Volume 15 Number 5 1987 Nucleic Acids Research

Correlation of the physical and genetic maps in the *lin-12* region of *Caenorhabditis elegans*

Iva Greenwald*, Alan Coulson, John Sulston and James Priess

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK

Received October 31, 1986; Revised and Accepted January 14, 1987

ABSTRACT

We describe the assembly of a set of overlapping clones from the lin-12 III chromosomal region that spans approximately 600 kb, and the identification of two restriction fragment length polymorphisms, eP6 and eP7, that flank the lin-12 locus. A comparison of the physical map and the genetic map yields approximate measurements of 930 kb/map unit for the eP6--lin-12 interval and 830 kb/map unit for the lin-12--eP7 interval. We interpret these values as supporting the proposal that the apparent clustering of genes observed for C. elegans autosomes results from decreased recombination frequency in clusters and not from nonrandom distribution of genes on the physical map.

INTRODUCTION

The intensive genetic analysis of diverse biological processes in Caenorhabditis elegans has resulted in the identification of over 500 genes (1,2). The five autosomal linkage groups display a striking feature: on each chromosome, most genes are localized in a cluster. For example, of the 50 genes that have been reasonably well positioned on linkage group (LG) III, 30 are located within the 3 map unit interval dpy-17--lin-12; the other 20 genes are distributed about equally on both sides of the cluster, over an additional 60 map units or so. The apparent clustering might reflect nonuniform recombination frequency along the autosomes, with a relative depression of crossing over in the cluster as compared to the chromosomal arms; alternatively, the clustering might reflect a real nonrandom distribution of genes on the physical map. A comparison of the physical map and the genetic map would address this question. If there is nonuniform recombination frequency along the autosomes, then for clusters, the amount of DNA/map unit should be greater than the average value of 266 kb/map unit [which is calculated by

dividing the genome size (8 x 10^4 kb; ref. 3) by the number of map units (approximately 300; ref. 2)]; for arms, the value should be similar to the average value. If there is a nonrandom distribution of the genes along the physical map, then the amount of DNA/map unit for genes in a cluster should be less than the genomic average. We describe here a correlation of the genetic and physical maps for a chromosomal region that provides direct evidence for nonuniform recombination frequency along C. elegans autosomes.

The data we describe derive from the assembly of a set of overlapping clones (termed a "contig") spanning approximately 600 kb in a region of linkage group (LG) III that includes lin-12, a developmental control gene (4,5). This contig is one of the intermediates in the assembly of a complete C. elegans genome map: the ultimate goal is a physical map of the genome that has been aligned with the genetic map (see ref. 6). To achieve this goal, the combined efforts of many laboratories will be required, and we hope that this account of our experience in the assembly of this contig will be useful for other C. elegans workers and for workers engaged in related projects involving other organisms.

MATERIALS AND METHODS

Genetic methods and strains

General methods for the maintenance and manipulation of C. elegans strains are described in ref. 1. The wild type strains used in this study were N2 (Bristol; ref. 1) and RW7000 (Bergerac; ref. 7). Mutant strains were derived from N2. The mutations used were dpy-19(e1259), unc-32(e189), unc-69(e587), unc-50(e306), which are recessive (refs. 1, 2); lin-12(n302 n865) and lin-12(n137 n720), which are recessive (4); and lin-12(n137), which is semidominant (4).

unc-50(e306) confers levamisole resistance; when this marker was used in three-factor crosses for congenic strain constructions or mapping, Unc-50 recombinants were selected by adding levamisole to a final concentration of 1 mM (1, 8) to plates containing animals.

The congenic strains described in this paper are CB3656 (dpy

19 eP6 unc-50), CB3657 (dpy-19 eP6 eP7 eP8 unc-50), and CB3658 (dpy-19 eP6 eP7 unc-50).

Restriction fingerprint matching

The methods used are described and discussed in ref. 6. Essentially, cloned DNAs are digested with HindIII, end-labelled, digested with Sau3A, and subjected to electrophoresis; the resulting patterns of bands are analyzed by computer for "matches" to the database (i.e. a match occurs when two or more clones share a significant number of bands).

Molecular cloning of eP7

General molecular methods are given in ref. 9. EcoRI-digested CB3658 DNA was size-fractionated on 0.75% LGT-agarose gels and an appropriate fraction was checked by Southern blotting for the presence of eP7, a 15 kb Tc1-hybridizing band. This fraction was cloned into lambda 2001 (10) and the primary library was screened by hybridization with ^{32}P-labelled pCe2002 (11) for Tc1-hybridizing phages. DNA was prepared from plaque-purified candidates, digested with EcoRI, and analyzed by Southern blotting to determine if the Tc1-hybridizing band comigrated with eP7. Phage D33-9i2 proved to contain an insert that corresponded to eP7. A unique HindIII-BamH1 fragment was subcloned and used to screen a 6000 clone cosmid collection from the ordered bank of the mapping project (6), thereby identfying C05F1 and C15H7 as containing eP7.

Construction of deletions for genomic walking

Cosmid DNA was prepared by a miniprep method (12), and then extracted with phenol/chloroform and precipitated with ethanol to improve the efficiency of religation. Approximately 100 ng of cosmid DNA was digested to completion with XbaI, precipitated with ethanol, and resuspended in 10 microliters of TE. We calculated that a DNA concentration of less than 20 micrograms/ml would favor circularization, based on the equation in ref. 9; typically, we used approximately 10 ng (1 microliter) of the digested cosmid DNA in a 20 microliter ligation reaction. DH1 (13) was transformed with the ligation reaction and ampicillin-resistant transformants were selected. Minipreps of putative deletion DNAs were prepared, digested with EcoRI, and the presence of small inserts verified; the inserts were prepared

from LGT-agarose gels to use for probing nematode cosmid or lambda libraries. In general, virtually all transformants examined proved to harbor the desired deletions.

RESULTS AND DISCUSSION

Identification and genetic mapping of RFLPs near lin-12

Restriction fragment length polymorphisms (RFLPs) are useful molecular genetic markers. In C. elegans, RFLPs have been identified by hybridizing cloned probes to genomic Southern blots of DNAs from different C. elegans strains (14, 15). Two of these strains are Bristol and Bergerac, both of which originated as wild isolates; the Bristol strain has been the wild type reference strain for almost all work done on C. elegans. RFLPs associated with the transposable element Tc1 have been particularly useful. There are only 30 copies of Tc1 in the Bristol strain, but approximately 300 copies in the Bergerac strain (16). Since the genome size is 8×10^4 kb (3), one might expect a Tc1-RFLP to occur every 300 kb in Bergerac, if Tc1 elements are randomly distributed. Tc1-RFLPs were first used to map cloned actin genes (17) and have subsequently been used to map other cloned genes and random restriction fragments. Tc1-RFLPs have also been cloned and used to identify genomic DNA sequences for genes defined genetically, which had not been previously cloned or associated with gene products (G. Ruvkun, V. Ambros and H.R. Horvitz, personal communication).

Two three-factor crosses were used to construct a set of congenic strains containing the lin-12 region of Bergerac in a Bristol genetic background (see Figure 1). The first cross generated recombinants in the dpy-19--unc-32 interval, resulting in a LG III of genotype dpy-19 Ber, where "Ber" indicates a segment of Bergerac DNA. Two independent dpy-19 Ber recombinants were then used in the second cross, generating recombinants in the lin-12--unc-50 interval; three recombinants were saved from each cross. In this way, a set of six congenic strains were generated, each with a LG III genotype of dpy-19 Ber unc-50. The congenic strains were backcrossed to Bristol three additional times to replace any remaining unlinked Bergerac sequences with Bristol sequences. We will describe here three strains--CB3656,

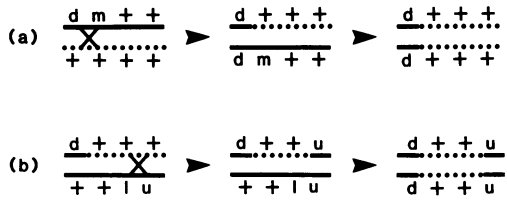

Figure 1. Schematic representation of the two three-factor crosses used to generate congenic strains containing the Bergerac lin-12 region in a Bristol genetic background. Straight lines indicate Bristol DNA, dotted lines indicate Bergerac DNA. d = dpy-19, m = unc-32, l = lin-12(n302 n865), u = unc-50.

(a) First cross, resulting in the replacement of a Bristol chromosomal region to the right of dpy-19 with the corresponding Bergerac region.

(b) Second cross, resulting in the replacement of part of the Bergerac-derived chromosomal region with Bristol sequences to the right of lin-12.

CB3657, and CB3658--which shared the same recombination event in the dpy-19--unc-32 interval but had different recombination events in the lin-12--unc-50 interval. The results from the other three strains are consistent with the data to be described below.

Genomic DNA was prepared from Bristol and from each of the congenic strains, digested with EcoRI, and probed with ^{32}P-labelled pCe2002 (11), which is essentially Tc1. A comparison of the hybridization patterns (Figure 2) reveals a 10.5 kb band in all three congenic strains that is not present in Bristol; this new band is designated eP6. Two of the three strains (CB3657 and CB3658) have a new 15 kb band, which is designated eP7. CB3657 also has two additional bands, eP8 (9.5 kb) and eP9 (8 kb). Because these strains were generated by defined recombination events, a map order of dpy-19--eP6--eP7--(eP8, eP9)--unc-50 may be deduced; in addition, from these data alone, one may also deduce that eP6 is to the left of or very close to lin-12 (since all three strains retain it) and that eP7 is about 0.5 map units to the right of lin-12 (i.e., 2/3 of the 0.8 map unit lin-12--unc-50 interval). Additional map data for eP6 and eP7, provided by additional crosses (Table 1) established the map order unc-32--eP6--lin-12--eP7--unc-50. The genetic map derived from these data is shown in Figure 3.

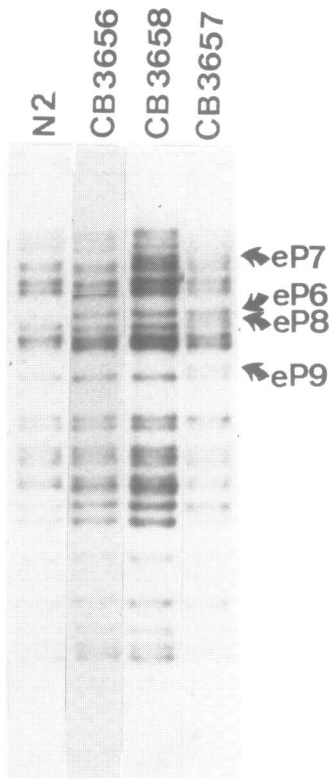

Figure 2. Southern blot of EcoRI-digested genomic DNA prepared from Bristol (N2), CB3656, CB3657 and CB3658, probed with pCe2002 (Ruan and Emmons, 1984). The positions of eP6, eP7, eP8, and eP9 are indicated.

There are potential advantages to constructing strains by three-factor crosses when possible, as opposed to repeated selection of the Bergerac region from trans heterozygotes containing a marked Bristol chromosome and an unmarked Bergerac chromosome. First, the genetic (and therefore physical) interval to be analyzed may be smaller, which would be especially suitable for genes located in chromosomal clusters (see below): on average, even after ten backcrosses involving trans heterozygotes, >5 map units of Bergerac DNA surrounding the Bristol marker may be retained (compare with the <1 map unit in the congenic strains described here). Second, mapping information--the location of polymorphisms within the interval of interest, and possibly with

TABLE 1 Genetic Map Data

A. Three factor cross data for eP6 and eP7

genotype of parent	recombinant phenotype[a]	number	segregated eP6	segregated eP7
dpy-19 eP6 eP7 unc-50/ unc-32 lin-12(n137)	Dpy	6	6	2
	Unc-50	3	3	3
	Unc-32 Unc-50	2	1	2
dpy-19 eP6 eP7 unc-50/ lin-12(n137) unc-69	Dpy Unc-69	5	5	2
	Lin Unc-50	2	0	2
	Unc-50	3	1	2
dpy-19 eP6 eP7 eP8 unc-50/ unc-32 lin-12(n137 n720)	Unc-32 Unc-50	14	3	14

B. Map distances

genotype of parent[b]	total progeny[c]	interval	recombinant class	number	p (m.u.)
dpy-19 eP6 eP7 unc-50/ unc-32 lin-12(n137)	3240	dpy-19--lin-12	Unc-50	5	0.3
		unc-32--lin-12	Unc-32 Unc-50	2	0.1
		lin-12--unc-50	Dpy	12	0.7
		eP6--lin-12	Unc-32 non-eP6 Unc-50	1	0.05
dpy-19 eP6 eP7 unc-50/ lin-12(n137) unc-69	1260	dpy-19--lin-12	DpyLin + Unc-50	3	0.2
		lin-12--unc-69	Dpy Unc-69	5	0.8
		lin-12--unc-50	Dpy + Lin Unc-50	11	0.9
		lin-12--eP7	Dpy Unc-69 non-eP7	3	0.5
dpy-19 eP6 eP7 eP8 unc-50/ unc-32 lin-12(n137 n720)	--	eP6--lin-12	Unc-32 non-eP6 Unc-50	--	0.08[d]

Table 1 Genetic map data. The congenic strains CB3657 and CB3658 were used for the mapping crosses shown here. Thus, the values calculated for the map distance p in map units (m.u.) are based on numbers derived for a trans heterozygote between a Bristol chromosome and a congenic chromosome that is mostly derived from Bergerac in the dpy-19--unc-50 interval. The values measured agree well with the established Bristol values (2, 22, and E. Ferguson, personal communication) and with our measurement of 1.5 m.u. for the dpy-19--unc-50 interval (31 Unc recombinants/4207 total progeny from Bristol heterozygotes of genotype dpy-19 unc-50/++). Note that the lin-12(n137) allele is semidominant, and the lin-12(n137 n720) allele is recessive. The recombinants analyzed by Southern blotting in section A are a subset of those presented in section B.

[a] recombinants were picked, and single segregants homozygous for the recombinant chromosome were used to establish strains, which were then analyzed by Southern blots as in Figure 2.

[b] some individuals were heterozygous for the unlinked mutation him-5(e1467).

[c] total progeny was estimated by multiplying the number of parents by 60, which is the average brood size of lin-12(n137)/+ hermaphrodites (Greenwald et al., 1983).

[d] calculated from the 11/14 recombinants in the 0.1 m.u. unc-32--lin-12 interval that failed to segregate eP6 (section A). Note that 0.1 m.u. is the established Bristol value (ref. 22 and E. Ferguson, personal communication) and the value observed in the congenic strain.

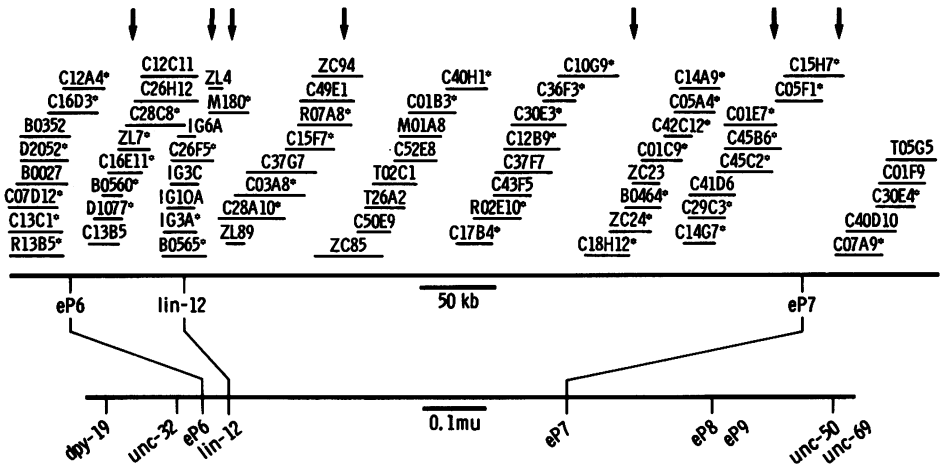

Figure 3. Correlation between the genetic and physical maps of the lin-12 region.

Genetic map: based on the data in Table 1.

Physical map: each clone is represented by a line proportional to the number of bands seen in the restriction fingerprint; overlapping lines indicate cosmids matched by random fingerprinting, and arrowheads indicate overlaps detected by walking. All clones prefaced with "Z" were found by probing.

Four steps were taken by constructing derivates of cosmids containing only end sequences. Of the methods we used, this strategy, when used in conjunction with probing a cosmid library, resulted in the largest and most direct steps. (1) p5a2 (insert size 2.3 kb), derived from C05F1, was used to probe 6000 cosmid clones and hybridized to C01E7. (2) p22a1 (insert size 2.5 kb), derived from C17A6 (which overlaps with C15H7) was used to probe 6000 cosmid clones and hybridized to C07A9. (3) p3a2 (insert size 4 kb), derived from C28C8, was used to probe a lambda library and hybridized to ZL7. (4) p1a2, derived from C26F5 (insert size 1 kb), was used to probe a lambda library and hybridized to ZL4.

Following a suggestion made by R. Plasterk, two steps were taken by digesting cosmid DNA with EcoRI, and then gel purifying en bloc all fragments smaller than 5.2 kb (the size of pJB8). (1) The insert from B0464 hybridized to ZC24 and ZC23. (2) The insert from C49E1 hybridized to ZC94 and ZC85.

One step was taken by using gel-purified restriction fragments from the lambda phage ZL4 to probe a lambda library; ZL89 was obtained.

respect to the gene of interest--may be obtained from the first Southern blot. Third, the congenic strains are already marked, which may facilitate subsequent mapping, especially when lethal or sterile mutations must be positioned.

Assembly of the 600 kb contig in the lin-12 region

Two strategies were used for the assembly of the contig shown in Figure 3: random restriction "fingerprint" matching was used to generate smaller contigs (6); chromosomal "walking" (18) was used to link some of these randomly assembled smaller contigs.

As part of the ongoing genome mapping project, random cosmid clones have been fingerprinted and compared with one another; thus far, 920 contigs, ranging from 35 kb to 350 kb and comprising 70% of the C. elegans genome, have been assembled by this random approach (6; A. Coulson and J. Sulston, unpublished observations). Two of these contigs were associated with lin-12 and eP7. A contig delimited by C28C8 and C26F5 was associated with the lin-12 locus: lin-12 was tagged with a transposon (5); lambda clones corresponding to lin-12 were isolated from a Bristol library, fingerprinted, and matched to the contig. A second contig, delimited by C05F1 and C15H7, was associated with eP7: the 15 kb eP7 EcoRI fragment was cloned from CB3658, and a unique subclone was used to probe 6000 cosmid clones, as described in Materials and Methods. (Repeated attempts to clone the 10.5 kb eP6 EcoRI fragment were unsuccessful, but subsequently the cosmids C12A4 and C16D3 were associated with eP6 by probing genomic DNA from CB3657 with cosmids; data not shown.)

Chromosomal walking was used to extend and join contigs in the lin-12 region. Our main walking strategy exploited features of the pJB8 cosmid vector (19) in which many of the cosmid clones used for the mapping project have been constructed: pJB8 contains an ampicillin-resistance marker, and has EcoRI sites flanking the BamH1 site into which nematode DNA was inserted. Four of the steps we have taken first involved the construction of derivative plasmids from which most of the insert DNA had been removed, leaving restriction fragments from the ends of the nematode insert: a cosmid clone was completely digested with an enzyme that does not cleave pJB8 but that does cleave the insert (typically, we used XbaI); the cleaved DNA was religated in a dilute reaction to favor recircularization, and a suitable E. coli host was transformed to ampicillin resistance. Minipreps of DNA from ampicillin-resistant colonies were prepared; the insert

was released by EcoRI digestion, gel-purified, and used to probe a nematode library. This probing resulted in relatively large steps taken in both directions from the original cosmid.

The early steps were monitored by probing Southern blots of Bristol and CB3658 genomic DNA with the end fragment probes. This monitoring was done to ensure that highly repetitive sequences were not present in the probe. Our experience suggests that highly repetitive sequences are not a major problem for this strategy, which is consistent with the observation that repetitive sequences are not a large component of the C. elegans genome (14). Low copy number repeats (which we encountered once) may be sorted out by restriction fingerprinting or by conventional restriction mapping methods. High copy number repeats (which we did not encounter) would result in an obvious increase in the frequency of clones after probing.

In all, seven steps were taken (see Figure 3). Concurrently, as part of the mapping project, random clones were fingerprinted (there are over 11,000 clones currently in the database). One of the steps, involving the lambda phage ZL4, was subsequently obviated by the fingerprinting of the cosmid M180 (Figure 3); the other overlaps would not have been detected by our computer methods (6). Walking has been an important component in the assembly of this 600 kb contig and will be essential for the completion of the physical map of the nematode genome. At this point in the mapping project, random clones will continue to be fingerprinted to reduce the number of contigs (currently 920); then, walking will be used to effect additional joins. The assembly of this large lin-12 contig encourages us to believe that other large contigs will be readily obtained from our extant libraries.

Correlation of the physical map and the genetic map

Figure 3 shows the positions of the genetic markers eP6, lin-12 and eP7 with respect to the 600 kb contig. The distribution of Tc1-RFLPs--two separated by 484 kb--is consistent with the estimate of one in 300 kb expected if Tc1 elements are distributed randomly (see above).

We may estimate the physical distance in each genetic interval by assuming an average insert size of 32 kb (6); we

thereby obtain a distance of 70 kb for the eP6--lin-12 interval and 414 kb for the lin-12--eP7 interval. Based on the map data of Table 1, the eP6--lin-12 distance is 0.08 map units (based on 16 recombinants in the 0.1 map unit unc-32--lin-12 interval) and the lin-12--eP7 map distance is 0.5 map units (based on 3 recombinants). Thus, we estimate metrics of 930 kb/map unit for the eP6--lin-12 interval and 830 kb/map unit for the lin-12--eP7 interval. We should like to emphasize that these values are only approximate, because they are based on small numbers of recombinants and an estimate of the physical distance; however, we think that they are useful as an indication of the relationship between the physical and genetic maps in this area.

These measurements are relevant to a consideration of the organization of the C. elegans genetic map. As described in the Introduction, it has been observed that the five autosomes all contain gene clusters, in which most of the genes located on a chromosome are contained within a relatively small (3-5 map unit) region (1). The apparent clustering might reflect nonuniform recombination frequency along the autosomes or the real nonrandom distribution of genes on the physical map. lin-12 is located at the right edge of the LG III cluster. Our estimate of 930 kb/map unit for the eP6--lin-12 interval is $3^1/_2$ times greater than the 266 kb/map unit estimated for the whole genome (i.e. 8×10^4 kb/300 map units), suggesting that the apparent clustering results at least in part from decreased recombination frequency in chromosomal clusters with respect to chromosomal arms. Perhaps the kb/map unit metric will be even greater in the heart of the cluster; we expect that on the autosomal arms, and on the X chromosome, which does not exhibit marked clustering, this value will be closer to the 266 kb/map unit average value. Even if the metric proves to vary somewhat among the different gene clusters, or at different positions within the clusters, the potentially high value of kb/map unit for genes within clusters should be considered when contemplating chromosomal walking strategies for the molecular cloning of C. elegans genes.

The original impetus behind identifying RFLPs and the correlation of the genetic and physical maps in this region was the molecular cloning of lin-12. Although this reason was

subsequently obviated by the successful use of transposon tagging to clone lin-12 (5), this general strategy, which was used successfully to clone the developmental control gene lin-14 (G. Ruvkun, V. Ambros and H.R. Horvitz, personal communication), is likely to remain useful for cloning genes that might be difficult to transposon tag: for example, in the absence of suitable genetic tricks, it might be tedious to isolate transposon-induced alleles when mutant phenotypes are not visible in the dissecting microscope, or are lethal or sterile. Two other interesting genes map within the genetic interval covered by the contig described here: dth-1, which is required for pharyngeal development and has maternal effect lethal alleles (J. Priess, unpublished observations) and glp-1, which is required for germ line development and has sterile alleles (20). By examining mutants for altered restriction fragments or by complementation after DNA transformation (21; A. Fire, personal communication), it should be possible to identify these genes within this contig.

ACKNOWLEDGEMENTS

We are grateful to R. Holmgren for suggesting the cosmid deletion walking strategy and for other cogent advice, to G. Ruvkun for illuminating discussions about the potential utility of RFLPs for cloning C. elegans genes, and to R. Baer, R. Dudler, S. Emmons, A. Fire, J. Hodgkin, J. Karn and R. Noethiger for advice, discussion and/or comments on this manuscript. I.G. was a fellow of the Jane Coffin Childs Memorial Fund for Medical Research; J.P. is a fellow of the National Institutes of Health (Grant GM 09751).

*To whom correspondence should be addressed at Biology Department, Princeton University, Princeton, NJ 08544, USA

REFERENCES

(1) Brenner, S. (1974) Genetics 77: 71-94.
(2) Swanson, M.M., Edgley, M.L. and Riddle, D.L. (1984) In Genetic Maps, Vol. 3 (ed. S.J. O'Brien), Cold Spring Harbor, New York.
(3) Sulston, J.E. and Brenner, S. (1974) Genetics 77: 95-105.
(4) Greenwald, I.S., Sternberg, P.W. and Horvitz, H.R. (1983) Cell 34: 435-444.
(5) Greenwald, I. (1985) Cell 43: 583-590.
(6) Coulson, A.R., Sulston, J.E., Karn, J. and Brenner, S. (1986) Proc. Natl. Acad. Sci. USA, in press.
(7) Moerman, D.G. and Waterston, R.E. (1984) Genetics 108: 859-877.
(8) Lewis, J.A., Wu, C.-H., Berg, H. and Levine, J.H. (1980) Genetics 95:905-928.

(9) Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York.
(10) Karn, J., Brenner, S., and Barnett, L. Meth. Enzymol. 101: 1-19.
(11) Ruan, K.-S. and Emmons, S.W. (1984) Proc. Natl. Acad. Sci. USA 81: 4018-4022.
(12) Birnboim, H.C and Doly, J. (1979) Nucleic Acids Res. 7: 1513.
(13) Hanahan, D. (1983) J. Mol. Biol. 155: 557-568.
(14) Emmons, S.W., Klass, M.R. and Hirsh, D. (1979) Proc. Natl. Acad. Sci. USA 76: 1333-1337.
(15) Rose, A.M., Baillie, D.L., Candido, E.P.M., Beckenbach, K.A. and Nelson, D. (1982) Mol. Gen. Genet. 188: 286-291.
(16) Emmons, S.W., Yesner, L., Ruan, K.-S., and Katzenberg, D. (1983) Cell 32: 55-65.
(17) Files, J.G., Carr, S. and Hirsh, D. (1983) J. Mol. Biol. 164: 355-375.
(18) Bender, W., Spierer, P. and Hogness, D. (1983) J. Mol. Biol. 168: 17-33.
(19) Ish-Horowicz, D. and Burke, J.F. (1981) Nucleic Acids Res. 9: 2989.
(20) Kimble, J., Barton, M.K., Schedl, T.B., Rosenquist, T.A. and Austin, J. (1986) In Gametogenesis and the Early Embryo, pp. 97-110 (ed. J.G. Gall), New York, Alan R. Liss.
(21) Fire, A. (1986) EMBO J., in press.
(22) Ferguson, E.L. and Horvitz, H.R. (1985) Genetics 110: 17-72.