain and *EcoRI* fragment of the original size minus 1600 bp. This was observed for six of the cloned TcIs. In the case of the two largest, which had additional *EcoRV* sites in the flanking sequence, one of them (pCe s237) has 700 bp of pUC13-attached flanking sequence. The other, pCe s238, has 100 bp of pUC13-attached flanking sequence plus the 1100-bp *EcoRV* fragment. *EcoRI* digests of these probes demonstrated that the expected fragment sizes had been cloned. In this manner, we obtained flanking sequence probes for RFLDs between the N2 and BO strains.

M 1 2 3 4 5 6 7 8 9 M

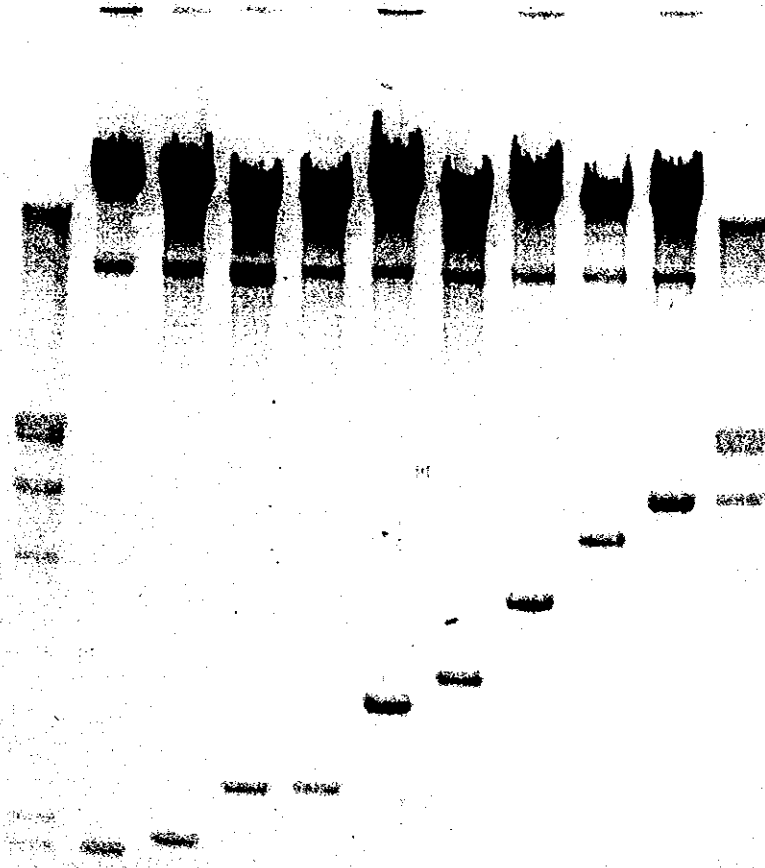


FIG. 4. Photograph of an ethidium bromide stained agarose gel of an *Eco*RI digest of gt10 clones. Phage arms can be seen at the top of the lanes. Phage giving rise to the subsequent subclones listed in Table 1 are shown: pCe s229 (lane 1); pCe s230 (lane 2); pCe s231 (lane 3); lane 4; pCe s233 (lane 5); pCe s234 (lane 6); pCe s235 (lane 7); pCe s236 (lane 8); and pCe s237 (lane 9). Size marker was *Eco*RI-*Hind*III digested *Cl857* DNA (21, 5.3, 5.1, 4.3, 3.4, 2.0, 1.9, 1.6, 1.4, 0.95, 0.83, 0.56 kb). Fragments below 1.9 kb are not seen on this gel.

mic blots were prepared using N2, BO, the "*unc-22*," and LG IV mapping DNA. The LG IV mapping DNA was N2 for LG IV in a mixed N2/BO genome and was prepared as described previously (Rose et al. 1982). These blots were probed with the plasmids containing flanking sequence DNA. Two of the probes were not linked to *unc-22*. One, pCe s230, was an insertion into the N2 strain. Another, pCe s238, a BO insertion, gave a complex banding pattern and did not map to LG IV. Further analysis of these two clones is not described in this paper. Five of the plasmids which were BO insertions were unique sequences, whereas one (pCe s235) was a low repetitive sequence. All of the plasmids generated RFLDs and were linked to *unc-22*. Tight linkage to *unc-22* is indicated by the presence of only an N2

DNA. No indication of a BO band was observed for experiments done with pCe s229 or pCe s233. Weak bands could be seen with the others.

In order to more accurately position these sites with regard to *unc-22*, blot hybridizations were done using pooled *Eco*RI-digested DNA from the recombinants of the three-factor crosses described in the Materials and methods. Homozygous recombinants from the 1 map unit interval either to the right or the left of *unc-22* were grown individually for DNA and equal amounts of each were pooled for genomic blot hybridization experiments. The pooled DNA was used for the initial positioning; then DNA that was prepared from individual recombinant events was tested. For example, the polymorphism *sP6* was mapped by using the flanking se-

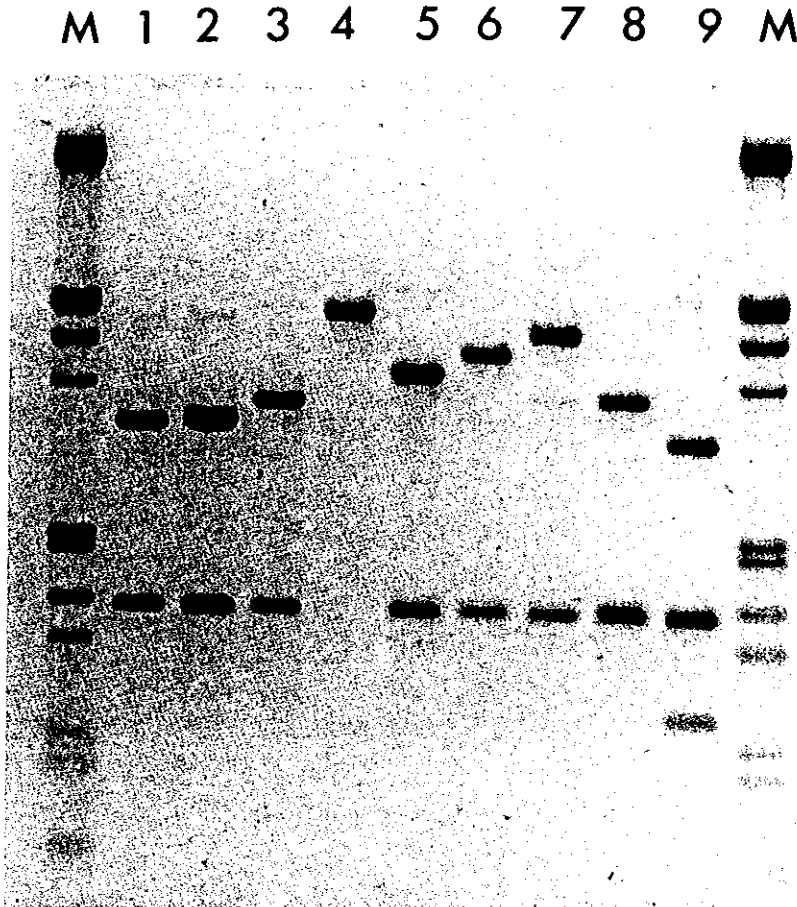


FIG. 5. *EcoRV* digest of the initial plasmid subclones obtained prior to *TcI* elimination. Lanes and markers as for Fig. 4. The subclone in lane 4 contained a single *EcoRV* site and was not analyzed further.

EcoRI-digested genomic DNA from nine *Unc-43* recombinants was probed. These *Unc-43* recombinants were derived from an *unc-43 unc-22* (N2) chromosome over a + + (BO) chromosome and the homozygous recombinant used to prepare DNA. The resulting autoradiograph is shown in Fig. 6. As can be seen, eight recombinants showed the N2 (1700 bp) and one showed the BO (3300 bp) fragment size. Thus, the *sP6* site lies close to *unc-43*, one-ninth of the way across the interval between *unc-43* and *unc-22*. In agreement with this position, the *Unc-31* recombinants clearly showed the *sP6* site to be to the left of *unc-22*. These results are summarized in Table 1. In a similar manner, *Unc-31* and *Unc-43* data placed the sites which were identified by pCe s234 (*sP5*), pCe s231 (*sP7*), and pCe s237 (*sP8*) clearly to the left of *unc-22*. Furthermore, *sP7* and *sP8* were very close to or to the left of *unc-43*. pCe s229 was used to position the *sP3* site. Data based on the *Unc-43* recombinants placed the RFLD site at or to

this site was half-way between *unc-22* and *unc-31*. The *Unc-31* mapping data places the site identified by pCe s233 (*sP4*) to the left of *unc-22*, whereas the *Unc-43* data places it to the right. Therefore, *sP4* must be very tight to *unc-22*. A summary of the results of the mapping data is given in Table 1 and illustrated in Fig. 7 which gives a detailed map of the *unc-22* region (Rogalski and Baillie 1985).

In addition to the above results, we hybridized the LG IV probes to a second *Bergerac* strain, FR. We had shown previously that FR had the same fragment size as N2 for the *sP1* site identified by pCe s18 on LG I (Rose et al. 1982). The results of genomic blot hybridizations using *EcoRI*-digested DNA from the FR strain are shown in Table 2. All of the RFLDs to the left of *unc-22* showed the BO pattern, whereas all those to the right including the 70 kd heat shock region RFLDs showed an N2 pattern. Thus in the FR strain, one portion of LG IV was N2-like and another BO-like.

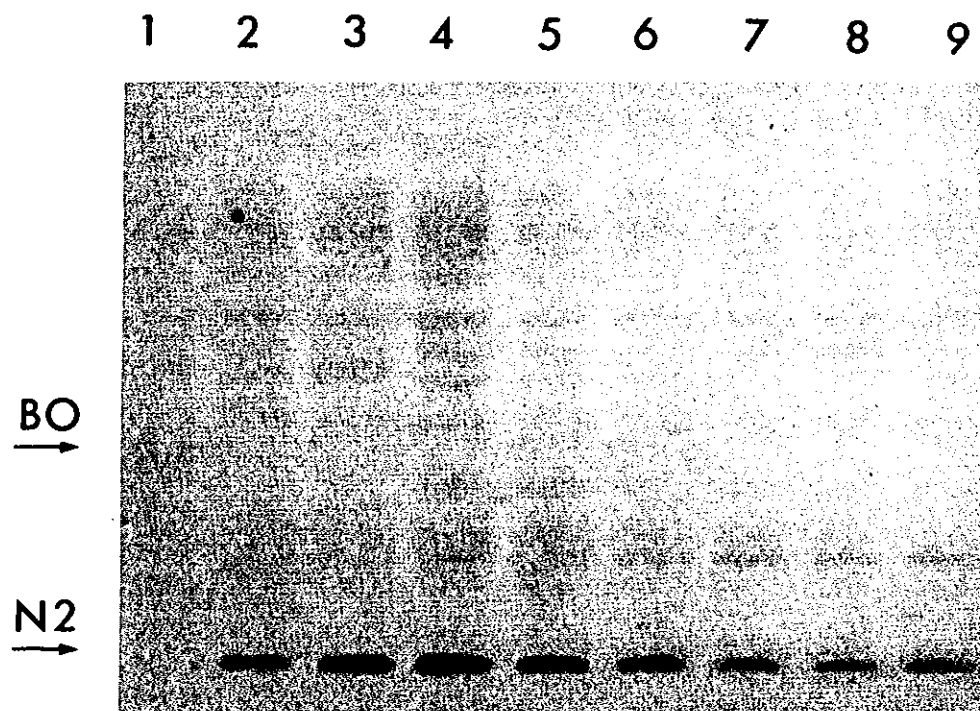


FIG. 6. Autoradiograph of a genomic blot hybridization probed with the flanking sequences from pCe s235. Each of the lanes contained DNA from one of nine *Unc-43* recombinants described in the Materials and methods. The upper arrow indicates the position of the 3300-bp BO fragment size. The lower arrow indicates the position of the 1700-bp N2 fragment size. Lane 1 is underloaded relative to lanes 2-9.

TABLE I. Summary of flanking sequence plasmids

Name of plasmid	Original fragment size	Both RV sites	Insert size (bp)	Inserted strain	Unc-31*	Unc-43*	Name of site
pCe s229	1900	+	300	BO	3/6	9/9	sP3
pCe s230	1950	+	350	N2			
pCe s231	2300	+	700	BO	6/6	0/9	sP7
pCe s232	2350	1 only					
pCe s233	2600	+	1000	BO	6/6	9/9	sP4
pCe s234	2800	+	1200	BO	6/6	2/9	sP5
pCe s235	3300	+	1700	BO	6/6	1/9	sP6
pCe s237	3900	+	700	BO	6/6	0/9	sP8
pCe s238	4300	+	1100	BO		Not on LG IV	

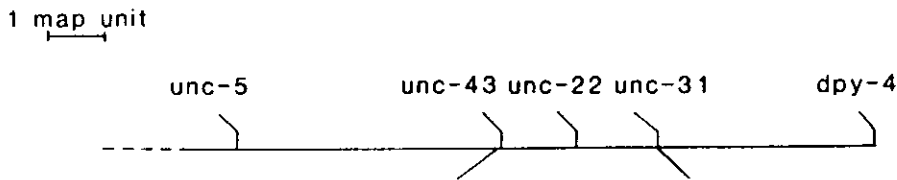
*Number of crossovers with the BO band size from either *Unc-31* or *Unc-43* crossover products derived, respectively, from either *unc-22 unc-31* (N2)/+ + (BO) or *unc-43 unc-22* (N2)/+ + (BO) heterozygotes.

to screen N2 genomic libraries and a set of overlapping Charon 4 phages have been obtained using these probes. Cosmids that overlapped these phage have been obtained compliments of J. Sulston, MRC Hills Road, Cambridge. At present, approximately 350 kb of DNA sequence covering the interval between *unc-43* and *unc-31* have been obtained. Extensive characterization of

Discussion

We describe here the targeted isolation of cloned DNA from the region flanking *unc-22*. We have used a rapid and general method that can be used for any genetically characterized region of the *C. elegans* genome. An outline of the method is shown in Fig. 2. Certain aspects deserve comment. First, the generation

A



B

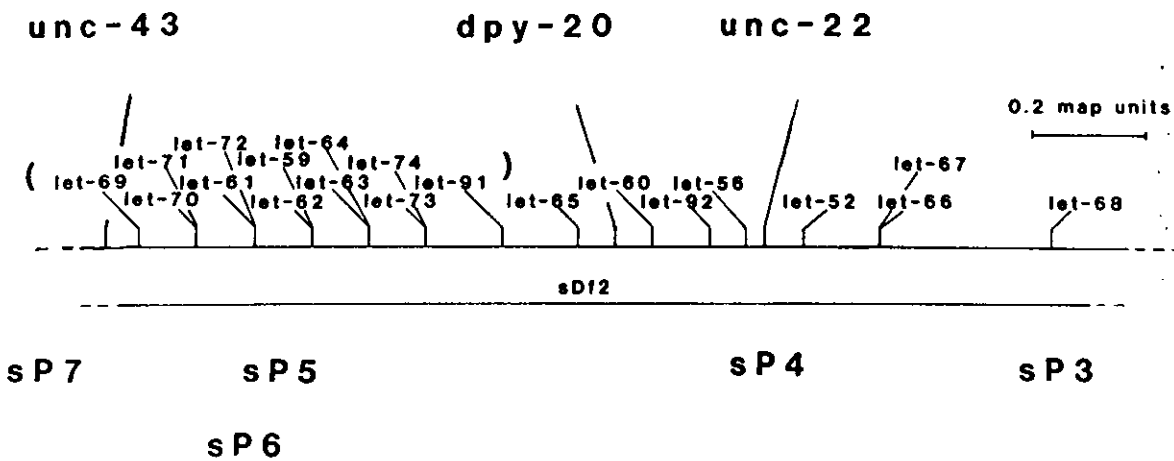


FIG. 7. (A and B) Genetic map of the *unc-22* region showing the positions of the RFLDs. The gene map is from Rogalski and Baillie (1985). Lethals to the left of *unc-22* (in parentheses) are not positioned relative to each other. The *sP8* site is inseparable from the position of *sP7* and is not shown.

TABLE 2. Fragment pattern in the FR strain

Chromosome position	FR band pattern
sP8	BO
sP7	BO
sP6	BO
sP5	BO
sP4	N2
sP3	N2
sP2*	N2
Heat shock region†	N2

*pCc s102 RFLD not due to Tc1 insertion.
 †Three non-Tc1 RFLDs tested (Snutch 1984).

fragments that are smaller than 7000 bp and indeed, the largest fragment we have identified in this library was 6000 bp. Further, the Tc1 hybridization pattern was examined on a genomic blot of *unc-22* DNA. As can be seen in Fig. 3, many additional Tc1-hybridizing bands were present as would be expected. Many, but not all, of the *unc-22* Tc1s cloned by us were readily distinguishable on this autoradiograph. Apparently there were cryptic Tc1 insertion fragments which were masked by the N2 bands. Fortunately of the nine Tc1 insertions isolated, seven were BO insertions and of these, six mapped to the *unc-22* region. The fact that all cloned Tc1 insertion sites examined were found to be occupied in one or the other of the parental lines indicated that none of these Tc1 insertions resulted from mobilization of the element during the construction of the strain. Although there are several *EcoRV* sites in λ gt10, it is possible by visual examination of agarose gels to determine whether or not a 1600-bp band is present in the purified λ gt10 phage. *EcoRV* sites exist

repeated backcrosses to N2 in order to construct the appropriate genetic strain. Second, the use of the inser-

band (more precisely 1572) by *EcoRV* digestion is a simple way of confirming the presence of an intact *TcI* in the cloned phage. In this study, all the insertions into the BO strain that we mapped had intact *EcoRV* sites. We suspect that the one lacking an *EcoRV* site is a N2 insertion.

Lastly, and more importantly for the purpose of this study, the presence of the *EcoRV* sites suggested a rapid method for obtaining flanking sequence probes from the cloned *TcI*-containing plasmids. The original cloned *EcoRI* fragments (containing *TcI*) can't be used for genetic mapping because of the many *TcI* inserts in BO which would result in a smear on an autoradiograph. Although the unique flanking sequence fragment could have been purified from phage preparations, subcloning into a plasmid was done in order to facilitate subsequent manipulations. In our approach the *EcoRI* band was easily subcloned into a plasmid vector and immediately grown using overnight plasmid cultures. *EcoRI* and *EcoRV* restriction digests provided confirmation that the desired fragment had been subcloned. The *EcoRV* digested DNA was immediately used to prepare flanking sequence probes. By digesting with *EcoRV*, diluting the digestion mix and religating, we were successful in obtaining flanking sequence probes from the plasmid subclones which could be used as [³²P]labelled probes for genomic blots. (Although these probes contained 38 bps of *TcI* terminal repeat sequence this did not cause a problem under the hybridization conditions described.) It is unlikely that we have identified all the *unc-22* *TcI*-insertion sites in BO. In a sample of 13 isolated *TcI*s, four were represented twice and five were represented only once. It is likely that a number of yet-unidentified *TcI*-containing BO sites are present in this region.

All the RFLDs which map to the left of *unc-22* showed a BO pattern in the FR strain, whereas all those to the right showed an N2 pattern. These results are consistent with the interpretation that FR was derived from a genetic hybrid of the N2 and BO strains of *C. elegans*. The phenotype of FR is also consistent with this suggestion. FR hermaphrodites are more coordinated and FR males more fertile than BO (A. M. Rose, unpublished results). We propose that the *Bergerac* FR strain is a mixture of N2 and BO and that on LG IV an exchange event occurred between these two genomes near the *unc-22* locus between *sP5* and *sP4*. The recombinant chromosome produced is composed of BO DNA to the left and N2 to the right of *unc-22*.

The focus of our analysis has been the *unc-22* region. This region has been the object of an extensive and on-going genetic analysis (Moerman and Baillie 1979; Moerman 1980; Rogalski et al. 1982; Rogalski 1983; Rogalski and Baillie 1985; L. Donati and D. L. Baillie,

flanking *unc-22* has been accomplished (Rogalski and Baillie 1985). The isolation of 350 kb of cloned and mapped DNA from this interval has initiated an intense investigation into the molecular organization of this region. The *sP3* site is located to the right of *unc-22* in the midst of a mutationally quiescent region. Analysis of cloned DNA around *sP3* shows, however, that this region is functionally active. The *sP4* site has provided a series of cosmids which have been used to identify the breakpoints of deletions flanking the *let-56* gene, immediately to the left of *unc-22*. Further to the left of *unc-22*, *sP5* and *sP6* are situated in the essential gene cluster described by Rogalski and Baillie (1985). Attempts are currently underway to identify coding regions and make assignments to the identified genes in this interval.

The method described in this paper, that of *TcI* linkage selection, is rapid, general, and may be targeted to any genetically characterized region of the *C. elegans* genome.

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