The Genetics and Molecular Biology of *unc-86*, a *C. elegans* cell lineage gene

by

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ABSTRACT

The *C. elegans* gene *unc-86* is analysed using genetic and molecular techinques. *unc-86* mutations have previously been shown to affect cell lineages. Two classes of *unc-86* alleles were known. We show that one of those classes very likely represents the null phenotype, and that the other class are deletions that probably affect at least one other gene. We determine the temperature-sensitive period of two *unc-86* defects and show that one of them does not result from a change in cell lineage, but may nevertheless represent a homeotic transformation. Two approaches toward molecular cloning of *unc-86* were tried. One approach, transposon tagging, did not work apparently because of the insertion site specificity of the transposon Tc1; when the gene was cloned by walking from linked polymorphisms, we found that some spontaneous *unc-86* alleles contained insertions of new transposable elements. We were unable to detect a transcript from *unc-86*. In an appendix, we describe the genetic mapping of a mutator activity from a strain in which transposable elements are known to be mobile.

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Introduction

Development and cell lineage

Development is the process by which a single cell becomes a multicellular organism. There are many reasons for studying development. It is one of the few remaining complex unsolved problems of biology, and as such is an intellectual challenge. An understanding of development could also lead to practical applications. Development can be interfered with in unwanted organisms; for example, insect juvenile and molting hormone analogs can be used as insecticide (Ware, 1986). Aberrant development might be cured in people—genetically identified molecules involved in development are related to molecules involved in cancer. For instance the nematode gene *lin-12* and the fruit fly gene *Notch* are related to transforming growth factor α (Greenwald, 1985; Wharton *et al.*, 1985) and the fruit fly gene *sevenless* is related to the tyrosine kinase family of oncogenes (Hafen, Basler, Edstroem and Rubin, 1987).

There are many ways of studying development and many organisms to study it in. Much recent work has been done in simple invertebrate animals, such as insects (grasshoppers, crickets and fruit flies), annelids (leeches) and the one we work on, the nematode worm *Caenorhabditis elegans*. Such animals are small enough and develop quickly enough that all or significant portions of development can be observed on a gross level, a cellular level, and even a subcellular level. For instance, *C. elegans* has an adult form that is not much more than a millimeter long and has only about a thousand cells; the

entire lineage of those thousand cells has been followed from the fertilized egg to the adult stage (Sulston and Horvitz, 1977; Kimble and Hirsh, 1979; Sulston, Albertson, and Thomson, 1980; Sulston, Schierenberg, White, and Thomson, 1983).

These animals share features with each other and with vertebrates that suggest that all have evolved from a common multicellular ancestor. Early embryos of animals in all of these groups undergo a complex series of morphogenetic events called gastrulation. All groups have basic cell types, such as nerve and striated muscle, in common. At least some members of all groups use common molecules which seem to be only of use to multicellular animals. For example, there is a large overlap in the neurotransmitters used by *C. elegans* and vertebrates: the nematode has neurons which produce molecules identical or very similar to dopamine, serotonin, acetylcholine, γ-amino butyric acid, and the neuropeptides FMRFamide and cholecystokinin (Sulston, Dew and Brenner, 1975; Horvitz et al., 1982; Rand and Russell, 1985; Li and Chalfie, personal communication; McIntire and Horvitz, personal communication). It seems unlikely that the use of these molecules as neurotransmitters evolved independently in both lines, so (unless there is an unknown selection pressure for the use of these particular molecules), then the ancestor of vertebrates and nematodes was multicellular.

If nematodes, insects and vertebrates did indeed evolve from a common multicellular ancestor, then it is possible that at least some of the mechanisms underlying development will have been conserved in all of them. Thus, studying development in any one group could help in the understanding of development in the other groups.

Two of the organisms mentioned above, the fruit fly *Drosophila melanogaster* and *C. elegans*, have the advantage that their development can be studied in the context of a powerful genetic system. The genetics of *Drosophila* goes back over 70 years (Morgan and Bridges, 1916); the genetics of *C. elegans* goes back less than 20 years (Brenner, 1974), but has advanced rapidly in that time. *C. elegans* has a number of advantages over *Drosophila* as a genetic system, including shorter generation time (3 days instead of 10), hermaphrodite genetics, and the fact that mutant strains can be frozen.

Genetic approach

The genetic approach to the study of development is to isolate mutations that perturb development and use those mutations to find out how genes and molecules act in normal development. The premise of this approach is that if a gene produces a product that plays an important role in development, then a mutation in that gene can cause abnormal development. What is "an important role in development"? That is not an easy question to answer, and in the end it may be a matter of taste. Our bias is that a gene plays an important role in development if its primary function is to decide the fate of a cell among several choices. Mutations in such a gene could well be homeotic. Homeosis is the transformation of a cell or group of cells that are normally one tissue or organ to another tissue or organ. Homeotic mutations have been found in both *Drosophila* and *C. elegans* (*e.g.* Lewis, 1978; Greenwald, Sternberg and Horvitz, 1983)

Since our goal is to study genes that play an important role in development, we need a guideline for deciding which genes, out the many in which mutations can and have been found, might tell us the most

about the mechanisms of development. It doesn't matter too much if the criteria are too strict and may exclude some genes with an important developmental role, because in the beginning we are only looking for a few genes that we can be reasonably confident are developmentally interesting. For this reason it is probably not a mistake to restrict ourselves to genes with homeotic mutations.

It may be difficult to find some homeotic genes. The most common class of alleles of most genes is complete loss-of-function (null) alleles (*e.g.* Greenwald and Horvitz, 1980). If a gene controls a developmental decision that affects any cell or group of cells necessary for the survival of the animal, then the most common class of alleles will result in a lack of the affected cells, and thus death. For this kind of gene, the only alleles that one could isolate easily are rare reduction-of-function or gain-of-function alleles. For example, the bithorax complex of *Drosophila* was first identified by rare recessive alleles (Lewis, 1963) and the Antennapedia complex was first identified by rare dominant alleles (Postlethwait and Schneiderman, 1971).

Some mutations in genes whose primary function is other than to decide among cell fates can cause changes that appear to be homeotic if the phenotype is not examined closely. For instance, if a neuron normally makes a particular neurotransmitter, a mutation in an enzyme required for the production of that transmitter could appear to transform that neuron into a different kind of neuron. Another example: imagine that the production of a particular cell type requires a great deal of energy, and that this energy is provided by glycolysis. If a mutation reduces the activity of a glycolytic enzyme

enough to block the production of that cell type but not enough to seriously affect other functions, then the mutation would seem to have a specific effect on the decision to produce that cell type. A similar case has occurred—the *rudimentary* mutations of *Drosophila*, which cause wings to be small, are actually mutations in genes of the pyrimidine biosynthesis pathway (Rawls and Fristom, 1975).

If some interesting genes are likely to be identified by reduction-of-function alleles, and reduction-of-function alleles of some developmentally uninteresting genes could appear interesting, how can these be sorted out? Possibly the best way is to ask that mutations in the gene be specific. That is, all alleles of a gene, even complete loss-of function alleles (although possibly excluding rare gain-of-function alleles), should not cause anything but a homeotic effect. Some alleles may well be lethal, and in that case it is important to examine the dead or dying animals to determine if the lethality could plausibly result from a homeotic transformation. For example, the *Drosophila* homeotic genes controlling the establishment of the dorso-ventral axis all have maternal effect lethal null phenotypes (Anderson and Nüsslein-Volhard, 1984), but it can be reasonably inferred that the cause of death in all cases is homeosis.

Of course, it is entirely possible that the null phenotype of a gene would not be lethal. In that case it is still important to determine the null phenotype to see if there are any other than homeotic effects.

unc-86

unc-86 is an example of a *C. elegans* homeotic gene identified on

the basis of non-lethal alleles. The first alleles of *unc-86* were isolated on the basis of their behavioral abnormalities, *e1416* by H. R. Horvitz because it is Egg-laying defective (Egl) and *e1507* by M. Chalfie because it is Mechanosensory defective (Mec) (Horvitz and Sulston, 1980). Both alleles had both phenotypes as well as a High incidence of males (Him) phenotype caused by meiotic nondisjunction of the X chromosome (Hodgkin, Horvitz and Brenner, 1979). It was subsequently shown that that *unc-86* animals have a number of anatomical defects, including lack of microtubule neurons (touch receptor cells), lack of the male-specific cephalic companion neurons, abnormal serotonin distribution, extra dopaminergic dereid and postdereid neurons, and frequently misplacement of the HSN neurons that drive egg laying (Horvitz and Sulston, 1980).

Furthermore it was shown that some of these phenotypes result from abnormal reiterative cell lineages (Chalfie, Horvitz and Sulston, 1981). Figure 1, reproduced from Chalfie, Horvitz and Sulston (1981), shows the alterations caused by *unc-86* in the postembryonic lineages and gives a representation of the common features of those lineages. The changes in these lineages are clearly homeotic--a daughter cell is transformed so that it has the same fate as its mother.

Sulston (personal communication) has since seen alterations in *unc-86* embryonic lineages. Some of these are consistent with the proposed pattern and some are not (figure 2). One phenotype of *unc-86* animals, the defect in the HSN neuron, is clearly not associated with a change in cell division patterns. HSNs are produced at the correct time by the correct lineage (J. Sulston, personal communication) and

Figure 1

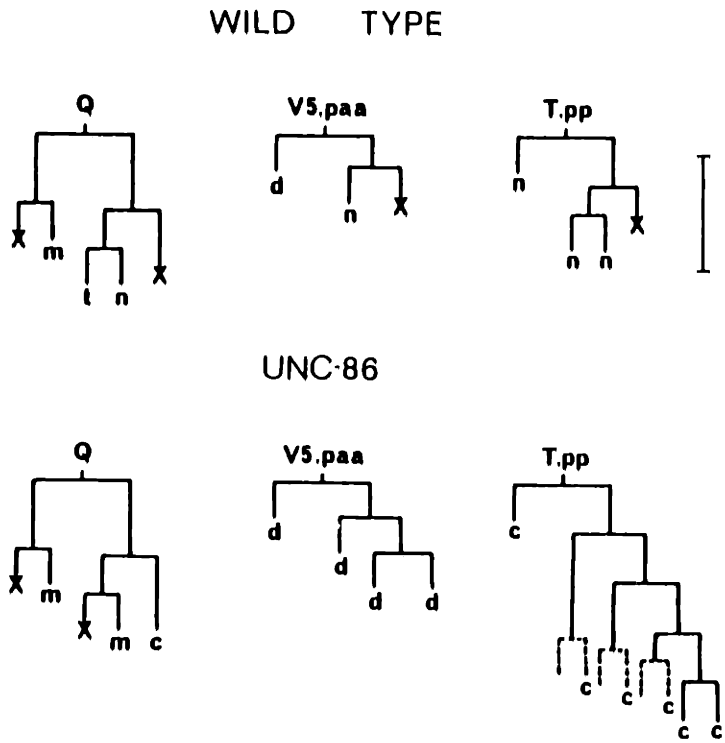
Alterations in postembryonic lineages in *unc-86* animals (reproduced from Chalfie, Horvitz and Sulston, 1981). Typical lineages, anterior drawn to the left.

A, wild type lineages compared to *unc-86* lineages. Abbreviations: c, compact nucleus (typical of neuron or neuron-associated structural cell); d, dopaminergic neuron; m, migrating neuron· n, neuron; t, touch receptor cell; X, programmed cell death.

B, common features of the lineages in A. N, neuroblast.

Figure 1

A


WILD TYPE
Q
V5.paa
T.pp
UNC-86
Q
V5.paa
T.pp

B

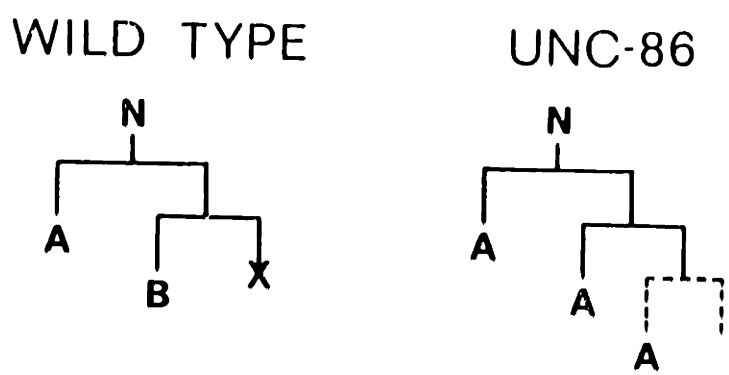
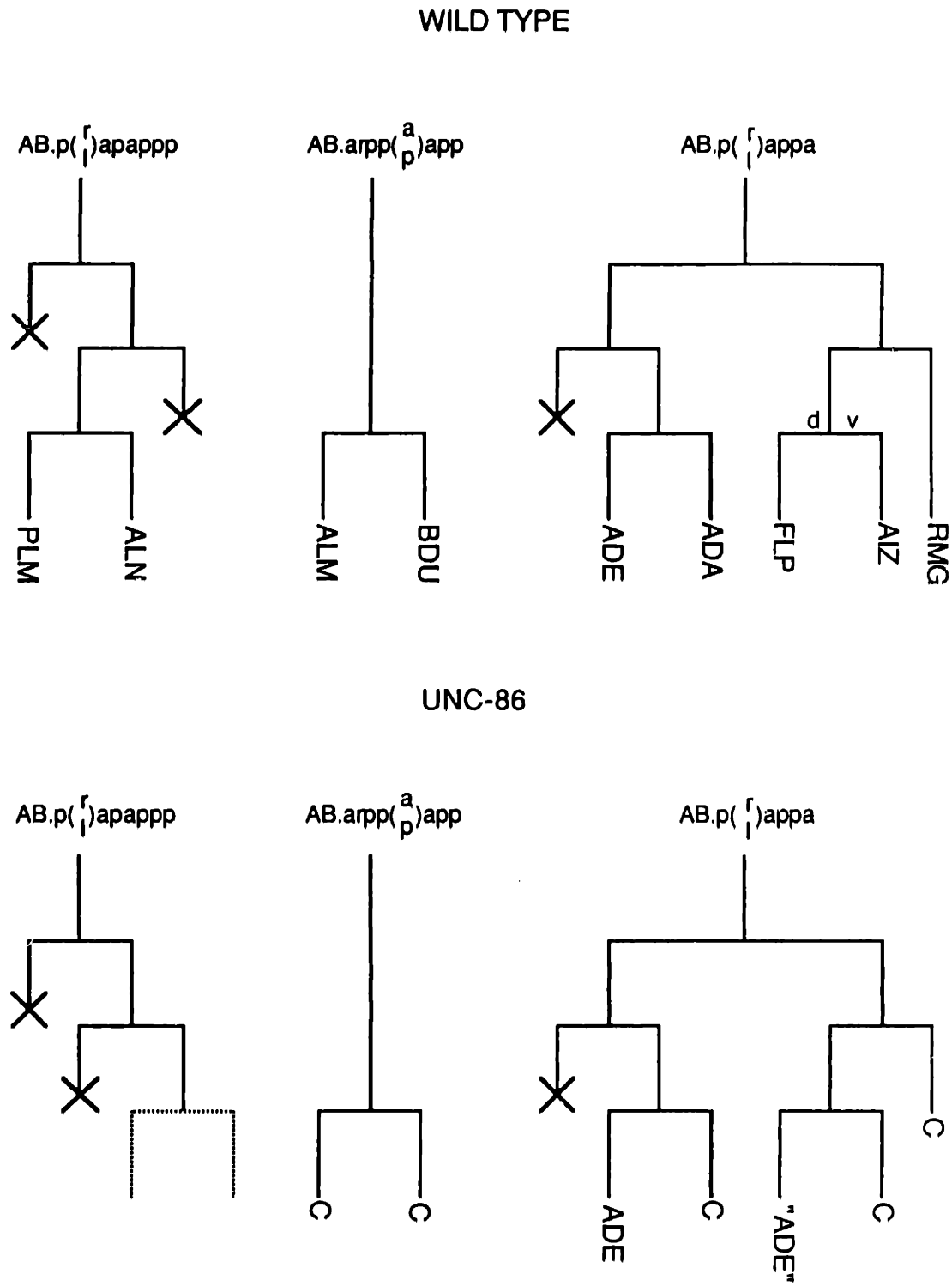

WILD TYPE
UNC-86
N
A
B
X
N
A
A
A

Figure 2

Alterations in embryonic lineages in *unc-86* animals (J. Sulston, personal communication).

Neurons produced by the wild type lineages are identified by their three-letter names. Unc-86 animals do not have PLM, ALN, ALM, BDU, or AIZ neurons but have two dopaminergic neurons similar to ADE. Abbreviations: "ADE", a neuron with a similar appearance to ADE; c, compact nucleus; X, programmed cell death.

Figure 2


WILD TYPE
AB.p(r/l)apappp
AB.arpp(a/p)app
AB.p(r/l)appa
PLM
ALN
ALM
BDU
ADE
ADA
d
v
FLP
AIZ
RMG
UNC-86
AB.p(r/l)apappp
AB.arpp(a/p)app
AB.p(r/l)appa
C
C
ADE
C
"ADE"
C
C

appear normal in the L1 stage. The question of whether this defect is homeotic is one of the issues addressed by this thesis.

Another phenotype of both original *unc-86* alleles was difficult to describe as homeotic—meiotic nondisjunction (Him). Somewhat surprisingly, the two alleles complemented for this phenotype; it was proposed that this might be an example of interallelic complementation (Chalfie, Horvitz and Sulston, 1981). V. Ambros showed (personal communication) that the *him* mutation in the original isolate of *e1507* was genetically separable from the *unc-86* mutation. Thus there were two classes of *unc-86* alleles, Him and non-Him. The next two alleles isolated, *n306* (E. Ferguson, personal communication) and *n412* (V. Ambros, personal communication) did nothing to resolve the dichotomy; the former had a Him phenotype and failed to complement the Him phenotype of *e1416*, and the latter was non-Him. The question of which, if either, is the null phenotype is another issue addressed by this thesis.

Genetic analysis

To determine the null phenotype of a gene it is essential to have an allele that eliminates all of the activity of that gene. It may be possible to identify an existing allele that does this, or it may be necessary to generate a new allele.

A simple first step toward the determination of the null phenotype of a gene is to compare the phenotypes caused by existing alleles. For instance, if most of the recessive alleles of a gene cause similar phenotypes, and those phenotypes are more severe than those caused by other alleles, then that group of alleles are most

likely null. On the other hand, if the recessive alleles of a gene have a range of severities with no two alike, then those alleles probably cause a reduction but not elimination of function and null alleles probably result in sterile or inviable animals.

No matter what the distribution of alleles is like, and especially if there are only a few alleles, it is important to be able to compare alleles with a chromosomal deficiency (multigenic deletion) which is known to delete the gene. Since a deficiency necessarily has no gene activity, heterozygotes with the deficiency can be compared to heterozygotes with a suspected null allele; if the phenotype is more severe in the former case, then the suspected null allele is probably not.

Deficiencies can answer other questions as well. For example, if a mutation has dominant effects, the mutation could either have a gain of function or could be haplo-insufficient. If a deficiency of the locus also has dominant effects, then haplo-insufficiency is indicated.

A deficiency can be used to tell whether any of the existing alleles in *trans* to a null allele results in a viable animal. If this is the case, then new alleles can be selected on the basis of failure to complement an existing allele without requiring that the new allele have a homozygous viable phenotype. This technique allows the isolation of null alleles of genes previously known only by reduction-of-function alleles (for example see Kornberg 1981).

Other genetic techniques, such as suppressibility by nonsense suppressors, can suggest but not prove that an allele is null, but strict proof can probably only come from molecular biology.

Null alleles are necessary to establish the function of a gene, but a class of non-null alleles, temperature-sensitive (ts) alleles, is also quite useful. In certain cases they can be used to establish the time of action of a gene and to investigate what happens when gene function is present for only part of development.

Shifting animals from a restrictive temperature to a permissive temperature can reveal a point before which gene activity is not required. The converse experiment, shifting animals from a permissive temperature to a restrictive temperature, does not necessarily reveal a point after which gene activity is no longer needed. For instance, if a mutation results in a gene whose product cannot be synthesized at the nonpermissive temperature, but which is stable once synthesized, then a shift to nonpermissive temperature after the product is synthesized but before it is used will result in a wild-type phenotype. However, if a shift to a restrictive temperature does result in a mutant phenotype then it may be concluded that the product is required after that point.

For the homeotic genes we are interested in, the time of action as determined by temperature shifts can be compared to the observed time at which development in the mutant becomes different from development in the wild type.

The phenotype resulting from mutations may be modulated not only by temperature, but also by the presence of mutant alleles of other genes. Mutations in other genes may either suppress or enhance the phenotype.

Suppressor could act in several ways. For instance they can act by restoring activity to the mutant gene (*e.g.* Waterston and Brenner 1978) or they act epistatically to the suppressed mutation and have a

different phenotype. This latter class can be a source of new homeotic mutations. For example, Anderson and Nüsslein-Volhard (1984) defined the genes *spätzle* and *pelle* on the basis of suppressors of dominant *Toll* mutations. An understanding of the processes affected by the new mutations might help clarify the effects of the original mutation.

Enhancers are a more difficult subject than suppressors because a mutation probably does not have to be as specific to make something worse as it does to make something better. Nevertheless, as in the case of suppressors, enhancers might give a clue as to what processes are affected by a mutation.

C. elegans molecular biology

Genetics can identify genes required for particular processes. There is a limit, however, to what genetics can say about what those genes are actually doing. Much more information can come from molecular biology. Molecular biology can tell us exactly what mutations do to a gene (*e.g.* Bender *et al.*, 1983). It can tell us about the structure of the wild-type gene and where, when, and how it is expressed (*e.g.* Beachy, Helfand and Hogness, 1985). It can tell us if related genes have been found elsewhere, and, by analogy give better hypotheses about function—for instance, Delotto and Spierer (1986) hypothesized on the basis of sequence data that the Drosophila gene *snake* encodes a serine protease. The slow step, at least in *C. elegans*, has been starting molecular biology on (*i.e.*, molecularly cloning) genes identified only by mutation.

C. elegans has a genome well suited to molecular biology. It has the smallest haploid genome size of any known metazoan, ($8X10^7$ base

pairs, 20 times the size of the *E. coli* genome) and 83% of the sequences are unique (Sulston and Brenner, 1974). This small size means that particular restriction fragments are present at a relatively high concentration in digests of *C. elegans* DNA, so when such digests are separated electrophoretically, transferred to solid support, and allowed to hybridize to radioactively labelled DNA (Southern blotted), resultant signals are quite strong even if only 1 μg of DNA is used. It also means that genomic clone libraries need not be large to contain most sequences. For instance, a cosmid library with an average insert size of 40 kilobases (kb) would need only 2000 clones to contain the entire *C. elegans* genome.

A set of overlapping cosmid clones covering the entire genome is currently being assembled by John Sulston and Alan Coulson at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England (Coulson, Sulston, Brenner, and Karn, 1986). Their technique is to digitize some easily-measured features of the restriction map of cosmid clones ("fingerprint" them), and store the digitization in a computer where it can be efficiently compared to the digitization of other clones. Since a computer compares the clones, a step which takes time proportional to the square of the number of clones, many more clones can be compared than could be done by any physical method. Contiguous sets of overlapping clones are called "contigs". Fortunately for *C. elegans* workers, Sulston and Coulson are happy to take any clones sent them, fingerprint the clones, and send back clones from any contig found. Thus, if a piece of DNA linked to a gene of interest can be cloned, it is relatively easy to get a large overlapping set of clones covering 100 or more kb.

An approach to finding such a linked clone was made feasible when the transposable element Tc1 was discovered (Emmons et al., 1983). Tc1 is present in 30 copies in *C. elegans* var. Bristol strain N2, the wild type strain used for most genetics, and in about 500 copies in *C. elegans* var. Bergerac strain RW7000, another wild type strain (see chapter 2). If the Bergerac Tc1 insertions are distributed at random, then there should be one every 160 kb; for any particular gene the distance to the nearest Bergerac Tc1 insertion should be on the order of tens to several hundred kb. This distance is feasible to "walk" by isolating overlapping clones (*e.g.* Bender *et al.*, 1983), and with the Sulston/Coulson physical map, might not even be too hard.

Cloning the nearest Bergerac Tc1 insertion to a gene can be done by a combination of the methods of genetics and molecular biology. The first step is to make a congenic strain whose entire genome is derived from the Bristol strain except for a small region linked to the gene of interest. This can be done by repeatedly crossing animals carrying the Bergerac region with Bristol animals mutant for that gene and selecting progeny carrying the Bergerac wild type allele. Once this congenic strain has been constructed, the Bergerac Tc1 insertions it carries can be genetically mapped, and then the closest Tc1 can be cloned on the basis of its hybridization to a cloned copy of Tc1 (Emmons et al., 1983). The sequences flanking this Tc1 insertion can then be used to begin a walk or find a contig.

Tc1 is useful in other ways as well. It can transpose in the Bergerac strain, causing insertional mutations in genes (Eide and Anderson, 1985). The mutation rate for Tc1 insertion in Bergerac was measured to be $5X10^{-7}$ (Eide and Anderson, 1985) and $2\text{-}5X10^{-5}$ (Greenwald,

1985); either rate is low enough that finding Tc1 insertions was practical only for genes for which there was a strong selection.

Obtaining Tc1 insertions in other genes became more practical when Anderson and coworkers isolated mutator (*mut*) mutations (P. Anderson, personal communication). These genes were isolated on the basis of their ability to increase the frequency of Tc1 excision, but they also cause other mutations, at least some of which are Tc1 insertions (P. Anderson, personal communication; S. Kim, personal communication; J. Way and M. Chalfie, personal communication).

The Tc1 insertions found are not inserted into random sites, but rather into sites with a consensus sequence of TNNPGPTPTARPT, where the element is inserted between the underlined nucleotides, which are always conserved (I. Mori, G. Benian, D. Moerman and R. Waterston, personal communication). This sequence is the minimum site specificity; there may be other factors which further restrict the choice of insertion sites. Therefore it is possible that Tc1 insertions in some genes could be very rare.

Given the approaches outlined above it is not unreasonable to attempt the molecular cloning of a *C. elegans* gene, like *unc-86*, identified only by mutations.

Materials and methods

Genetics

C. elegans strains were handled and ethylmethanesulphonate mutagenized according to Brenner (1974) except that plates contained 2% agar to prevent the animals from burrowing, and animals were often grown on lawns of *E. coli* strain HB101 which grows into a thicker lawn than the commonly used strain OP50.

Standard genetic nomenclature is used (Horvitz, Brenner, Hodgkin and Herman, 1979).

unc-86 alleles used and their sources are listed in table 1; other alleles are listed in table 2.

γ-ray mutagenesis was done using 7000 to 9000 rads from a cobalt-60 source generously made available by Anthony Sinskey.

The free duplication *nDp2* was generated by mating γ-ray mutagenized males of the genotype *+ unc-86(e1416)/unc-36(e251) +* with *unc-36(e251) unc-86(e1507)* hermaphrodites and selecting wild-type cross progeny (most progeny from this cross are either Unc-86 or Unc-36; wild-type progeny can only be generated by recombination in the male, reversion or suppression of one of the mutations, or the presence of two copies of the region in a single sperm). *nDp2* carries the allele *unc-86(e1416)*. *unc-86(n1042)* was also isolated serendipitiously in this experiment; since it has different genetic characteristics from either *e1416* or *e1507*, it was assigned a new allele name. Molecular evidence (chapter 3) indicates that *n1042* does not carry *e1416*, but the possibility remains that it is a partial revertant of *e1507*.

Table 1

Sources of *unc-86* alleles.

Allele	Isolated by	Isolation phenotype	Class
e1416	H. R. Horvitz	Egl	Him
e1507	M. Chalfie	Mec	Common
n306	E. Ferguson	Egl	Him
n412	V. Ambros	Unc-86	Common
n843	M. Finney	Unc-86	Him
n844	M. Finney	Unc-86	Common
n845	M. Finney	Unc-86	Common
n846	M. Finney	Unc-86	Common
n847	M. Finney	Unc-86	Him
n848	N. Tsung	extra dopaminergic neurons	Weak
n946	C. Desai	Egl	Common
n992	C. Desai	Egl	Common
n993	C. Desai	Egl	Common
n994	C. Desai	Egl	Common
n1041	C. Desai	Egl	Common
n1042	M. Finney	Unc-86	Weak
n1071	C. Desai	Egl	Common
n1132	C. Desai	Egl	Common
n1351	S. Kim	Egl	Common
rh1029	E. Hedgcock	Unc-86	Common
u5	M. Chalfie	Mec	Weak
u21	M. Chalfie	Mec	Common
u31	M. Chalfie	Mec	Common
u44	M. Chalfie	Mec	Common
u68	M. Chalfie	Mec	Common
u83	M. Chalfie	Mec	Common
u168	M. Chalfie	Mec	Weak
u261	M. Chalfie	Mec	Common
u301	M. Chalfie	Mec	Weak
u371	M. Chalfie	Mec	Common

Table 2

Alleles used

Allele	Gene	Chromosome
eT1(III;V)	*unc-36*	III;V
e27	*dpy-3*	X
e51	*unc-13*	I
e55	*unc-2*	X
e61	*dpy-5*	I
e164	*dpy-17*	III
e185	*lon-1*	III
e189	*unc-32*	III
e251	*unc-36*	III
e306	*unc-50*	III
e307	*unc-47*	III
e364	*dpy-18*	III
e382	*unc-49*	III
e491	*sma-3*	III
e502	*sma-2*	III
e587	*unc-69*	III
e729	*sma-4*	III
e937	*bli-4*	I
e1091	*unc-13*	I
e1259	*dpy-19*	III
e1364	*daf-4*	III
e1464	*sup-5*	III
e1467	*him-5*	V
e1489	*him-8*	IV
e1490	*him-5*	V
e1743	*lin-16*	III
e1777	*lin-1*	IV
nDf16		III
nDf17		III
nDf20		III
nDf21		III
nDf22		III
n363	*sup(n363,n1134)*	I
n387	*lin-13*	III
n411	*him(n411)*	III
n892	*sup(n892)*	X
n1131	*mab(n1131)*	III
n1133	*him(n1133)*	III
n1134	*sup(n363,n1134)*	I
n1347	*dpy-19*	III
n1348	*dpy-19*	III
r323	*unc-54*	I
r459	*mut-2*	I
st311	*unc(st311)*	III
u282	*lin-32*	X

nDf16 was generated by Victor Ambros (unpublished data). Other deficiencies were generated by crossing γ-ray mutagenized N2 males with *dpy-19 unc-32* hermaphrodites and selecting Dpy cross progeny.

Nondisjunction (Him) phenotype was scored as in Hodgkin, Horvitz, and Brenner (1979).

Mechanosensory defective (Mec) phenotype (Chalfie and Sulston, 1981) was scored by touching worms gently on the head and tail with a fine platinum wire coated with bacteria. Mec animals that are still will often start moving in response to such a touch, but once moving will not reverse direction. Wild type animals will reverse direction five to ten times in response to alternate touches on the head and tail. Presence of mechanosensory neurons was occasionally scored using Nomarski optics.

Male mating defective (Mmd) phenotype was scored similarly to Hodgkin (1983), except that sometimes four males were mated with four *sma-3(e491)* or *sma-3(e491) unc-32(e189)* hermaphrodites; *dpy-11(e224)* hermaphrodites are no longer used because about 10% of them die as young adults.

Response to serotonin (5-hydroxytryptamine) was tested by placing animals on NGM agar plates containing 5 or 10 mg/ml serotonin.

Molecular biology

Standard procedures were used (*e.g.* Maniatis et al. 1982).

Enzymes were obtained from New England Biolabs; radioactive nucleotides were obtained from Amersham.

Preparation of *C. elegans* DNA

For small amounts (100 μg or less) worms were grown on a single NGM agarose plate containing 1% peptone and 0.5% yeast extract (Difco); for larger amounts worms were grown in liquid culture. In either case, *E. coli* HB101 was used as food because it grows well and has no plasmids; OP50 has a large plasmid with homology to commonly used plasmid cloning vectors and NA22 has at least five plasmids, at least one of which has homology to commonly used plasmid cloning vectors. Worms were washed free of excess bacteria in M9 salts (Brenner 1974) in microcentrifuge tubes or 15 ml disposable centrifuge tubes and suspended in a solution of 100mM NaCl, 50mM tris pH 8, 10mM Na_3EDTA. SDS was added to 1%, proteinase K to 100 μg/ml, and the mixture incubated at 65° for 15 to 30 minutes with occasional mixing. Samples were then extracted with phenol and phenol/chloroform, precipitated with 2 volumes of ethanol and wound on glass rods. DNA was then suspended in 10 mM tris pH 8, 0.5mM Na_3EDTA (TE) with 10 μg/ml RNase A, incubated at 37° for 30 minutes, extracted with phenol/chloroform and chloroform and precipitated with ethanol. DNAs were suspended at a concentration of approximately 500 μg/ml.

Agarose gels

Two methods were used to run agarose gels that can resolve the maximum number of Tc1 bands. First, gels were run for very long times at very low voltages: 1 V/cm or less and 60-72 hr. for a 22 cm 0.8% agarose gel run in 0.5X TBE buffer. Second, the higher molecular weight bands were spread out using field inversion electrophoresis (Carle, Frank and Olson, 1986). The field inversion controller was donated by MJ Research, Inc.

High specific activity DNA probes

DNA was labelled using the "oligo labelling" technique of Feinberg and Vogelstein (1983).

Chromosomal walking

Walking was done in collaboration with John Sulston and Alan Coulson at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England, who are in the process of constructing a complete physical map (*i.e.*, set of overlapping clones) of the *C. elegans* genome (Coulson, Sulston, Brenner and Karn, 1986; see introduction). Much of the walking was done in a 13,000-clone cosmid library that had been plated into microtitre dishes. 7000 clones from a *Sau*3A I partial digest genomic library in the vector pHC79 made by Guy Benian (personal communication) were plated in a cooperative effort at MIT and a duplicate set sent to Sulston and Coulson; 6000 clones from a *Sau*3A I partial digest genomic library in the vector pJB8 (Ish-Horowicz and Burke, 1981) made by Sulston were plated in England and sent to us. The rest of the walking was done in a *Sau*3A I partial digest genomic phage library made by John Sulston in the vector λ2001 (Karn *et al.*, 1984).

Rnase protection

RNase protection experiments were performed as in Zinn *et al.* (1983).

Chapter 1

Mapping

Establishing the correct map position for a gene is essential for genetic analysis. For instance, generation of new alleles by complementation screens, generation of deficiencies and proof that they span the locus, separation of linked mutations, and strain constructions using linked markers all required knowledge of the correct map position of *unc-86*.

unc-86 and other markers near the center of linkage group III (LG III) were mapped using three-factor crosses and complementation tests with deficiencies (table 1) These data conflict with published map positions (e.g. Hodgkin, Horvitz, and Brenner, 1979; Chalfie, Horvitz, and Sulston, 1981; *Caenorhabditis* Genetics Center genetic map 1981, 1983) for *unc-86*, *sma-3*, and *sma-4*; we believe our map positions to be correct. We mapped *lin-16*, *unc(st311)*, *nDf16*, *nDf20*, *nDf21* and *nDf22* more precisely than previously. Figure 1 shows a map of the markers on LG III close to *unc-86*, constructed from these and other data.

Generation of deficiencies

A deficiency of a locus is valuable because it is a "reference null allele," an allele that is known to produce no gene product, against which other alleles can be compared. Because we did not know if animals carrying the existing *unc-86* alleles in *trans* to a deficiency would be viable, we obtained deficiencies by selecting for γ-ray-induced mutations failing to complement *dpy-19(e1259)* but complementing *unc-32(e189)*: γ-ray mutagenized N2 males were crossed to

Table 1

A, three-factor mapping data. Only one class of recombinant was picked from the heterozygote if 1) only that class could be easily distinguished from the parental double mutant, or 2) the other class of recombinants would not give scorable results. Results are given as gene orders, with parentheses around unordered genes, with number of recombinants given for each interval.

B, deficiency complementation results. Heterozygous or homozygous mutant males were crossed into deficiency heterozygotes and the F1 progeny were scored for the presence of animals with the mutant phenotype. If none were found, Wild-type hermaphrodites were cloned and their progeny scored to confirm that some were heterozygotes for the mutation and the deficiency.

A

Heterozygote	Animals picked	Results
unc-36(e251)/dpy-17(e164) unc-86(e1416)	5 Unc-86, 11 Dpy-17	*dpy-17* (*unc-86 unc-36*)
unc-86(e1416)/dpy-17(e164) unc-32(e189)	10 Dpy-17	*dpy-17* (*unc-32 unc-86*)
daf-4(e1364)/lon-1(e185) lin-16(e1743)	9 Lon-1	*lon-1* (3) *daf-4* (6) *lin-16*
sma-3(e491)/lon-1(e185) lin-16(e1743)	7 Lon-1	*lon-1* (6) *sma-3* (1) *lin-16*
unc-86(e1416)/lon-1(e185) unc-36(e251)	10 Lon-1	*lon-1* (*unc-36 unc-86*)
unc-36(e251)/lon-1(e185) unc-86(e1507)	15 Unc-86, 1 Lon-1	*lon-1* (15) *unc-36* (1) *unc-86*
unc-36(e251)/lon-1(e185) unc-86(e1416)	15 Unc-86, 4 Lon-1	*lon-1* (*unc-86 unc-36*)
lin-16(e1743)/lon-1(e185) dpy-19(e1259)	14 Lon-1, 12 Dpy-19	*lon-1* (12) *lin-16* (14) *dpy-19*
unc-86(e1507)/lon-1(e185) dpy-19(e1259)	10 Lon-1, 6 Dpy-19	*lon-1* (14) *unc-86* (2) *dpy-19*
unc-86(e1416)/lon-1(e185) dpy-19(e1259)	8 Lon-1, 11 Dpy-19	*lon-1* (16) *unc-86* (3) *dpy-19*
unc-86(n848)/lon-1(e185) dpy-19(e1259)	10 Lon-1, 10 Dpy-19	*lon-1* (16) *unc-86* (4) *dpy-19*
unc-86(n1042)/lon-1(e185) sma-2(e502)	15 Sma-2	*lon-1* (12) *unc-86* (3) *sma-2*
unc-86(e1416)/lon-1(e185) unc-32(e189)	6 Lon-1	*lon-1* (4) *unc-86* (2) *unc-32*
sma-3(e491)/lin-16(e1743) unc-86(e1507)	10 Unc-86	(*sma-3 lin-16*) *unc-86*
unc-86(e1416)/unc-36(e251) dpy-19(e1259)	10 Unc-36, 5 Dpy-19	(*unc-86 unc-36*) *dpy-19*
unc-86(e1507)/unc-36(e251) dpy-19(e1259)	9 Unc-36, 5 Dpy-19	(*unc-86 unc-36*) *dpy-19*
unc-36(e251)/unc-86(e1416) dpy-19(e1259)	4 Dpy-19, 2 Unc-86	(*unc-36 unc-86*) *dpy-19*
unc-36(e251)/unc-86(e1507) dpy-19(e1259)	4 Unc-86, 1 Dpy-19	(*unc-36 unc-86*) *dpy-19*
unc-86(e1416) sma-2(e502)/dpy-19(e1259) unc-32(e189)	13 Sma-2	*unc-86* (8) *dpy-19* (5) *sma-2*
unc(st311)/unc-86(e1416) unc-32(e189)	2 Unc-86	*unc-86* (1) *unc(st311)* (1) *unc-32*
dpy-19(e1259)/unc-86(e1416) unc-32(e189)	8 Unc-86	*unc-86* (6) *dpy-19* (2) *unc-32*
unc-47(e307)/unc-86(e1416) dpy-19(e1259)	1 Unc-86, 2 Dpy-19	*unc-86* (*dpy-19 unc-47*)
unc-50(e306)/unc-86(e1416) dpy-19(e1259)	4 Unc-86, 2 Dpy-19	*unc-86* (*dpy-19 unc-50*)
dpy-19(e1259)/unc-86(e1416) unc-69(e587)	16 Unc-86, 2 Unc-69	*unc-86* (15) *dpy-19* (3) *unc-69*
unc-69(e587)/unc-86(e1416) unc-47(e307)	9 Unc-86, 10 Unc-47	*unc-86* (*unc-47 unc-69*)
unc-50(e306)/unc-86(e1416) unc-69(e587)	13 Unc-86	*unc-86* (*unc-69 unc-50*)
unc-69(e587)/unc-86(e1416) unc-50(e306)	5 Unc-86, 9 Unc-50	*unc-86* (*unc-50 unc-69*)
unc-47(e307)/unc-86(e1416) unc-69(e587)	9 Unc-86	*unc-86* (8) *unc-47* (1) *unc-69*
unc-49(e382)/unc-86(e1416) unc-69(e587)	13 Unc-86	*unc-86* (*unc-69 unc-49*)
lon-1(e185) sup-5(e1464)/ dpy-19(e1259) sma-2(e502); lin-1(e1777)	1 Dpy-19, 3 Sma-2	*dpy-19* (2) *sup-5* (2) *sma-2*
lon-1(e185) sup-5(e1464)/ dpy-19(e1259) unc-32(e189); lin-1(e1777)	2 Dpy-19, 2 Unc-32	*dpy-19* (1) *sup-5* (3) *unc-32*
sma-2(e502)/dpy-19(e1259) unc-32(e189)	12 Unc-32	*dpy-19* (9) *sma-2* (3) *unc-32*
unc-50(e306)/dpy-19(e1259) unc-49(e382)	13 Unc-49, 8 Dpy-19	*dpy-19* (15) *unc-50* (6) *unc-49*
unc-47(e307)/unc-69(e587) dpy-18(e364)	29 Dpy-18	(*unc-47 unc-69*) *dpy-18*
unc-47(e307)/unc-50(e306) dpy-18(e364)	20 Unc-50, 19 Dpy-18	(*unc-47 unc-50*) *dpy-18*
unc-47(e307)/unc-49(e382) dpy-18(e364)	24 Dpy-18	(*unc-47 unc-49*) *dpy-18*
unc-50(e306)/unc-69(e587) dpy-18(e364)	45 Dpy-18	(*unc-50 unc-69*) *dpy-18*
unc-50(e306)/unc-49(e382) dpy-18(e364)	8 Unc-49, 8 Dpy-18	(*unc-50 unc-49*) *dpy-18*
unc-49(e382)/unc-69(e587) dpy-18(e364)	20 Dpy-18	*unc-69* (8) *unc-49* (12) *dpy-18*
unc-49(e382)/unc-50(e306) dpy-18(e364)	8 Unc-50, 12 Dpy-18	*unc-50* (7) *unc-49* (13) *dpy-18*

B

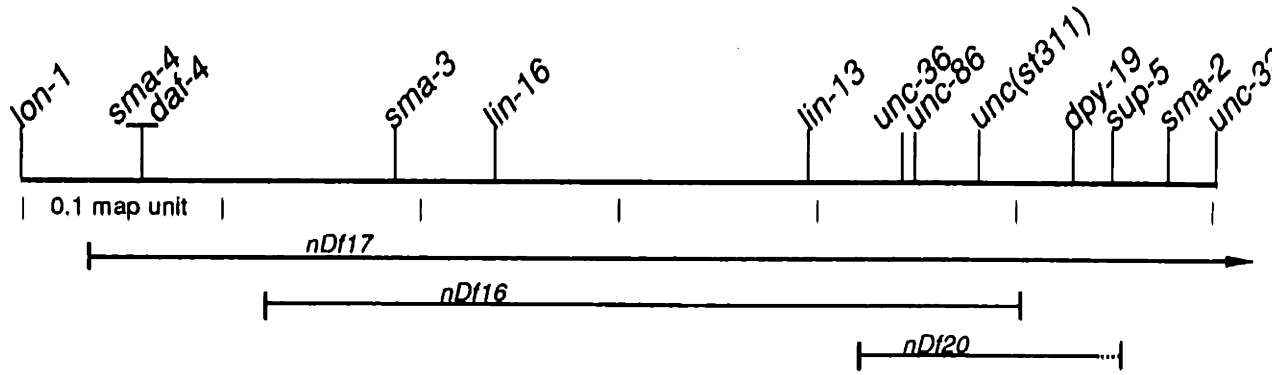
nDf16 does not delete *sma-4(e729)*
nDf16 deletes *sma-3(e491)*
nDf16 deletes *lin-16(e1743)*
nDf16 deletes *lin-13(n387)*
nDf16 deletes *unc(st311)*
nDf16 does not delete *sma-2(e502)*
nDf17 deletes *sma-4(e729)*
nDf17 deletes *sma-3(e491)*
nDf17 deletes *unc(st311)*
nDf17 does not delete *unc-50(e306)*
nDf17 does not delete *unc-69(e587)*

nDf20 does not delete *sma-3(e491)*
nDf20 does not delete *lin-13(n387)*
nDf20 deletes *unc-36(e251)*
nDf20 deletes *unc-86(e1416)*
nDf20 deletes *unc(st311)*
nDf20 deletes *dpy-19(e1259)*
nDf20 does not delete *sma-2(e502)*
nDf21 does not delete *unc-86(e1416)*
nDf21 deletes *dpy-19(e1259)*
nDf21 does not delete *sma-2(e502)*
nDf22 does not delete *unc-86(e1416)*
nDf22 does not delete *unc(st311)*
nDf22 deletes *dpy-19(e1259)*
nDf22 does not delete *sma-2(e502)*

Figure 1

Map of the center ("cluster") of LG III. Extent of the deficiencies is shown below the line. *nDf20* can not be genetically mapped with respect to *sup-5* because suppressor alleles are dominant.

Figure 1


lon-1
sma-4
daf-4
sma-3
lin-16
lin-13
unc-36
unc-86
unc(st311)
dpy-19
sup-5
sma-2
unc-32
0.1 map unit
nDf17
nDf16
nDf20

dpy-19(e1259) unc-32(e189) hermaphrodites and progeny that were Dpy but not Unc were selected. More than 22,000 F1 progeny were screened, and *nDf20*, *nDf21* and *nDf22* were isolated; of these, only *nDf20* uncovers *unc-86*. *nDf20* also uncovers at least one marker on each side of *unc-86*, proving that it deletes the whole locus (table 1). All *unc-86* alleles are viable in *trans* to *nDf20*.

Generation of new alleles by complementation screens

We wished to make new alleles of *unc-86* without selecting for viability, in case there were a class of alleles which were not viable as homozygotes. Animals heterozygous for either *unc-86(e1416)* or *unc-86(e1507)* in *trans* to a deficiency are viable. Therefore animals carrying a complete loss-of-function allele generated in a mutagenesis in *trans* to either allele would be viable, even if a homozygote for the new allele were inviable. Screens for mutations failing to complement *e1416* and *e1507* were performed as described in table 2. Five new EMS-induced alleles were found, at a frequency of one per 3000 mutagenized chromosomes. Homozygotes of all alleles are viable; two alleles cause a Him phenotype and three do not.

Phenotypes resulting from *unc-86* alleles

unc-86 mutations cause several defects which lead to independently scorable phenotypes. Some of the defects can be attributed to alterations in cell lineages, but others can not (Chalfie et al., 1981). We wanted to know how *unc-86* alleles affect the function of the gene, and especially if any of the alleles resulted in elimination of gene function. We examined homozygous *unc-86* animals

Table 2

Complementation screens for *unc-86* alleles.

dpy-19; him-5 strains were grown at 15°, so that they had a wild type phenotype. Hermaphrodites were mated with mutagenized males for one day and then transferred to new plates daily for two more days. Progeny were scored for Unc-86 phenotype twice, when they were three and four days old.

Protocol	Number of F1 cross progeny	Alleles generated
EMS mutagenized *dpy-19(e1259); him-5(e1467)* males × *unc-86(e1507) unc-69(e587)* hermaphrodites	8400	*n843* Him *n844* non-Him *n845* non-Him
EMS mutagenized *dpy-19(e1259); him-5(e1467)* males × *unc-86(e1416) unc-32(e189)* hermaphrodites	7200	*n846* non-Him *n847* Him

and animals heterozygous for two *unc-86* alleles or an *unc-86* allele and a deficiency for phenotypes which appeared to have different causes. We wished to discover whether the alleles formed a graded series of increasing severity and whether any caused an enhanced phenotype in *trans* to a deficiency. We hoped to discover whether any functions were independently mutable, or whether the phenotypes resulting from different mutations resulted from different mutational states of a single activity.

Four phenotypes were scored as described in Materials and Methods: Mec (Mechanosensory defective, caused by defects in lineages), Egl (Egg-laying defective, caused by failure in cell differentiation), Him (High incidence of males, caused by meiotic nondisjunction), and Mmd (Male mating deficient, unknown cause).

All alleles result in recessive phenotypes. Mutant animals carrying each allele alone and in certain heteroallelic combinations were scored for the phenotypes of Mec, Egl, Him, and Mmd. Three categories of alleles were found.

Weak alleles (see table 3A): *n848* and *n1042* are temperature-sensitive (ts). *n848* is Mec and Egl at 25° but not at 15° or 20°. *n1042* is variably Mec but not Egl at 15°, Mec but not Egl at 20°, and both Mec and Egl at 25°. Neither allele is Him or Mmd at any temperature tested. Three alleles, *u5*, *u168*, and *u301*, behave similarly—they are Mec but not Egl, Him, or Mmd at all temperatures tested.

Common alleles (see table 4A): 21 alleles behave similarly. They are Mec and Egl but not Him or Mmd at all temperatures.

Table 3

Phenotypes of weak *unc-86* alleles. Phenotypes were scored as described in Materials and Methods. +, Wild-type; −, mutant; ±, variable; nt, not tested. A, homozygotes; B, heterozygotes.

A

Genotype	Temperature	Mec	Egl	Him	Mmd
n848	15°	+	+	+	+
n848	20°	+	+	+	+
n848	22.3°	–	–	+	nt
n848	25°	–	–	+	+
n1042	15°	±	+	+	nt
n1042	20°	–	+	+	+
n1042	22.3°	–	+	+	nt
n1042	25°	–	–	+	+
u5	15°, 20°, 25°	–	+	+	+
u168	15°, 20°, 25°	–	+	nt	nt
u301	15°, 20°, 25°	–	+	nt	nt

B

Genotype	Temperature	Mec	Egl	Him	Mmd
n848/e1416	15°, 20°, 25°	–	–	+	nt
n848/e1507	15°, 20°, 25°	–	–	+	nt
n848/nDf20	20°	–	–	+	+
n848/nDf20	15°, 25°	–	–	+	nt
n848/e1507	10°	–	–	nt	nt
n848/n412	10°	–	–	nt	nt
n848/n844	10°, 15°	–	–	nt	nt
n848/n845	10°, 15°	–	–	nt	nt
n848/n846	10°, 15°	–	–	nt	nt
n848/n946	10°, 15°	–	–	nt	nt
n848/n992	10°, 15°	–	–	nt	nt
n848/n993	10°, 15°	–	–	nt	nt
n848/n994	10°, 15°	–	–	nt	nt
n848/n1041	10°	–	–	nt	nt
n848/n1132	10°	–	–	nt	nt
n1042/e1507	20°	–	–	nt	nt
n1042/e1416	20°, 25°	–	–	nt	nt
n1042/nDf20	20°	–	–	nt	+
n1042/nDf20	25°	–	–	nt	nt

Table 4

Phenotypes of common *unc-86* alleles. A, homozygotes; B, heterozygotes. For explanation see table 3 legend.

A

Genotype	Temperature	Mec	Egl	Him	Mmd
e1507	20°, 25°	–	–	+	+
n412	20°, 25°	–	–	+	+
n844	20°, 25°	–	–	+	+
n845	20°, 25°	–	–	+	+
n846	20°, 25°	–	–	+	+
n946	20°, 25°	–	–	+	+
n992	20°, 25°	–	–	+	+
n993	20°, 25°	–	–	+	+
n994	20°, 25°	–	–	+	+
n1041	20°, 25°	–	–	+	+
n1071	20°	–	–	nt	+
n1132	20°, 25°	–	–	+	+
n1351	20°	–	–	+	+
u21	20°, 25°	–	–	+	+
u31	20°, 25°	–	–	+	+
u44	20°, 25°	–	–	+	+
u68	20°, 25°	–	–	+	+
u83	20°, 25°	–	–	+	+
u261	20°	–	–	nt	+
u371	20°	–	–	+	+
rh1029	20°	–	–	nt	+

B

e1507/e1416	20°	–	–	+	nt
e1507/nDf20	20°	–	–	nt	+
n412/e1416	20°	–	–	nt	+
n412/n306	20°	–	–	nt	+
n412/n843	20°	–	–	nt	+
n412/n847	20°	–	–	nt	+
n412/nDf20	20°	–	–	nt	+
n844/e1416	20°	–	–	+	nt
n844/nDf20	20°	–	–	nt	+
n845/e1416	20°	–	–	+	nt
n845/nDf20	20°	–	–	nt	+
n846/e1416	20°	–	–	+	nt
n846/nDf20	20°	–	–	nt	+
n946/e1416	20°	–	–	nt	+
n946/n306	20°	–	–	nt	+
n946/n843	20°	–	–	nt	+
n946/n847	20°	–	–	nt	+
n946/nDf20	20°	–	–	nt	+
n992/nDf20	20°	–	–	nt	+
n993/nDf20	20°	–	–	nt	+
n994/e1416	20°	–	–	nt	+
n994/n306	20°	–	–	nt	+
n994/n847	20°	–	–	nt	+
n994/nDf20	20°	–	–	nt	+
n1041/n306	20°	–	–	nt	+
n1041/nDf20	20°	–	–	nt	+
n1132/nDf20	20°	–	–	nt	+

Table 5

Phenotypes of Him *unc-86* alleles. For explanation see table 3 legend. A, homozygotes; B, heterozygotes.

A

Genotype	Temperature	Mec	Egl	Him	Mmd
e1416	15°, 20°, 25°	-	-	-	-
n306	15°, 20°, 25°	-	-	-	-
n843	15°, 20°, 25°	-	-	-	-
n847	15°, 20°, 25°	-	-	-	-

B

Genotype	Temperature	Mec	Egl	Him	Mmd
e1416/n306	20°	-	-	-	-
e1416/nDf20	20°	-	-	-	-
n306/nDf20	20°	-	-	nt	-
n843/n306	20°	-	-	nt	-
n843/nDf20	20°	-	-	nt	-
n843/e1416	20°	-	-	-	nt
n847/n306	20°	-	-	nt	-
n847/nDf20	20°	-	-	nt	-
n847/e1416	20°	-	-	-	nt

Him alleles (see table 5A): four alleles, *e1416*, *n306*, *n843*, and *n847*, are Mec, Egl, Him and Mmd at all temperatures. The Him and Mmd phenotypes were either both present in or both absent from all single mutants and heteroallelic combinations for which we tested both phenotypes; therefore we tested some mutants only for one of the two phenotypes.

One possible explanation for the range of phenotypes of the *unc-86* alleles is that they represent three levels of reduction of function: the weak alleles have the smallest reduction in function, the common alleles a greater reduction, and the Him alleles the largest reduction. To test this we examined the phenotype of each class of alleles in *trans* to alleles of a putatively stronger class and in *trans* to a deficiency.

The phenotype of *n848* is indeed enhanced in *trans* to common alleles, Him alleles, and a deficiency (see table 3B). *n848* in *trans* to any of the three results in the phenotypes characteristic of a common allele at all temperatures tested. These data suggest that *n848* and the other alleles are mutations that reduce the function of *unc-86* and that there is a threshold in the requirement for *unc-86* function so that a two-fold reduction in the level of *n848* gene dosage makes the mutant phenotype completely penetrant. *n1042* is also enhanced in *trans* to common alleles, Him alleles, and a deficiency. In no case, however, does either allele have a Him or Mmd phenotype.

In contrast, the phenotypes of the common *unc-86* alleles are not enhanced at higher temperatures or in *trans* to Him alleles or deficiency (see table 4B). In no case did any of these alleles have a Him or Mmd phenotype. The original isolates of *e1507* and *u5* were Him

and the original isolate of *n412* was Mmd, but these phenotypes proved to be due to the genetically separable mutations *him(n411)* (V. Ambros, personal communication), *him(n1133)*, and *mab(n1131)*, respectively.

The Him alleles fail to complement each other and the deficiency *nDf20* for the Him and Mmd phenotypes (see table 5B). Neither phenotype is enhanced in *unc-86* over *nDf20* heterozygotes.

Interactions with *him-5*

The Him phenotype of the *unc-86* Him alleles is relatively weak—2.2% of viable progeny are male, while in wild type 0.2% are male (Hodgkin, Horvitz, and Brenner, 1979). Conditions of stress such as heat shock result in increased nondisjunction in *C. elegans* (Meneely and Herman, 1981). We wondered whether the Him phenotype of some *unc-86* alleles could be due to stress resulting from some other uncharacterized defect, or whether it was caused by a direct effect on the machinery necessary for chromosome disjunction (see Discussion for discussion of direct and indirect effects).

By counting the number of XX hermaphrodite, XXX hermaphrodite, male, and inviable progeny in the broods of Him hermaphrodites, it is possible to see whether the ratios are consistent with nondisjunction of the X chromosome alone (*i.e.*, if all of the inviable zygotes are nullo-X animals (resulting from fertilization of a nullo-X egg by a nullo-X sperm), then the frequency of inviable zygotes should be equal to the square of the frequency of nullo-X gametes, or, if that frequency is low, to the square of half the frequency of males). For instance, both *him-5* and *unc-86(e1416)* mutations cause more inviable zygotes than can be accounted for by nondisjunction of the X chromosome

alone, suggesting that there is also some nondisjunction of autosomes. In contrast, *him-8(e1489)* does not seem to cause nondisjunction of the autosomes (Hodgkin, Horvitz, and Brenner, 1981; Table 6).

In the course of other work the double mutation *unc-86(e1416); him-5(e1490)* was constructed. To our surprise, its brood size was extremely low and it produced many more inviable zygotes than expected.

Double mutations were then constructed between several alleles of *unc-86* and *him-5* and *him-8* alleles (table 6A). All had viability and male frequency similar to the *him* mutation alone except those that had *him-5* with a Him allele of *unc-86*. All six such double mutations constructed with three *unc-86* alleles and two *him-5* alleles had the same phenotype: animals appeared healthy and grew normally, but were almost sterile. For instance *unc-86(e1416); him-5(e1490)* produced 71% inviable zygotes, while *unc-86(e1507); him-5(e1490)* produced only 20% inviable zygotes.

Several lines of evidence suggest that the reduced viability of *e1416; e1490* is caused by autosomal nondisjunction. First, the lethality has a maternal effect—there is no decreased viability of *e1416; e1490* progeny of an *e1416 +/+ unc-32(e189); e1490* parent (see table 6 B), implying that an *e1416; e1490* parent produces defective sperm or ova. Second, the lethality can be partly but not completely rescued by mating with wild-type males (see table 6 C), implying that both sperm and ova are defective. Third, *e1416; e1490* produces progeny likely to be abnormal in chromosome number. Some are almost certainly polyploids (Madl and Herman, 1979)—Long animals that segregate both Long and WT-length progeny. Others may be aneuploids—animals that

Table 6

unc-86 interactions with *him* mutations. A, percent viability and brood size of double mutations and single mutations. Percent viability was calculated as follows: parental hermaphrodites were transfered to a new plate each day and the total number of eggs and larvae recorded; after 24 hours, larvae and unhatched eggs were counted; after 48 hours, viable animals were counted. The number of viable animals was divided by the highest total number recorded on day 1 or day 2. The percent viable is likely to be an overestimate for all Unc-86 animals because they are Egg-laying defective and many inviable zygotes are never laid, but instead degenerate internally. L means less than about 25% and H means more than about 50%. For brood size, L means less than about 25 and high means more than about 50.

B, segregants from animals homozygous for *him-5* and heterozygous for a Him allele of *unc-86*.

C, % viable progeny and brood size of *unc-86*(Him); *him-5* double mutants mated with wild-type males.

A

unc-86 genotype \ *him* genotype	% viable / brood size *him-5* *e1467* 15°	*him-5* *e1467* 20°	*him-5* *e1467* 25°	*him-5* *e1490* 20°	*him-8* *e1489* 20°
+	70 / 100	nt	69 / 148	66 / 190	H / H
e1416/+	68 / 129	78 / 137	72 / 115	nt	nt
e1507	104 / 49	nt	70 / 53	80 / 71	H / H
e1416 (Him)	nt	22 / 21	11 / 3.5	29 / 10	H / H
n306 (Him)	nt	L / L	nt	21 / 11	H / H
n843 (Him)	nt	L / L	nt	21 / 5	nt
e1416/nDf20 (Him)	nt	nt	nt	39 / 22	nt

B

Parental genotype	Progeny phenotype (%) Unc-86	WT	Unc-32	
e1416 + / *+ unc-32(e189)* ; e1467	25	51	24	(n=164)

C

Cross	% viable	brood size
N2 males × *e1416; e1490*	54	30
N2 males × *n306; e1490*	45	55

appear sick, grow slowly, and produce few or no eggs; some of these eggs hatch into healthy-appearing individuals.

him-5 mutations, which cause preferential but probably not completely specific nondisjunction of the X chromosome, are enhanced by Him alleles of *unc-86* to affect all chromosomes. A *him-8* mutation, which causes nondisjunction that is probably specific for the X, is not enhanced by Him alleles of *unc-86*. These facts together suggest (see Discussion) that the *unc-86* Him alleles have specific effects on the process of meiotic chromosome segregation.

Temperature-sensitive period

The *unc-86* allele *n848* is ts for the Mec and Egl phenotypes, allowing determination of the temperature-sensitive period (tsp) of *unc-86*. The tsp was determined by growing the animals for one generation at the starting temperature, then transferring animals of a chosen stage to new plates pre-equilibrated to the second temperature. Results for Mec and Egl phenotypes are given in figure 2.

The mechanosensory neurons necessary for touch response are generated before hatching (Sulston, Schierenberg, White and Thomson, 1983); consistent with this, the tsp for Mec is embryonic.

The HSN neurons, which drive egg laying and which fail to mature in *unc-86* mutants, are generated embryonically and undergo further differentiation and process outgrowth in the fourth larval stage. *unc-86* animals produce normal or near-normal presumptive HSN neurons: HSNs in *unc-86* animals are generated at the right time, migrate to the correct place, appear normal in the first larval stage, and are affected by mutations specific for the HSN (J. Sulston, personal

Figure 2

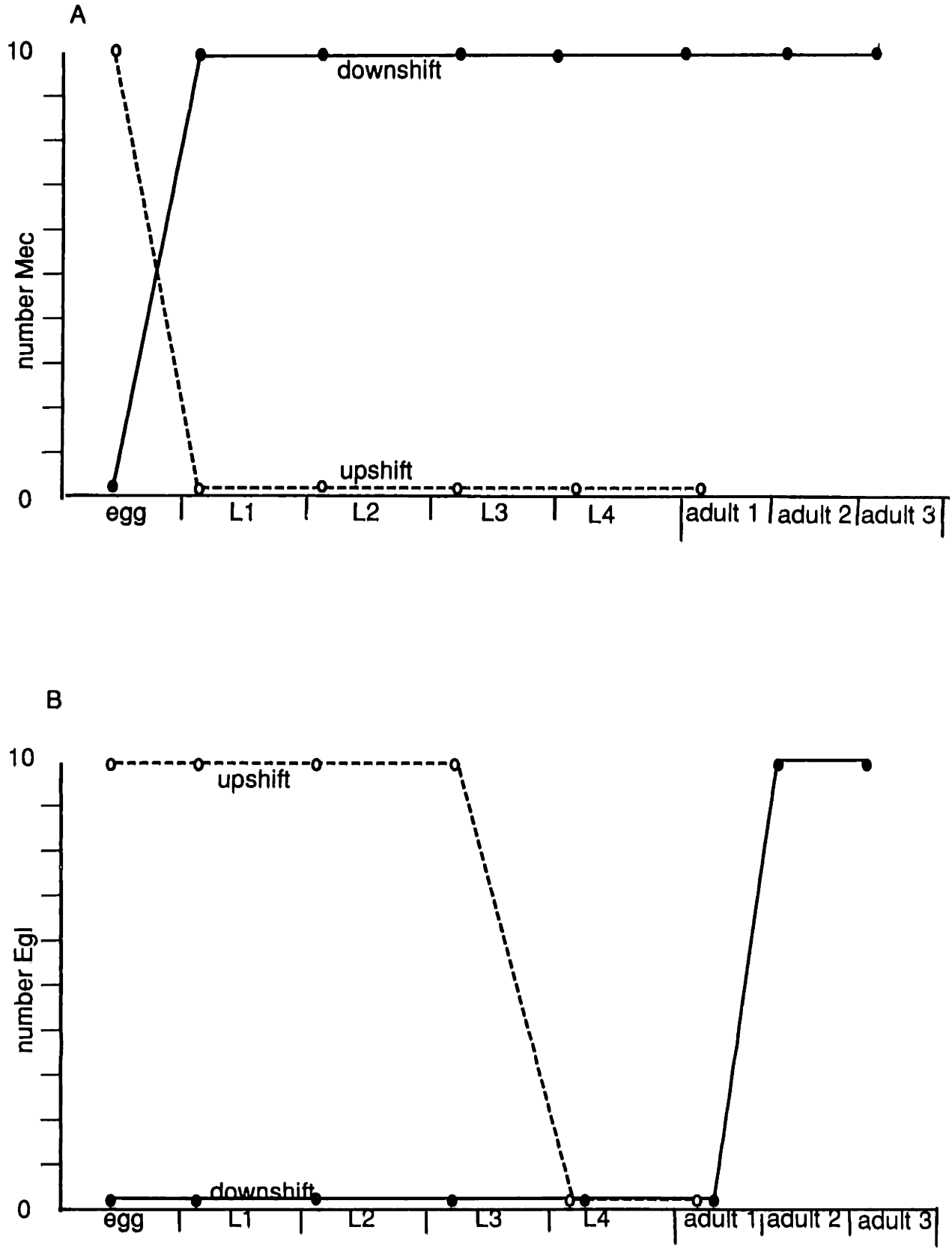
Temperature shifts of *unc-86(n848)*.

Animals were grown at 15° for one generation and transferred to 25° (upshift) or were grown at 25° and transferred to 15° (downshift). Stages were scored by eye; all animals were young animals of the stage indicated except: adult 1 refers to young adults that have not yet produced any eggs; adult 2 refers to adults that have produced a few (3-6) eggs; adult 3 refers to adults that have produced enough eggs (15-20) that they are visible as two rows in each half gonad. Ten animals of each stage were scored.

A, Mec.

B, Egl

Figure 2


A
10
0
number Mec
downshift
upshift
egg
L1
L2
L3
L4
adult 1
adult 2
adult 3
B
10
0
number Egl
upshift
downshift
egg
L1
L2
L3
L4
adult 1
adult 2
adult 3

communication; H. Ellis, personal communication). Adult *unc-86* animals, however, do not have serotonin-containing HSN neurons (C. Desai, personal communication). The tsp for Egl is in the fourth larval stage, suggesting that HSN differentiation is the affected step. The data in figure 2B indicate that an *unc-86* mutant in the fourth larval stage has HSNs which are capable of differentiating into functional neurons if *unc-86* product is provided.

The tsp for HSN differentiation has another interesting feature. Shifting to low temperature in the early adult stage is sufficient for an Egl^+ phenotype, but shifting to high temperature during the fourth larval stage does not cause an Egl phenotype. There are two possible explanations for this observation. First, *n848* could be ts for synthesis of the *unc-86* product; *unc-86* would have to be synthesized during the fourth larval and adult stages but required only in the adult stage. Second, at the restrictive temperature the incompletely differentiated HSNs could remain in a state that the *unc-86* gene product is capable of acting on, for some time after the *unc-86* gene product would normally have acted.

Isolation of suppressors

n363 was isolated by Victor Ambros as a weak recessive suppressor of *unc-86(e1416)*. *n363* animals are thin; egg-laying constitutive (Egl^C), *i.e.*, they lay their eggs at earlier stages than wild-type animals do and in some circumstances that wild-type animals do not (E. Wolinski, personal communication); hyperactive; and bend their bodies more sharply than wild-type when moving. *n363; e1416* animals are Mec and retain more eggs than *n363* alone, but move more than *e1416* alone.

Reversion experiments were carried out on *unc-86(n848ts)*. *n848* was chosen because it is a weak allele and therefore might be easier to suppress. Animals were mutagenized with EMS and placed at 25° and the F1 and F2 generations were screened for Mec^+ and/or Egl^+ animals.

n892 was isolated in the F1 generation of the above screen. The original isolate was a semidominant suppressor of *n848*, and caused a phenotype similar to but more extreme than that of *n363* animals. It also caused animals to grow slowly and have a small brood size. Frequent partial revertants that weakened all phenotypes of the suppressor mutation appeared and quickly overgrew the original mutation; the original isolate could not be maintained, but a partial revertant that still had significant, albeit recessive, suppressing activity was rescued and named *n892*. *n892* animals are similar in phenotype to *n363* animals; *n892* complements *n363*.

Bob Horvitz noticed a resemblance between the phenotypes of the two mutants and the phenotype of a mutant, *n1134*, found by M. Chalfie to be partly resistant to the effects of exogenously added serotonin (5-hydroxytryptamine; see below). *dpy-5(e61) n363* hermaphrodites mated with *n1134/+* males produced some non-Dpy cross progeny that displayed the thin, hyperactive phenotype, showing *n1134* to be allelic to *n363*.

Both *n892* and *n363* are partly resistant to serotonin: when wild-type worms are placed on media containing serotonin, their pharynges pump rapidly, they lay eggs abnormally quickly, and they stop moving; when *n892* and *n363* worms are exposed to serotonin, they pump and lay eggs, but do not stop moving.

Both genes were mapped using their thin, hyperactive phenotype. *n363* and *n1134* map to LG I and *n892* maps to LG X (figure 3). *n892* maps

Figure 3

Map positions of *unc-86* suppressors.

A. Map data for *sup(n363,n1134)*.

B. Genetic map of part of LG I showing position of *sup(n363,n1134)*.

C. Map data for *sup(n892)*.

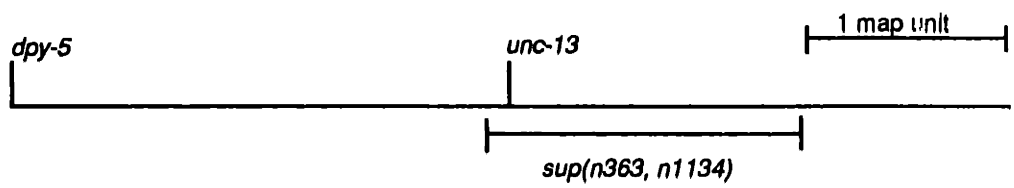
D. Genetic map of part of LG X showing position of *sup(n892)*. Mapping *sup(n892)* with respect to *lin-32* is difficult because mutations in both genes lead to subtle phenotypes that are difficult to distinguish from each other, especially in the presence of other mutations.

Figure 3

A

Heterozygote	Animals picked	Results
sup(n363) / *dpy-5(e61)unc-13(e1091)*	20 Sup(n363)	*dpy-5 sup(n363)* 2.5% *unc-13 sup(n363)* 0%
sup(n1134) / *dpy-5(e61)unc-13(e1091)*	22 Sup(n1134)	*dpy-5 sup(n1134)* 4.5% *unc-13 sup(n1134)* 0%
sup(n1134) / *dpy-5(e61)unc-13(e1091)*	20 Dpy-5 10 Unc-13	*dpy-5 (unc-13 sup(n1134))*

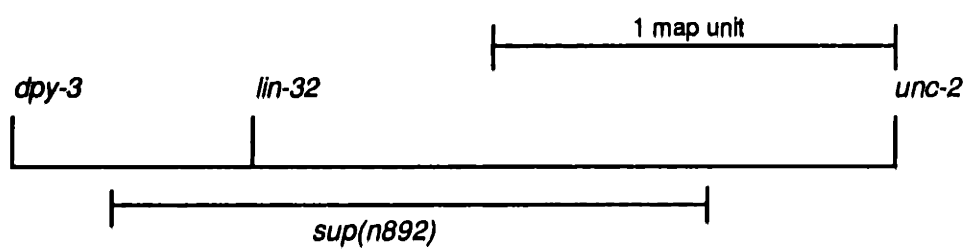
B



C

Heterozygote	Animals picked	Results
sup(n892) / *dpy-3(e27) unc-2(e55)*	4 Unc-2	*dpy-3* (1/4) *sup(n892)* (3/4) *unc-2*

D



to the same interval as *lin-32*, which was previously defined by two recessive alleles (C. Kenyon, personal communication; M. Chalfie, personal communication). *lin-32(u282)*, the stronger of the two alleles, has a strongly-bent phenotype similar to *n892*, but it is sluggish instead of hyperactive, Egl instead of Egl^c, variably Mec, and sensitive to serotonin. *n892* complements *lin-32(u282)*. Because the phenotypes of both mutants are subtle and somewhat similar, it is not feasible to map them with respect to each other directly. *lin-32(e1926)* has been shown to affect a number of neural cell lineages, many of which are also affected by *unc-86* (C. Kenyon personal communication). It is possible that *n892* is a gain-of-function allele of *lin-32*. This possibility could be tested if null alleles of *sup(n892)* could be found by screening for loss of suppression of *n848*, but the suppression of *n848* by *n892* is not sufficiently penetrant.

Suppression of *unc-86* by *n892* and *n363* is summarized in table 7. The suppression of *e1416* in the *n363; n892* triple mutant was confirmed by examining animals with Nomarski optics. Two of two adults and one of two animals in the second larval stage had an ALM (the anterior lateral mechanosensory neuron) on the side examined; in none of the animals was a PLM (posterior lateral mechanosensory neuron) seen and in neither adult was an HSN neuron recognizable (V. Ambros and M.F.).

Table 7

Suppression of *unc-86*. +, at least 80% wild type; −, mutant; ±, about 50% mutant; nt, not tested. The Egg-laying phenotype of animals carrying *n363* or *n363; n892* is difficult to score because of the thin body shape and low brood size of these animals.

unc-86 genotype	suppressor genotype	temp.	Mec	Egl
+	*n892*	20°,	+	+
	n363	25°	+	+
n848	+	25°	–	–
	n892		+	+
	n363		+	+
n848/e1416	+	20°	–	–
	n892		+	+
	n363		+	nt
n848/e1416	+	25°	–	–
	n892		+	+
	n363		+	nt
e1416	+	20°	–	–
	n892		–	–
	n363		–	–
	n363; n892		±	nt

Chapter 2

Complementation screens for mutator-derived *unc-86* alleles

We attempted to get Tc1 insertions into *unc-86* by looking for spontaneous *unc-86* mutations in Mutator strains (see Introduction). We chose to use complementation screens—mating *unc-86* males into Mutator hermaphrodites and looking for *unc-86* F1 progeny—to avoid some of the problems of simple screening of Mutator strains. Direct screens can be foiled if null mutations in the gene being screened for have a lethal or sterile phenotype. Mutant animals from direct screens are also likely to have mutations in other genes that interfere with fertility, because those animals are likely to be homozygous for a large number of mutant loci. This is especially important for mutator strains because homozygotes of some strains (e.g. TR679 *(mut-2(r459))*) produce so few eggs that the most useful phenotype of *unc-86*, Egl, can not be scored.

Table 1 lists the complementation screens performed. More than 22,000 hermaphrodite cross progeny of six mutator strains were examined. No *unc-86* alleles were found. We suspect that the site specificity of Tc1 may make Tc1 insertions in *unc-86* very rare.

Mutator-derived *unc-86* alleles from other sources

While maintaining, backcrossing, mapping and constructing mutator strains we have had quite a bit of opportunity to screen those strains for Unc-86 animals. During the construction of MT2878 (see appendix) we isolated *unc-86(n1432(lost))* in the F2 progeny from a cross of N2 males with TR679.

Table 1

Complementation screens with mutator strains. 8 or more *unc-86 dpy-19* males were mated with 4 or 5 of the indicated hermaphrodites for one day, and the hermaphrodites were transferred to a new plate each day for three days. The number of hermaphrodite cross progeny was estimated by counting the number of male progeny.

TR679: *mut-2(r459)*

NJ81: has mutator activity (E. Hedgcock, personal communication).

TR679 SK1: an isolate of TR679 carrying a *dpy* mutation, found by S. Kim.

MT2879: *mut-2(r459)* crossed three times into Bristol (see appendix).

MT2879 *dpy-19(n1347) unc-49(n1324)*: an isolate of MT2879 carrying the indicated spontaneous mutations.

MT2975: *mut-2(r459); sma-3(e491) unc-32(e189)* crossed four times into Bristol.

unc-86(e1507) dpy-19(e1259) males ×	approx. number of hermaphrodite cross progeny scored
TR679	2300
NJ81	2500
TR679 SK1	900
MT2879	3950
MT2879 *dpy-19(n1347) unc-49(n1324)*	1300
MT2975	11,600

Very large numbers of animals from mutator strains have been examined in screens that would pick up *unc-86* animals. Stuart Kim screened approximately 200,000 TR679 animals for mutants defective in egg laying and found one *unc-86* allele, *n1351*. Martin Chalfie and coworkers found one *unc-86* allele, *u371* in a screen of TR679 animals for Mec mutants. Edward Hedgecock found one *unc-86* allele, *rh1029*, in a screen of TR679.

Tc1s in mutator-derived *unc-86* alleles

To remove the many extraneous copies of Tc1 present in the mutator parent strains, we repeatedly crossed the two *unc-86* alleles, *n1432(lost)* and *n1351*, into Bristol strains using a scheme similar to that in figure 5. We then constructed strains having mutation each in *trans* to double mutations spanning *unc-86*, and picked recombinants. For instance, the heterozygote *n1351/sma-3(e491) unc-32(e189)* was constructed and Sma and Unc-32 recombinants were picked. Each recombinant was made homozygous, scored for *n1351*, and analyzed by Southern blots of DNA cut with *Eco* RI, *Hind* III, and both enzymes together, probed with Tc1 (for construction of probe see below). Representative gels are shown in figures 1 and 2.

n1432(lost), which came from an F1 heterozygote between N2 and TR679, seems to have been generated on a Bristol chromosome. It has only one extra Tc1, which maps near or to the right of *dpy-19*. *n1351* came from TR679 and has five Tc1s linked to it; however, there is no Tc1 that is present in every strain that has *n1351* and absent in every strain that does not.

Figure 1

Tc1 insertions near *unc-86(n1351)*. *unc-86(n1351)*, a spontaneous mutation from the mutator strain TR679, was crossed 10 times into Bristol and made heterozygous with *sma-3(e491) unc-32(e189)*, a double mutation spanning *unc-86*. Sma non-Unc and Unc non-Sma recombinants were picked, and their progeny were scored for the presence of *unc-86(n1351)*. DNA was prepared from homozygous recombinant strains, digested with restriction endonucleases, transfered to nylon filters, and probed with ^{32}P labelled Tc1.

Each lane is an independent recombinant. Lanes A-H cut with *Hind* III. A, *eT1* (identical pattern to N2); B, *sma-3(e491) unc-86*(+); C, *sma-3(e491) unc-86(n1351)*; D, *sma-3(e491) unc-86(n1351)*; E, *unc-86(n1351)* ten times crossed into Bristol; F, *unc-86(n1351) unc-32(e189)*; G, *unc-86(n1351) unc-32(e189)*; H, *unc-86*(+) *unc-32(e189)*. Lanes I-P cut with *Hind* III and *Eco* RI. I, N2; J-P as in B-H.

A Tc1 insertion in *unc-86* would appear in lanes C, D, E, F, and G, but not A, B, and H; and also in K, L, M, N and O, but not I, J and P.

Figure 1

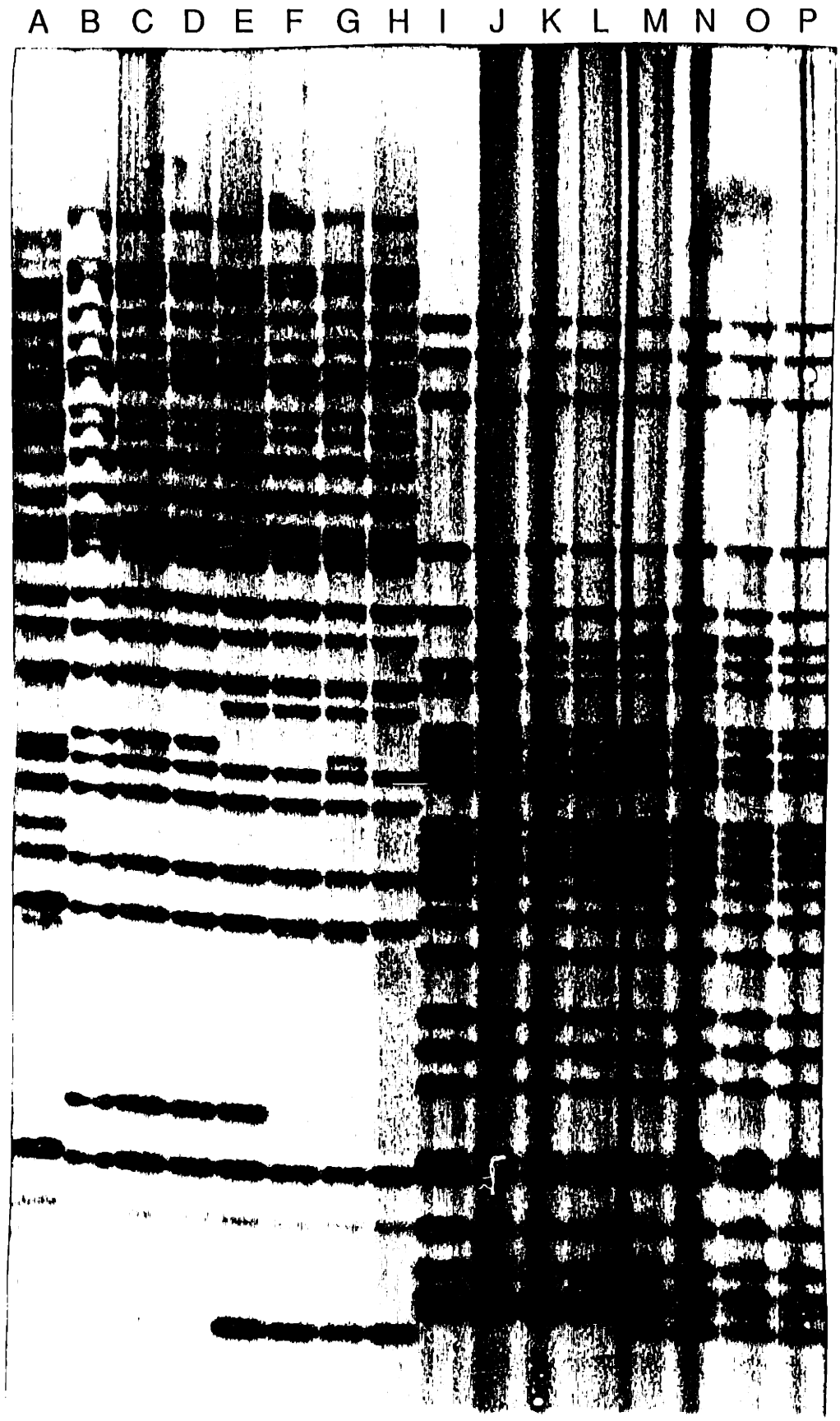


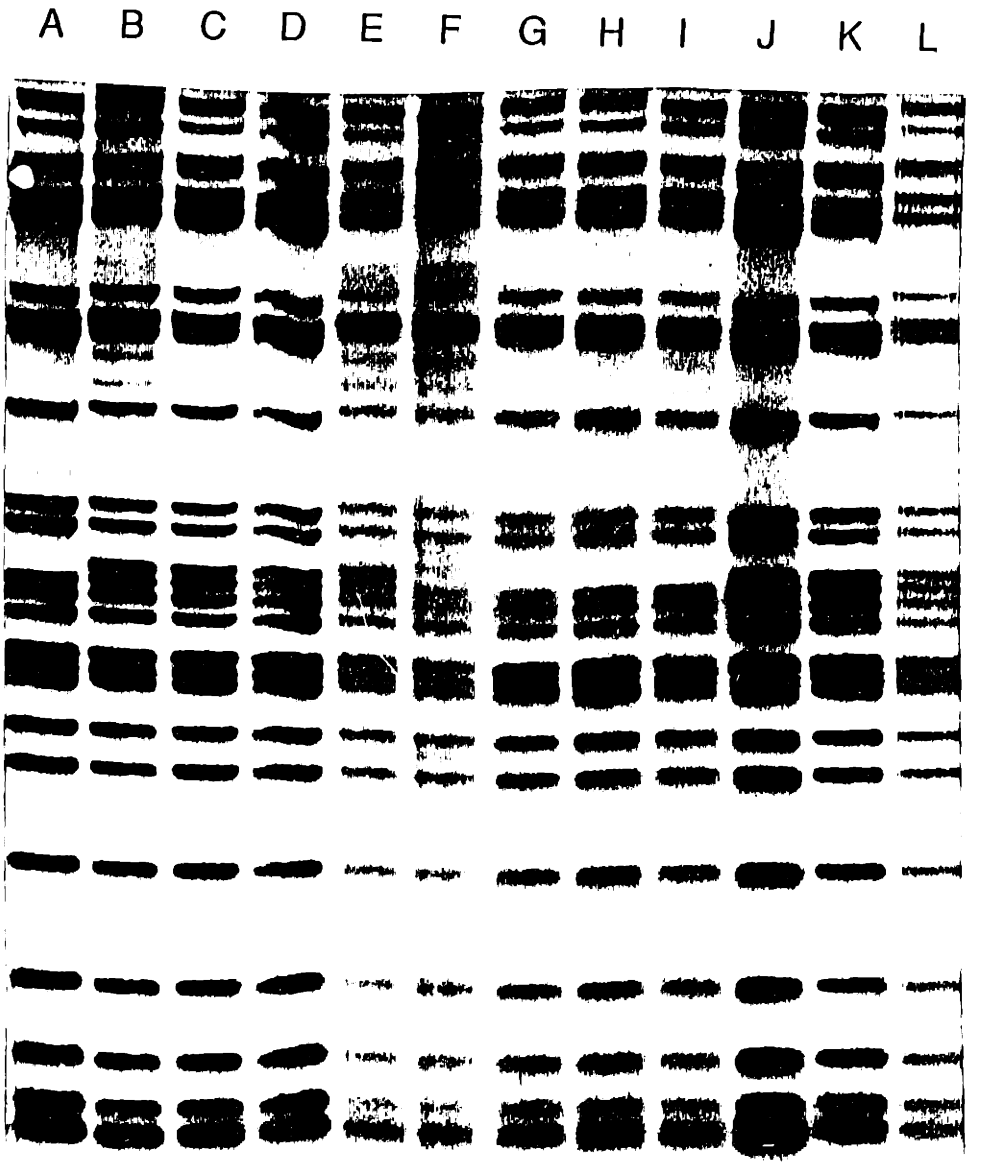
Figure 2

Tc1 insertions around *unc-86(n1432)*. *unc-86(n1432)*, a spontaneous allele isolated as described in the text, was crossed 10 times into Bristol and made heterozygous with *lon-1(e185) dpy-19(e1259)*, a double mutation spanning *unc-86*. Lon non-Dpy and Dpy non-Lon recombinants were picked and scored for the presence of *unc-86(n1432)*. Southern blots were performed as described in figure legend 1.

All lanes are independent backcrosses or recombinants. All lanes digested with *Eco* RI. A, N2; B, *unc-86(n1432)* crossed 10 times into Bristol; C, *unc-86(n1432)* crossed 10 times into Bristol; D, *lon-1(e185) unc-86(n1432)*; E, *lon-1(e185) unc-86(+)*; F, *unc-86(+) dpy-19(e1259)*; G, *unc-86(+) dpy-19(e1259)*; H, *unc-86(n1432) dpy-19(e1259)*; I, *unc-86(+) dpy-19(e1259)*; J, *lon-1(e185) unc-86(n1432)*; K, *lon-1(e185) unc-86(+)*; L, *lon-1(e185) unc-86(n1432)*.

A Tc1 insertion in *unc-86* should be visible as a band in lanes B, C, D, H, J and L, but no others.

Figure 2



u371 and *rh1029* were received after *unc-86* had already been cloned by walking (see below), so they were not subjected to the analysis described above.

Recombinant congenic strains

Most *C. elegans* wild-type strains have about 30 copies of the transposable element Tc1, but a few strains have many more copies (Emmons *et al.*, 1983). The wild-type strain used for genetics, var. Bristol strain N2, has exactly thirty copies of Tc1 (see figures 7 and 8); var. Bergerac strain RW7000 has about 500 copies (figure 3). Given that *C. elegans* has a haploid genome size of 8×10^7 base pairs (Sulston and Brenner, 1974), if the extra Tcls in Bergerac were distributed evenly over the whole genome, there would be one every 160 kilobases (kb). Thus if one could obtain a genomic clone for the nearest Tc1 insertion to a gene, it might well be possible to clone that gene using the technique of chromosomal walking (*e. g.* Bender *et al.*, 1983).

The restriction enzyme *Eco* RV cuts in the inverted repeats of Tc1 19 base pairs from each end (Rosenzweig *et al.*, 1983). The *Eco* RV fragment containing almost all of Tc1 was subcloned from pCe2001 (Emmons et al., 1983) into the *Eco* RV site of pBR322 to produce pCeT1 for use as a hybridization probe (see figure 4).

The first step in identifying the closest Tc1 insertion to *unc-86* was to construct a recombinant strain that is entirely Bristol except for a Bergerac region on LG III around *unc-86*. This strain should have only a few more Tcls than the Bristol parent, but all of them should be linked to *unc-86*. The region of the Bergerac strain RW7000 around *unc-86* was crossed into Bristol 10 times as shown in figure 5. Each

Figure 3

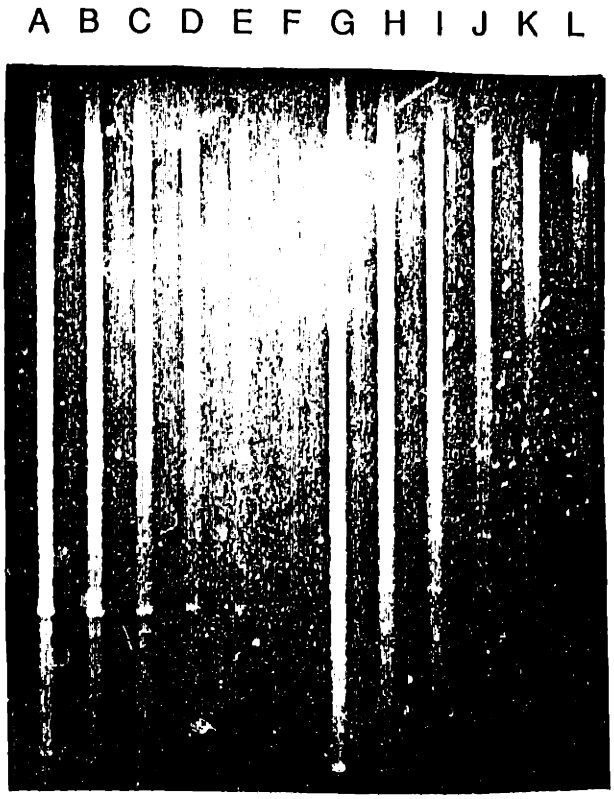
Comparison of the Tc1 copy number of N2 (Bristol) and RW7000 (Bergerac). N2 and RW7000 DNA was cut with *Eco* RV, which cuts in the inverted repeats of Tc1, and analyzed by Southern transfer and probing with Tc1.

A, photograph of gel stained with ethidium bromide. Lanes A-F, 2-fold serial dilutions of RW7000 DNA; lanes G-L, 2-fold serial dilutions of N2 DNA. There is slightly more DNA present in the N2 lanes than in the corresponding RW7000 lanes.

B, Autoradiogram of Southern blot probed with Tc1. Lanes A-L, as above. The intensity of the single 1.6 kb band containing almost all Tc1-hybridizing material was compared among lanes. There was slightly more radioactivity present in the RW7000 lanes containing nominally 16-fold less DNA than the N2 lanes. Shorter exposures of the same filter confirm this result (not shown), indicating that film saturation has not biased the results. Combined with the information in part A, this indicates that there are about 20 times more copies of Tc1 in RW7000 than in N2.

Figure 3

A



B

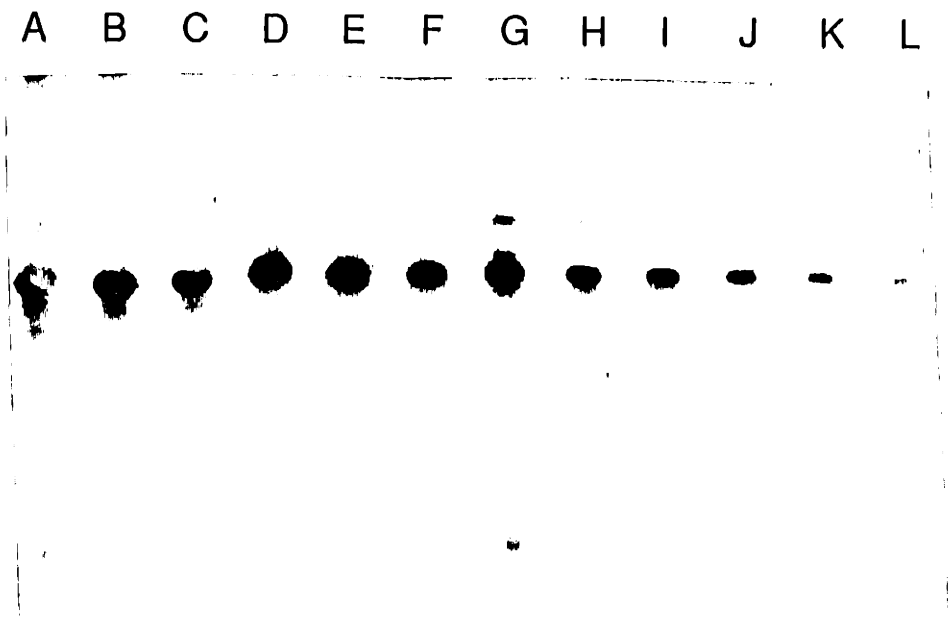


Figure 4

A. Restriction map of Tc1 (Rosenzweig et al., 1983). *Eco* RV cuts 19 bp from each end.

B. pCe2001 (Emmons et al., 1983) has an 8.7 kb insert containing Tc1.

C. pCeT1 and pCeT2 were constructed by cutting pCe2001 with *Eco* RV, preparing the 1.6 kb doublet from an agarose gel by electrophoresis onto diethylaminoethyl paper (Schleicher and Schuell), elution at pH 11, and ligation with *Eco* RV-cut pBR322. DNA was prepared from Amp^{r} Tet^{s} colonies and cleaved with *Sal* I to identify the correct clones and establish their orientations.

Figure 4

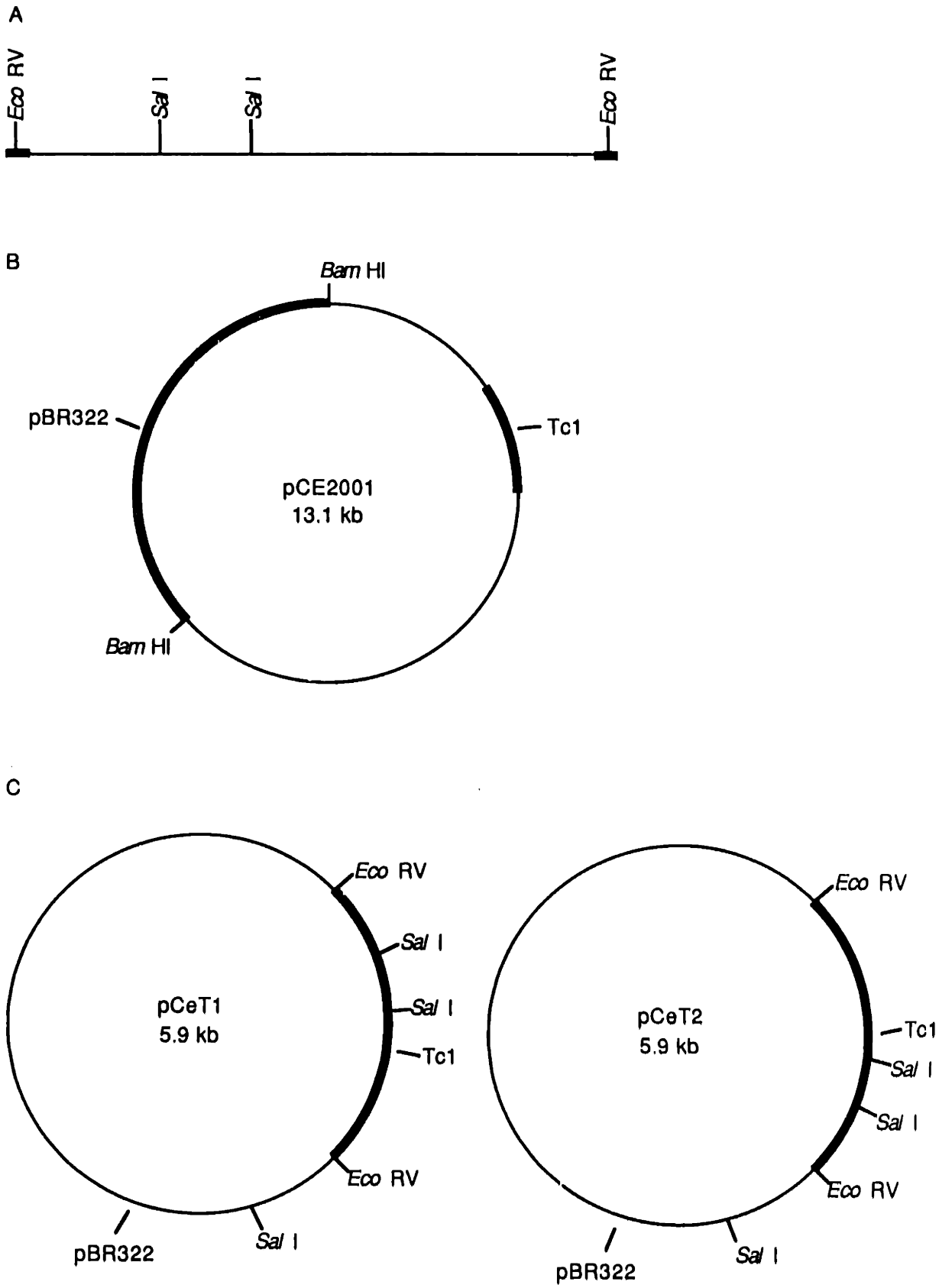
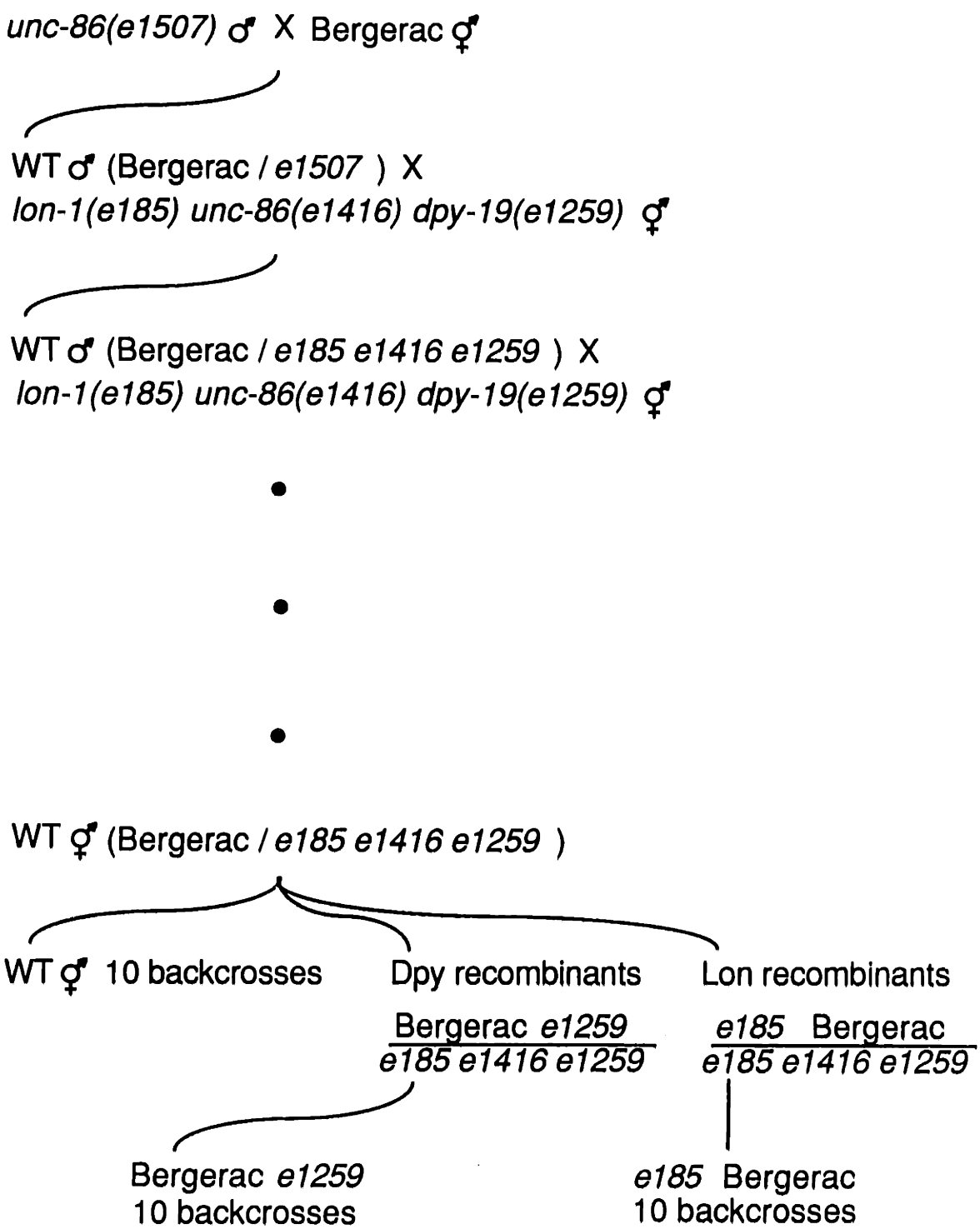

A
Eco RV
Sal I
Sal I
Eco RV
B
Bam HI
pBR322
Tc1
pCE2001
13.1 kb
Bam HI
C
pCeT1
5.9 kb
Eco RV
Sal I
Sal I
Tc1
Eco RV
pBR322
Sal I
pCeT2
5.9 kb
Eco RV
Tc1
Sal I
Sal I
Eco RV
pBR322
Sal I

Figure 5

Moving the region of chromosome III genetically linked to *unc-86* from a Bergerac background into a Bristol background. Bergerac wild-type alleles of *lon-1*, *unc-86*, and *dpy-19* were selected at every generation.

Figure 5


unc-86(e1507) ♂ X Bergerac ♀
WT ♂ (Bergerac / e1507) X
lon-1(e185) unc-86(e1416) dpy-19(e1259) ♀
WT ♂ (Bergerac / e185 e1416 e1259) X
lon-1(e185) unc-86(e1416) dpy-19(e1259) ♀
WT ♀ (Bergerac / e185 e1416 e1259)
WT ♀ 10 backcrosses
Dpy recombinants
Bergerac e1259
e185 e1416 e1259
Lon recombinants
e185 Bergerac
e185 e1416 e1259
Bergerac e1259
10 backcrosses
e185 Bergerac
10 backcrosses

cross was expected to reduce by half the number of extra Tcls unlinked to *unc-86*, so after 10 crosses there should be 2^{-10} of the starting number of unlinked Tcls, or fewer than one. Tc1 insertions within a few map units on either side of *unc-86* should all be retained.

Two congenic strains independent from the fifth generation on were made. Both contained extra copies of Tc1 as assayed by Southern transfer and probing with labeled Tc1 (figure 6). The set of 30 Bristol Tcls made convenient internal markers for the foreign Tcls. Bristol Tcls were named according to their positions on a gel of *Eco* RI-cut DNA, and Bergerac Tcls were named in relation to them. Figure 8 gives the sizes of Bristol bands and the relative positions of Bergerac bands that are present in the congenic strains.

The second step in finding the closest Bergerac Tcls to *unc-86* was to map the extra Tcls using multifactor crosses. The only difference between these mapping experiments and standard three-factor crosses (e.g. chapter 1 table 1) is that the markers are scored using Southern blots. Chromosomes containing Bergerac regions were put in *trans* to Bristol multiple mutations (see table 2) and recombinants were picked. Recombinant strains were analyzed using Southern blots (e.g. figure 7) and the Tc1 insertions were ordered with respect to each other and to selected genetic markers. For instance, in figure 7, the band 26C is present in 8 of 8 B recombinants (which have a Bristol allele of *unc-36* and a Bergerac allele of *lin-13*) and 2 of 8 C recombinants (which have a Bristol allele of *unc-86* and a Bergerac allele of *lin-16*). Thus Tc1 26C maps close to *lin-13* and between *lin-16* and *unc-86*.

Figure 6

Extra copies of Tc1 in Bristol/Bergerac congenic strains. DNA was cut with *Eco* RI, Southern blotted, and probed with Tc1.

A, N2; B, X10-1, a ten-times backcrossed congenic strain; C, X10-2, an independently ten-times backcrossed congenic strain.

Figure 6

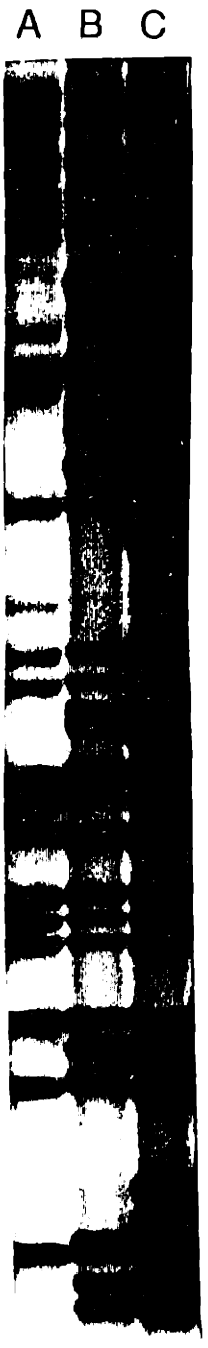


Figure 7

An example of recombinant strains used to map Bergerac Tcls. Strains are named in sequence from the cross used to generate them (the crosses are listed in table 2). For instance B8 is the eighth recombinant found in cross B. DNA was cut with *Eco* RI, Southern blotted, and probed with Tc1. A key to the names of the bands is in figure 8 and an interpretation of the bands present in each lane is in figure 9.

A, A1R-O; B, N2; C, B8; D, B9; E, B11; F, B12; G, B13; H, B14; I, B15; J, B16; K, C8; L, C9; M, C10 N, C11; O, C12; P, C13; Q, C14; R, C15; S, N2; T, A1R-O.

Figure 7

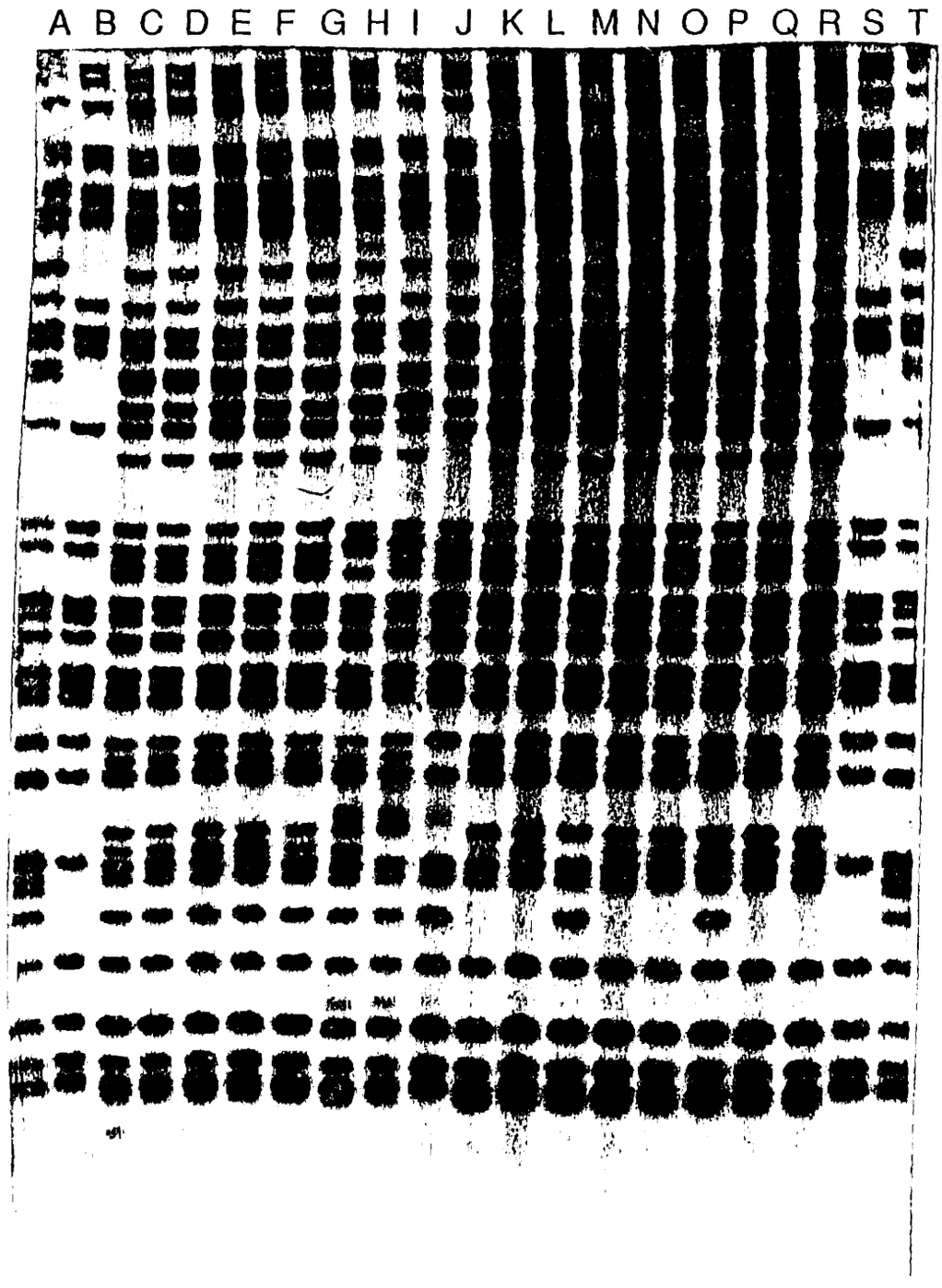


Figure 8

Sizes and gel positions of *Eco* RI fragments containing the 30 Tcls in N2 and the 29 extra Tcls in our Bristol/Bergerac congenic strains.

Figure 8

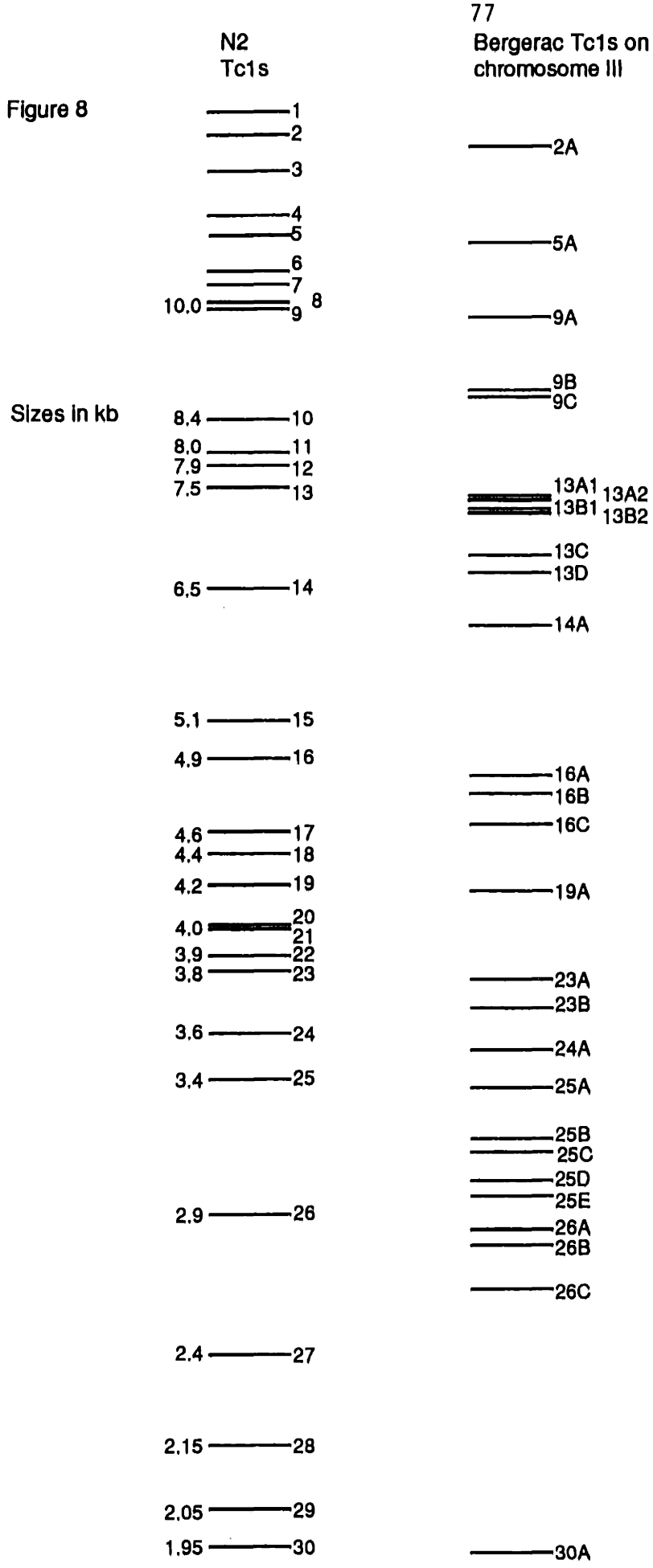

N2
Tc1s
Bergerac Tc1s on
chromosome III
Sizes in kb
1
2
3
4
5
6
7
10.0
8
9
8.4
10
8.0
11
7.9
12
7.5
13
6.5
14
5.1
15
4.9
16
4.6
17
4.4
18
4.2
19
4.0
20
21
3.9
22
3.8
23
3.6
24
3.4
25
2.9
26
2.4
27
2.15
28
2.05
29
1.95
30
2A
5A
9A
9B
9C
13A1
13A2
13B1
13B2
13C
13D
14A
16A
16B
16C
19A
23A
23B
24A
25A
25B
25C
25D
25E
26A
26B
26C
30A

Table 2

Genetic mapping of Bergerac Tcl polymorphisms. Strains resulting from the AR and AL experiments (see figure 9) were used as the Bergerac chromosomes in the B through H experiments.

Expt.	Bristol/Bergerac heterozygote	Genotype of recombinant strain
AL	X10/*lon-1(e185) unc-86(e1416) dpy-19(e1259)*	*lon-1 Ber*
AR	X10/*lon-1(e185) unc-86(e1416) dpy-19(e1259)*	*Ber dpy-19*
AlRL	AlR-N/*unc-36(e251) unc-86(e1507)*	*Ber unc-86*
B	AlR-N/*lin-13(n387) unc-36(e251)*	*Ber unc-36*
C	AlR-N/*lin-16(e1743) unc-86(e1507)*	*Ber unc-86*
D	A9L/*lin-13(n387) unc-36(e251)*	*lon-1 Ber unc-36*
E	A9L/*lin-16(e1743) unc-86(e1507)*	*lon-1 Ber unc-86*
F	AlR-N/*unc-86(e1416) unc-32(e189)*	*unc-86 Ber dpy-19*
G	A9L/*unc-86(e1416) dpy-19(e1259)*	*unc-86 Ber*
G'	A9L/*unc-86(e1416) unc(st311)*	*unc-86 Ber*
H	A3L/*unc-86(e1416) dpy-19(e1259)*	*lon-1 Ber dpy-19*

This analysis was more difficult than it might appear for several reasons. First, the backcrossed region has a total of 28 non-Bristol Tcls besides the 30 from Bristol. For mapping to work perfectly, one would need to distinguish 58 randomly distributed bands on a genomic Southern blot. Of course this is impossible—frequently Bergerac bands comigrated with each other or with Bristol bands, resulting in Tcl insertions that seemed to map in two places or that were visible only as 2:1 intensity differences.

Second, a strain often had recombination points other than the one that was selected. This caused two classes of problems. A strain selected for a recombination point on the right side could recombine a second time on the left before the chromosome could be made homozygous. This results in a strain that is homozygous for the breakpoint on the right but heterozygous for the breakpoint on the left; if two animals are cloned from the strain, they may have different Bergerac regions. This actually happened to strain A1R (see figure 9). The first animal picked from this strain (A1R-O in figure 9) and grown up for DNA had only ten extra Tcls, so the strain was used as the Bergerac chromosome in experiments B and C. When the B and C recombinants proved to have far more Tcls than expected, A1R was grown up again, and the new DNA contained 24 Tcls (A1R-N in figure 9).

Unselected recombination also complicates the genetic analysis. For instance in the B and C experiments (table 2) the Bergerac chromosome used as the source for all recombinants is the same (A1R-N), so one would expect the left end of the Bergerac region would be the same in all. Instead, fifteen out of the 50 had a second recombination on the left (figure 9). To order these Tcls they must be arranged so

Figure 9 (Two pages)

Bergerac Tcls in recombinant strains. Names of Bergerac Tcls (see figure 8) are written across the top and names of recombinant strains (see table 2) are written along the right side. A black bar indicates the presence of that Tcl; a hatched bar indicates that the Tcl could not be scored.

Figure 9

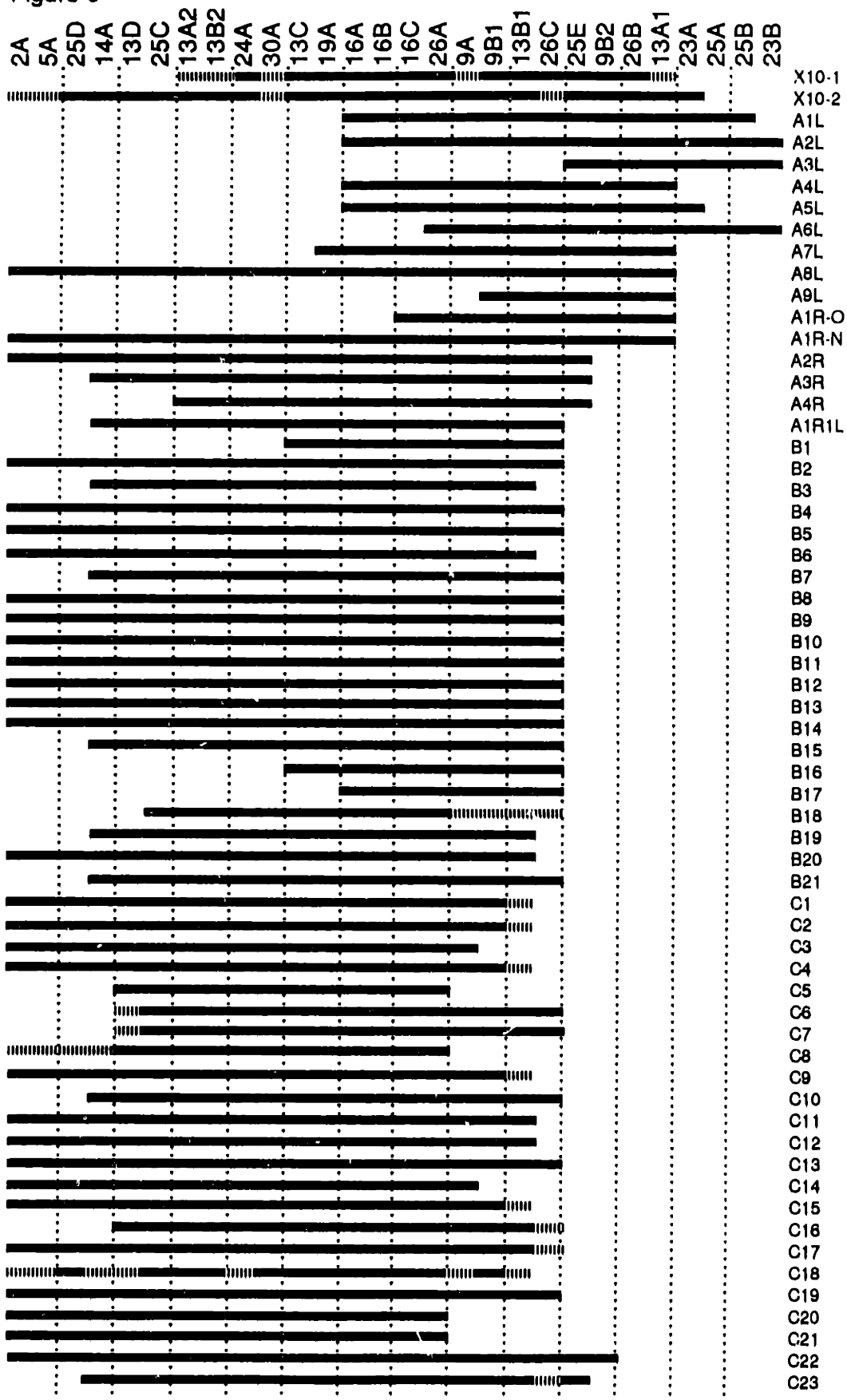
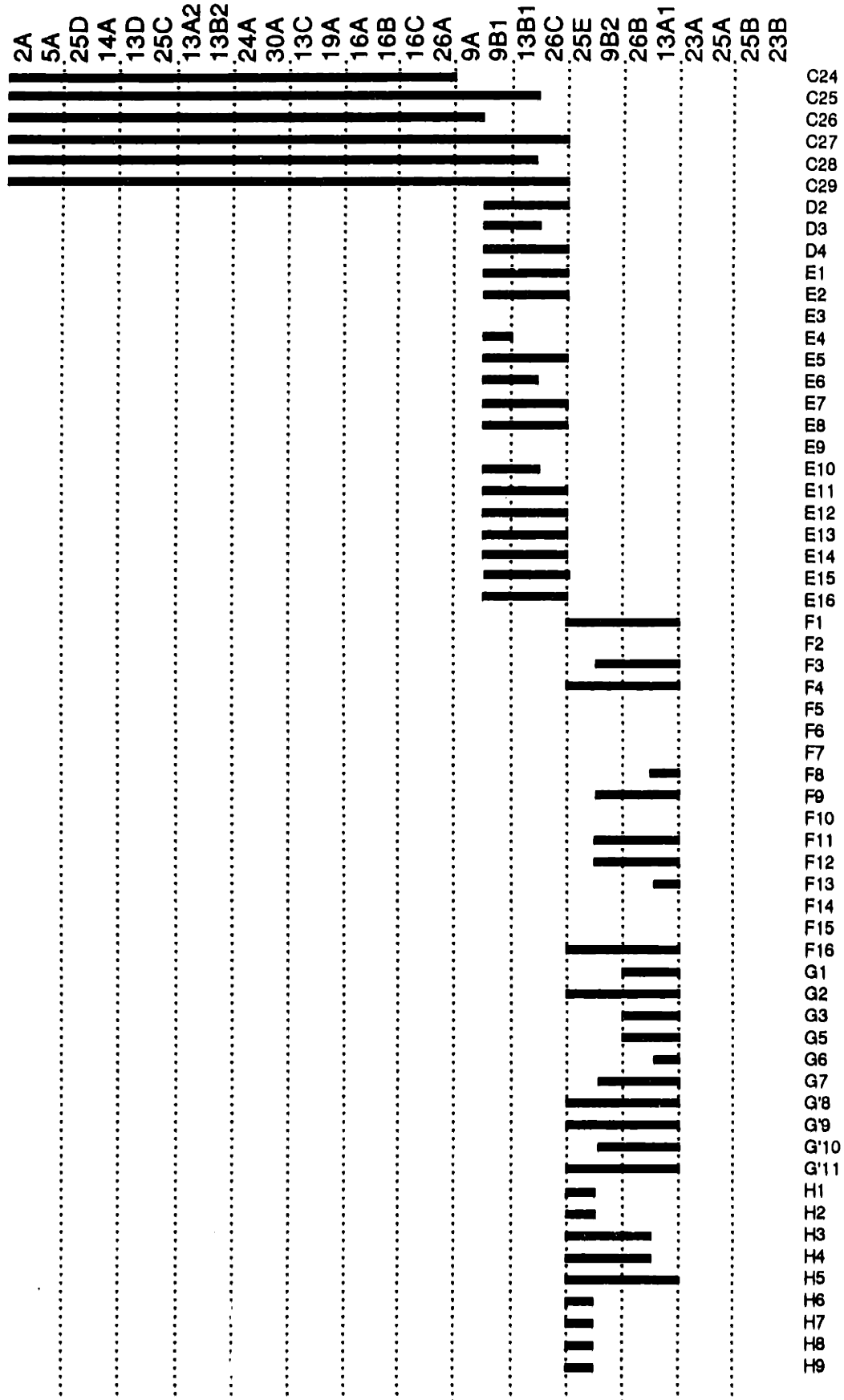

2A
5A
25D
14A
13D
25C
13A2
13B2
24A
30A
13C
19A
16A
16B
16C
26A
9A
9B1
13B1
26C
25E
9B2
26B
13A1
23A
25A
25B
23B
X10-1
X10-2
A1L
A2L
A3L
A4L
A5L
A6L
A7L
A8L
A9L
A1R-O
A1R-N
A2R
A3R
A4R
A1R1L
B1
B2
B3
B4
B5
B6
B7
B8
B9
B10
B11
B12
B13
B14
B15
B16
B17
B18
B19
B20
B21
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
C11
C12
C13
C14
C15
C16
C17
C18
C19
C20
C21
C22
C23

Figure 9 (continued)



that each strain may lose Tcls from the left side, the right side, or both, but not from the middle.

Mapping results from experiments A, B, and C (table 2 and figure 9) were confirmed and extended (table 2 and figure 9, experiments D, E, F, G, and H) by starting with recombinant strains which had lost most of the Tcls on one side and selecting further recombinants so as to lose most of the Tcls on the other side as well. These experiments resulted in recombinant strains with four or fewer extra Tcls, making mapping straightforward. Tc1 insertions were ordered so that a minimum number of recombinations are required to account for the data (figure 9) and a map was constructed (figure 10).

Molecular cloning of Tc1 insertions.

The closest Tc1 insertions to *unc-86* on the right and left sides, respectively, are 25E (a 2.9 kb *Eco* RI fragment) and 26C (a 2.6 kb *Eco* RI fragment). 25E was cloned from strain H1 and 26C was cloned from strain A7L: size-selected *Eco* RI fragments were ligated into *Eco* RI-cut dephosphorylated pUC13 (Yanisch-Perron, Vieira and Messing, 1985) and transformed into *E. coli*; plasmids containing Tc1 were selected by colony hybridization.

Tc1-containing clones from strain A7L could have been any of several different Tcls. 31 independent clones were analyzed by cleavage with *Eco* RI, mixing with *Eco* RI-cut N2 DNA and Southern blotting (figure 11). Three of the clones migrated at the position expected of 26C.

Tc1-containing clones from strain H1 should have been either 25E or the Bristol Tc1 26. 16 independent clones were analyzed by

Figure 10

Genetic map of Bergerac Tcls near *unc-86*.

A. Tcls outside the *lon-1* to *dpy-19* interval. Not to scale.

B. Tcls between *lon-1* and *dpy-19*.

Figure 10

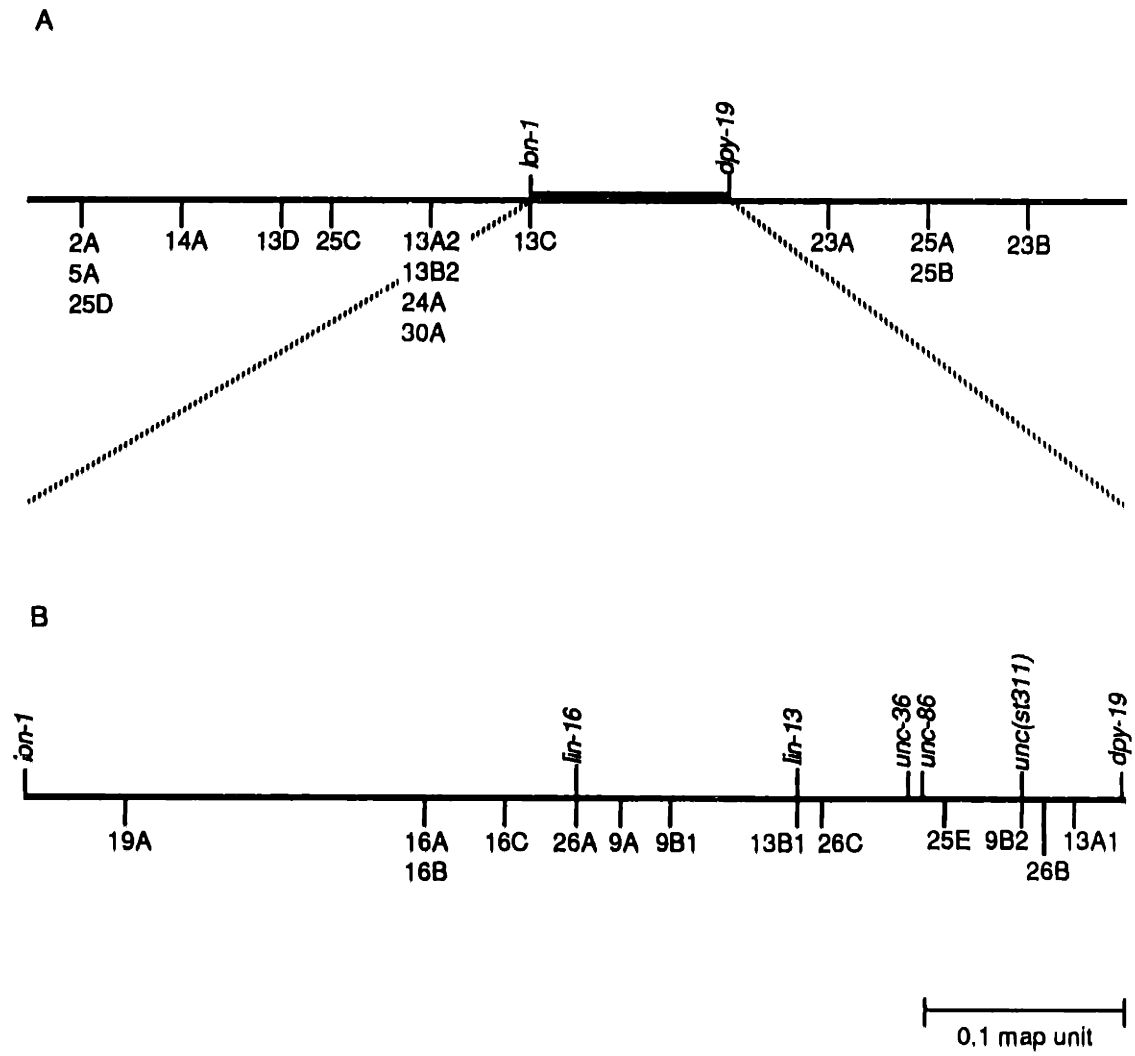
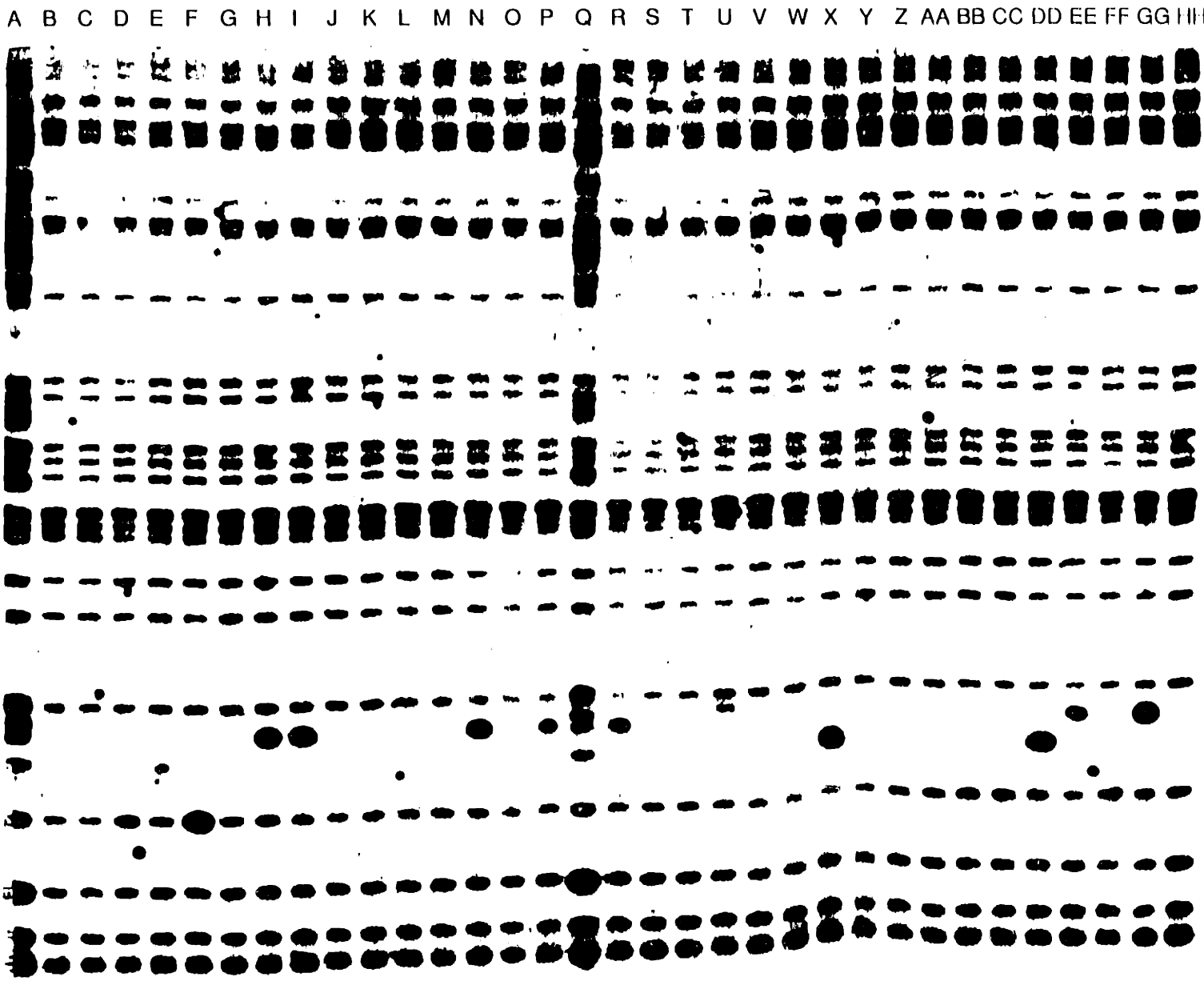

A
bn-1
dpy-19
2A
5A
25D
14A
13D
25C
13A2
13B2
24A
30A
13C
23A
25A
25B
23B
B
ion-1
lin-16
lin-13
unc-36
unc-86
unc(st311)
dpy-19
19A
16A
16B
16C
26A
9A
9B1
13B1
26C
25E
9B2
26B
13A1
0.1 map unit

Figure 11

Identification of cloned Tcls. Plasmids from bacterial strains identified by hybridization to Tc1 were cut with *Eco* RI and mixed with *Eco* RI-cut N2 DNA, Southern blotted, and probed with Tc1.

A, A7L; B, N2 + clone 1; C, N2 + clone 2; D, N2 + clone 3; E, N2 + clone 4; F, N2 + clone 5; G, N2 + clone 6; H, N2 + clone 7; I, N2 + clone 8; J, N2 + clone 9; K, N2 + clone 10; L, N2 + clone 11; M, N2 + clone 12; N, N2 + clone 13; O, N2 + clone 14; P, N2 + clone 15; Q, A7L; R, N2 + clone 16; S, N2 + clone 17; T, N2 + clone 18; U, N2 + clone 19; V, N2 + clone 20; W, N2 + clone 21; X, N2 + clone 22; Y, N2 + clone 23; Z, N2 + clone 24; AA, N2 + clone 25; BB, N2 + clone 26; CC, N2 + clone 27; DD, N2 + clone 29; EE, N2 + clone 30; FF, N2 + clone 31; GG, N2 + clone 32; HH, N2.

Figure 11



restriction mapping; they indeed fell into two classes. One member of each class was analyzed by Southern blotting as described below.

To confirm the identity of che cloned fragments and for walking experiments, subclones of sequences flanking the insertion sites were needed. These were made by cutting plasmids with *Eco* RV, which does not cut in pUC13, but does cut very near both ends of Tc1. The cut plasmids were religated and cloned, resulting in plasmids (p26Cends, p26ends, and p25Eends) carrying flanking sequences from both sides of the insertion but nothing that will hybridize to Tc1.

p25Eends was distinguished from p26ends by hybridizing each to Southern blots of *Eco* RI-cut DNA from strains that contain Tc1 25E and strains that do not (figure 12B). p26C also hybridized to a unique band of the expected size in Bergerac and to bands approximately 1.6 kb smaller in N2 (figure 12A).

Walking from Tc1 26C

Purified insert from p26Cends hybridized to one cosmid in our library, C33C3 (see materials and methods for a description of the cosmid library). The number was sent to John Sulston and Alan Coulson, who fingerprinted the clone and entered it in the data base for their emerging physical map (Coulson, Sulston, Brenner and Karn, 1986; see introduction). They sent back a "contig" of overlapping cosmids spanning approximately 100 kb (figure 13).

We physically mapped clones by probing Southern blots of strains carrying chromosomal deficiencies. Genetic mapping (see figure 1 in chapter 1 and figure 10 in this chapter) placed Tc1 26C between *lin-13* and *unc-36*, both of which are deleted by *nDf16*, indicating that

Figure 12

Flanking sequences of Tc1 insertions hybridize to unique sequences with the expected characteristics.

A. p26Cends hybridizes to a unique sequence that is on an *Eco* RI restriction fragment that is smaller in Bristol-derived strains than in Bergerac-derived strains: Southern blot of *Eco* RI-cut DNA probed with p26Cends. A, N2; B, Bergerac; C, *nDf20; nDp2*.

B. p25Eends hybridizes to a unique sequence that is polymorphic between Bristol and Bergerac, and that polymorphism maps to a small region to the right of *unc-86*. Southern blot of *Eco* RI-cut DNA probed with p25Eends. A, N2; B, Bergerac; C, recombinant E1; D recombinant G'11. The Bergerac allele is present in strain G'11, which has only a small Bergerac region right of *unc-86*.

Figure 12

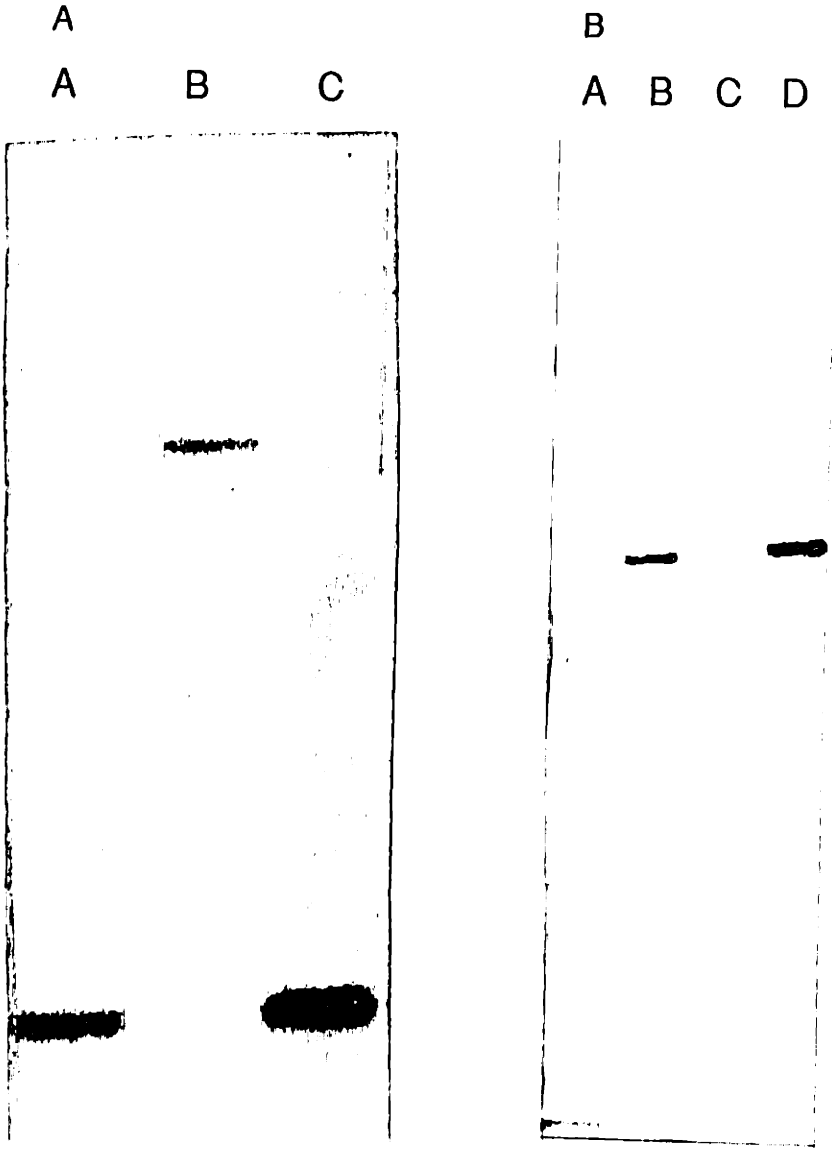
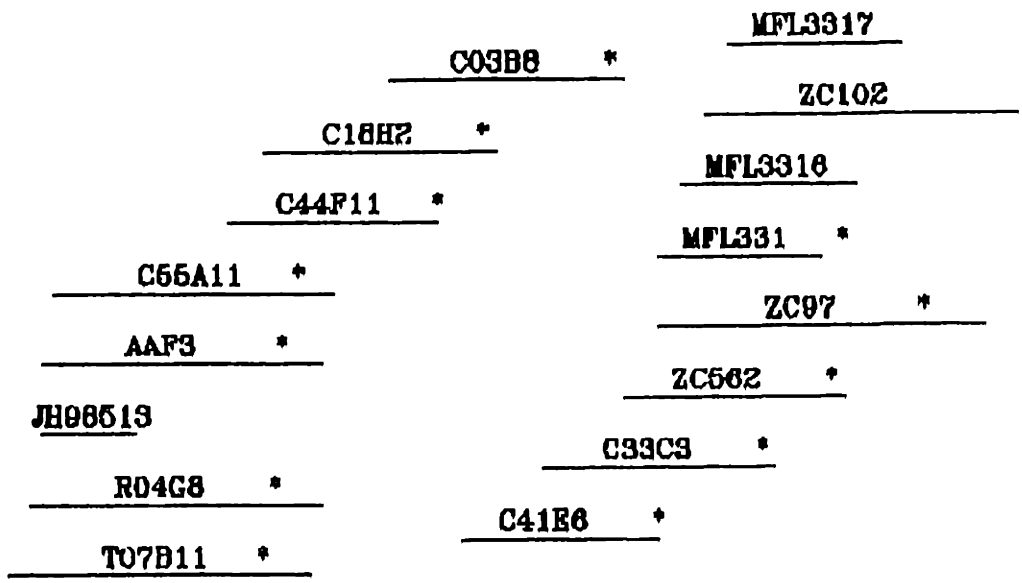


Figure 13

Contig of overlapping clones found by computer matches by John Sulston and Alan Coulson (see introduction). C33C3 contains the insertion site for Tc1 26C. MFL331 and MFL3316 are λ clones found by us and sent to Sulston and Coulson.

Figure 13


MFL3317
C03B8 *
ZC102
C18H2 *
MFL3316
C44F11 *
MFL331 *
C55A11 *
ZC97 *
AAF3 *
ZC562 *
JH96513
C33C3 *
R04G8 *
C41E6 *
T07B11 *
AAF3prob conld; lft<>rt
R02C2>?

p26Cends should hybridize to sequences deleted by *nDf16*. *unc-36* but not *lin-13* is deleted by *nDf20*, so p26Cends could have hybridized to sequences deleted by or not deleted by *nDf20*.

The source of *nDf20* DNA was a strain homozygous for *nDf20* and carrying the duplication nDp2 (*nDp2* is a free duplication covering much of LG III; see Materials and Methods). Thus there should be a 3:1 ratio of signals on Southern blots for a probe hybridizing to sequences on LG III but outside *nDf20* versus a probe hybridizing to sequences deleted by *nDf20*. *nDf16* DNA was from the strain carrying *nDf16* balanced by *nDf22*; this strain provided only a 2:1 signal ratio between probes outside and inside the deficiency.

The cosmid C33C3 was at one end of the contig; the other end was the cosmid AAF3. Southern filters bearing equal amounts of DNA from N2 and deficiency strains were made, and the amount of DNA in the lanes checked by hybridization to Tc1 probe. Both cosmids were used to probe the filters and both gave band intensities indicating that they hybridized to sequences deleted by *nDf16* but not by *nDf20* (figure 14A); the original probe, p26Cends, also hybridized to sequences not deleted by *nDf20* (figure 12A, lanes A and C). Because of the presence of repeated sequences in the cosmid probes, if the end of *nDf20* were within either cosmid that fact could have been missed by this experiment. The outer end of each cosmid was subcloned and used to probe the same filters; the end of C33C3 hybridized to sequences deleted in the *nDf20* strain, thus establishing the direction of the walk (figure 14B).

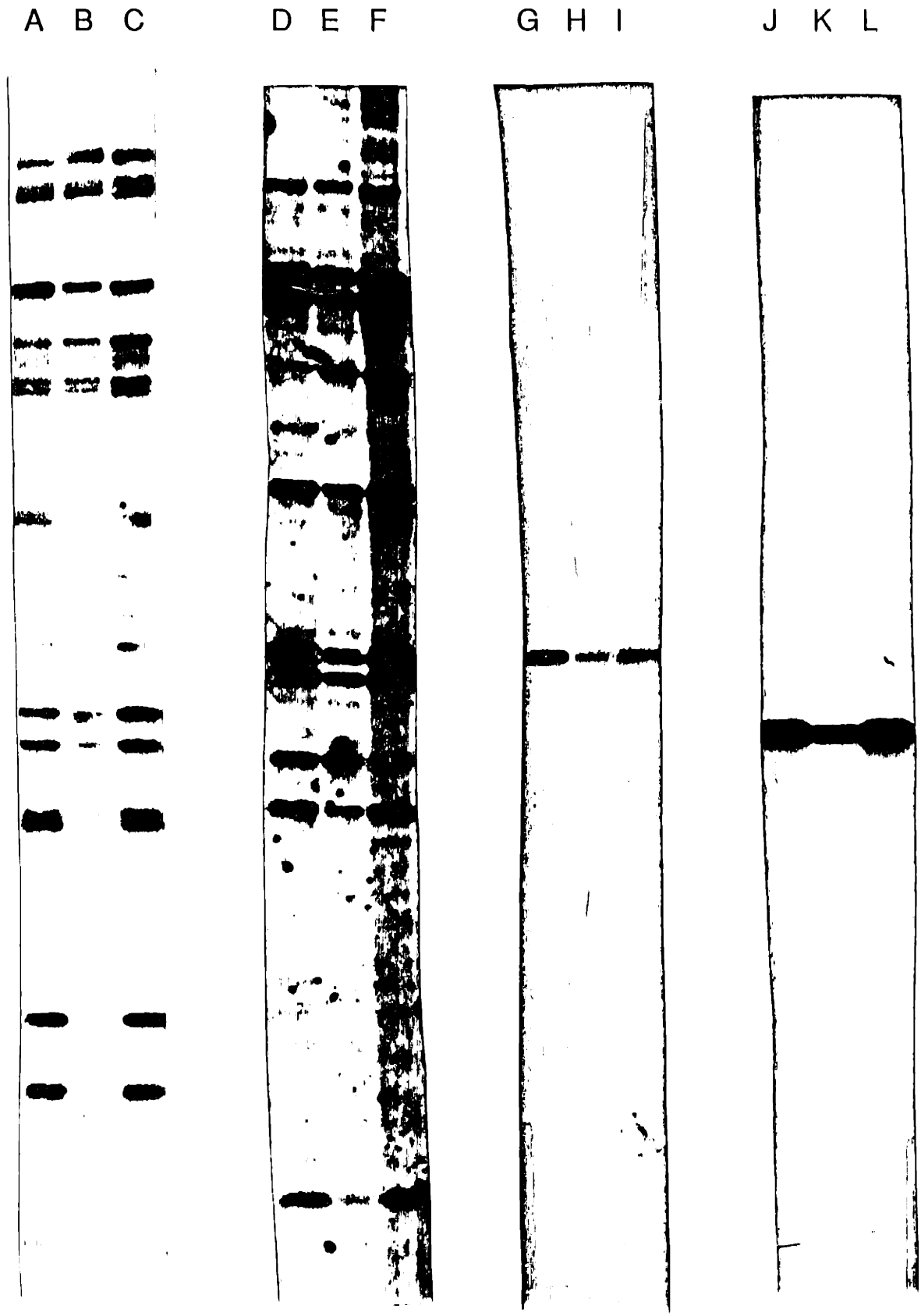
The end of C33C3 was used to probe both the cosmid library and the phage library. In the cosmid library, the only colony that

Figure 14

Deficiency location of clones from the 26C contig. Southern blots of *Eco* RI-cut DNA from N2 and strains carrying chromosomal deficiencies of *unc-86* and nearby genes (see chapter 1 figure 1 for map). All lanes contain approximately equal amounts of DNA, so differences in hybridization signal reflect differences in copy number of the sequences corresponding to the probes. The strain *nDf16/nDf22* gives half as much signal as N2 if the probe corresponds to sequences deleted by *nDf16*; the strain *nDf20; nDp2* gives half as much signal as N2 if the probe corresponds to sequences deleted by *nDf20* and 1.5 times as much signal for sequences nearby but not deleted.

Lanes A-C probed with those Eco RI fragments of the cosmid C33C3 that are larger than 2 kb (the smaller fragments contain highly repeated sequences), D-F probed with the cosmid AAF3, G-I probed with the outer end fragment of C33C3 (subcloned by digestion of the cosmid with *Xba* I and religation), and J-L probed with the outer end fragment of AAF3 (subcloned by digestion of the cosmid with *Pvu* II and religation). Lanes A, D, G and J are N2; B, E, H and K are *nDf16/nDF22*; and C, F, I and L are *nDf20; nDp2*.

Figure 14



hybridized was C33C3 itself. Phage clones were, however, found to extend the walk, and the end fragment from the clone that extended the farthest (clone MFL331 in figure 13) was used to probe deficiency blots and to take a second step (clones MFL3316 and MFL3317 in figure 13). Phage DNAs were sent to Sulston to be fingerprinted, but they failed to link the contig to another contig. Sulston and Coulson eventually extended the contig somewhat more (clones ZC97 and ZC102 in figure 13); surprisingly, the cosmid ZC102 is not in *nDf20* by deficiency blots. We do not have a good explanation for this result. It is possible that a small deletion closely linked to *nDf20* was generated in the same mutagenesis that generated *nDf20*, and had not ever segregated away.

Walking from Tc1 25E

Tc1 25E maps between *unc-86* and *unc(st311)*, both of which markers are deleted by both *nDf20* and *nDf16*. Therefore we expected that p25Eends would hybridize to sequences deleted by both deficiencies. p25Eends was used to screen the phage library. Resulting clones (MF251 and MF252 in figure 16) were shown to hybridize to sequences deleted by *nDf16* and *nDf20* (figure 15) and were sent to England for fingerprinting. The phages were part of a small contig. Later Sulston and Coulson, using libraries constructed with new techniques (Coulson *et al.*, 1986), were able to link the clones into a much larger contig (figure 16). This contig contained *unc-86*.

Figure 15

Deficiency location of a clone from the 25E contig. For explanation see figure 14 legend.

λ clone MFL252 (see figure 16) used as probe. A, N2; B, *nDf16/nDf22*; and C, *nDf20; nDp2*.

Figure 15

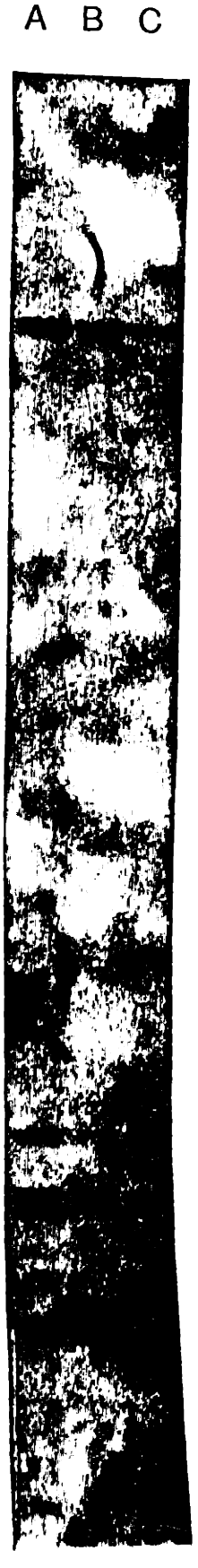


Figure 16

Contig of overlapping clones found by computer matches by John Sulston and Alan Coulson (see introduction). MFL251 and MFL252 are λ clones found by us and sent to Sulston and Coulson; these contain the insertion site for Tc1 25E.

Figure 16

(III)
10 HindIII sites
C04H11
C06G4
C58B6
F28G2
ZK581
E05I8
C09A12
C44C9
R01F2
C04D4
T01C8
F25D6
M03F2
ZK23
E03C5
C14B3
T16F11
C28G5
E02B3
ZK17
C34B10
C50C3
T05G3
D2007
AA1
C07C6
C30A5
M128
W06F7
C53G10
C02F5
C38H3
MF251
MF252
T20F3
W05H8
ZC282
ZK29
R05D3
F10E8
F10E9
C05G1
ZC251
C11F8
C12C10
C25F1
SG25/58
ZK94
ZK37
C08G3
C38C9
C13C7
ZK353
ZK345
ZC257
C54B2
R10A5
C48F11
C04D8
C22H10
ZK363
M207
ZK421
C30C11
C06E1
C03B9
C43G3
C02D5
M24
ZL53
ZC3
ZC21
B0523
W01D8
C32G8
C19G5
ZK370
C55G3
W06B4
ZL67
D2086
C01A7
SB13
C05C11
C17G3
C10D6
B0303
SUP5
TCUNC86B

Chapter 3

Location of *unc-86*

Identification of *unc-86* within a large contig of overlapping clones (see Chapter 2, figure 16) relied on three kinds of physical markers. First, as mentioned above, chromosomal deficiencies gave a rough location. Second, the reciprocal translocation *eT1(III;V)* is believed for two reasons to break in or near *unc-36* (which maps very close to *unc-86*—see figure 8): *eT1* homozygotes have an Unc-36 phenotype, and in *eT1* heterozygotes genetic recombination is suppressed in the region to the right of *unc-36* but not in the region to the left (Rosenbluth and Baillie, 1981). The breakpoint of *eT1* could be expected to produce a restriction fragment size difference visible on Southern blots and mapping slightly to the left of *unc-86*. The third kind of physical markers for *unc-86* are *unc-86* alleles themselves. 29 EMS-induced alleles exist, and a small fraction of EMS-induced alleles of *C. elegans* genes are known to be small deletions (MacLeod, Karn and Brenner, 1981; G. Ruvkun, personal communication).

Cosmids from the 25E contig (chapter 2 figure 16), which represent sequences deleted by both *nDf16* and *nDf20* (for map see chapter 1 figure 1), were used to probe Southern blots of N2 and *eT1* DNA cut with several enzymes. Restriction fragment size differences were found for the cosmid C34B10 (figure 1) but not for cosmids C04D4, T05G3, AA1, C38H3, M126, C11F9, or C12C10 (map of contig, chapter 2 figure 16). Since the contig had started with Tc1 25E (right of *unc-86*), if the new polymorphism was indeed the *eT1* breakpoint (left of *unc-86*), then *unc-86* must lie somewhere in between.

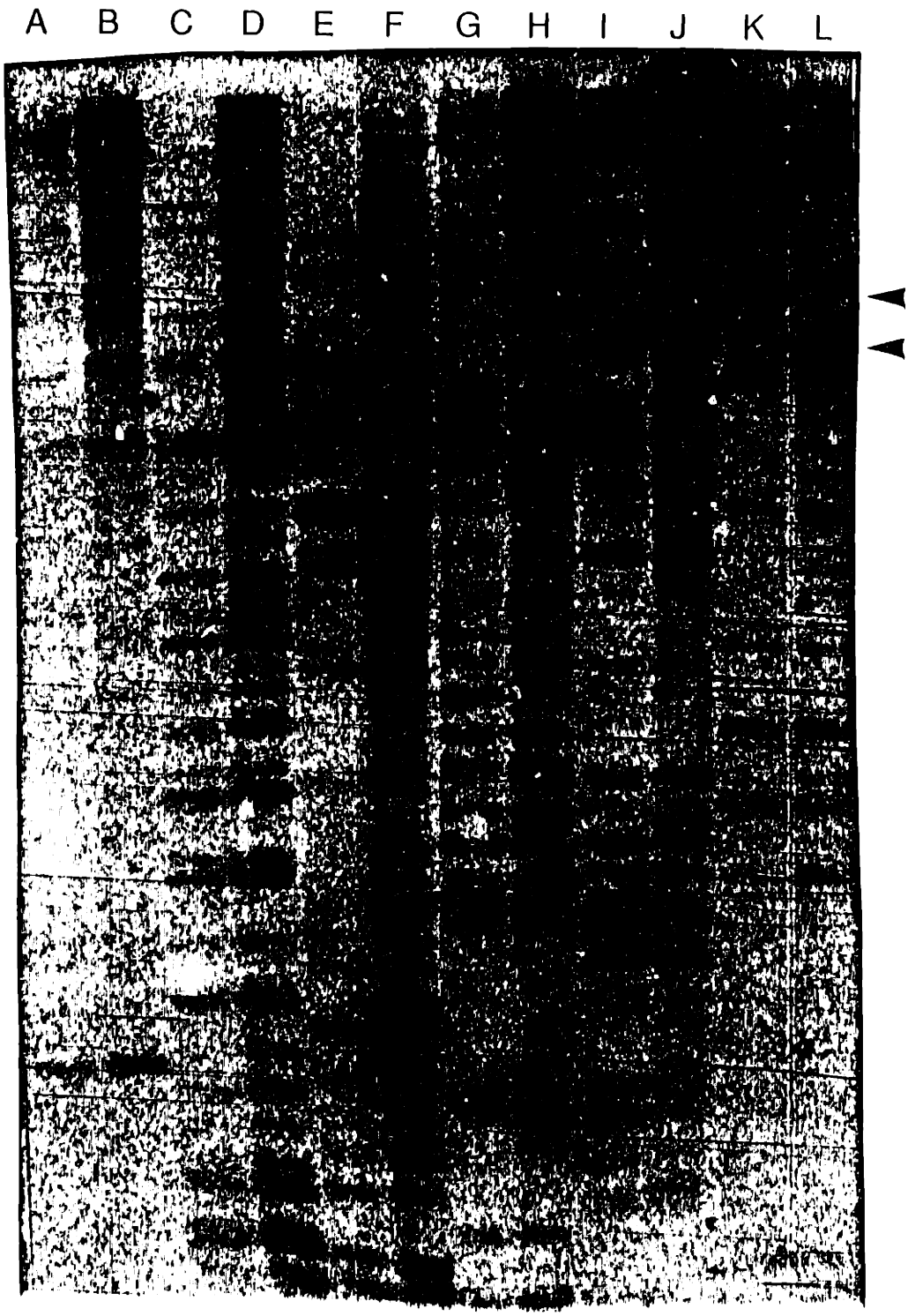
Figure 1

Restriction fragment size differences associated with the reciprocal translocation *eT1(III;IV)*. Southern blot of N2 and *eT1* DNA probed with the cosmid C34B10.

Lanes A, C, E, G, I and K, N2; lanes B, D, F, H, J and L, *eT1*. A and B, cut with *Bam* HI; C and D, cut with *Eco* RI; E and F, cut with *Eco* RV; G and H, cut with *Hin*d III; I and J, cut with *Xba* I; K and L, cut with *Xho* I.

Arrows point to size a difference visible in the *Xho* I digest.

Figure 1



Deletions in Him alleles

The cosmid C38H3, which contains about half of the DNA between Tc1 25E and the eT1-associated polymorphism, was used to probe a Southern blot of *unc-86* alleles (figure 2). The sequences hybridizing to the cosmid have a strikingly different arrangement in the *unc-86* Him alleles (see Chapter 1) than in the wild type. The four Him alleles seem to be identical. They are missing *Eco* RI fragments of approximately 8, 6.7, 6.3, 6.0, 2.5, and 1 kb and have a new *Eco* RI fragment of about 12-13 kb, suggesting that the missing fragments are deleted in these alleles and that the new large fragment is formed by joining the fragments at the ends of the deletion.

The cosmid C38H3 contains only part of the 6.0 kb *Eco* RI fragment, so it was possible that the deletion extended to sequences not contained in the cosmid. To test this possibility, the cosmid AA1, which overlaps C38H3 but is slightly to the left on the genetic map and contains the entire 6.0 kb fragment, was used to reprobe the same filter (figure 3). No more bands present in wild type but missing in the *unc-86* Him alleles were found. Thus the *unc-86* Him alleles are missing bands whose sizes total over 30 kb and have in their place a band whose size is about 12 or 13 kb, suggesting there is a deletion of about 18 kb in these alleles.

The 6.3 and 6.0 kb fragments were subcloned from the cosmid AA1 into a pUC-related vector, Bluescribe M13+. Probing a Southern blot with the 6.3 kb fragment shows that it is indeed deleted in Him alleles (figure 4A); probing with the 6.0 kb fragment shows that it hybridizes to the deletion junction fragment.

Figure 2

DNA rearrangement associated with *unc-86* Him alleles. Southern blot probed with the cosmid C38H3.

A, *unc-86(e1416)*; B, *unc-86(n306)*; C, *unc-86(n843)*; D, *unc-86(n847)*; E, *unc-86(n1042)*; F, Bergerac/Bristol recombinant A3L; G, Bergerac/Bristol recombinant A1R-O; H, *dpy-19(e1259) sup-5(e1464)*; I, N2 J, Bergerac. *e1416*, *n306*, *n843* and *n847* are Him alleles of *unc-86*; *n1042* is a non-Him allele.

Figure 2

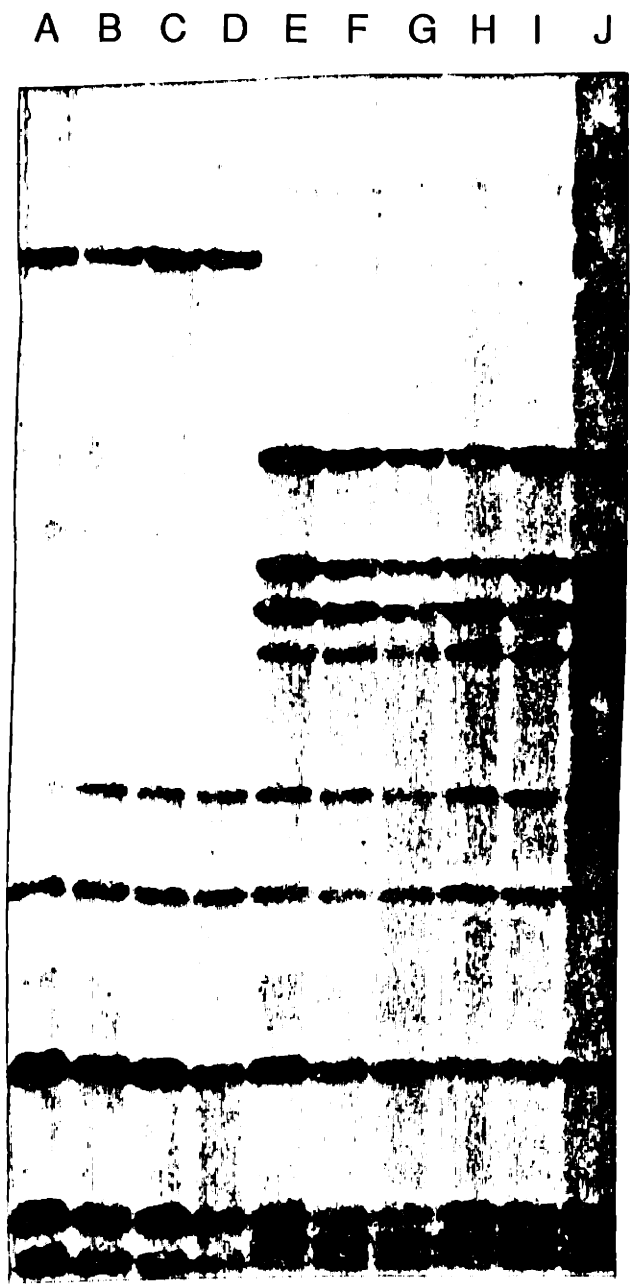


Figure 3

DNA rearrangements in *unc-86* Him alleles. Same Southern blot as in figure 2, reprobed with the cosmid AA1.

Lanes A-J, same as figure 2. All polymorphic bands line up precisely with those in figure 2.

Figure 3

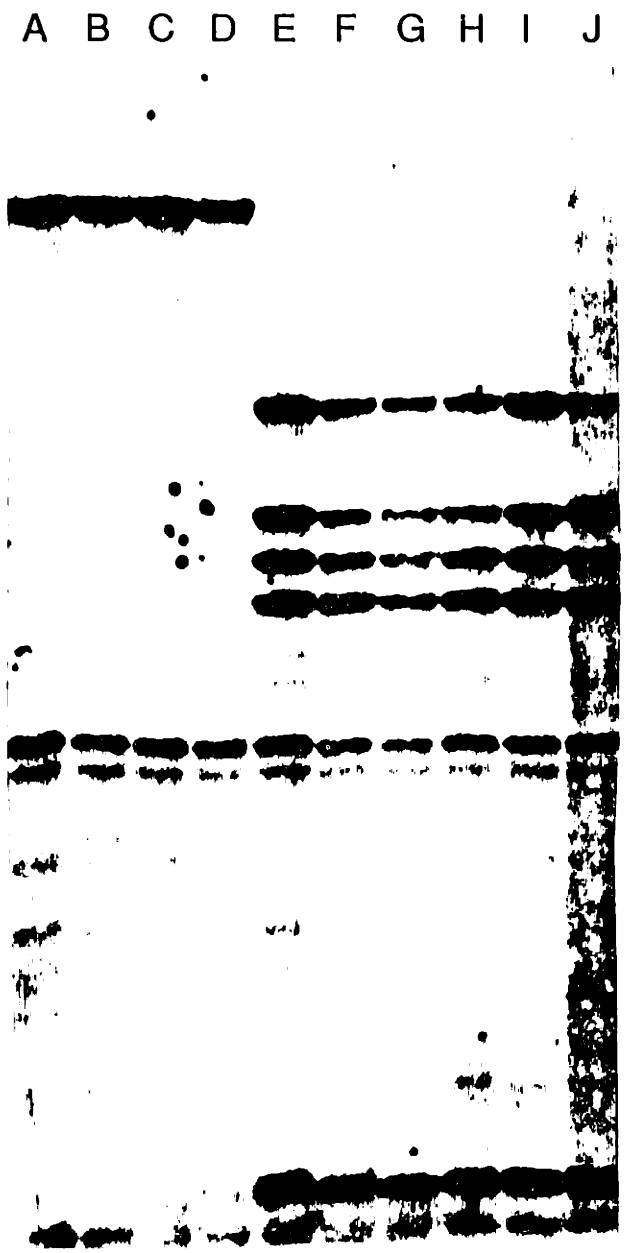


Figure 4

The rearrangement in *unc-86(e1416)* is a deletion with one endpoint in a 6.0 kb *Eco* RI fragment. Southern blot of *Eco* RI-cut N2 and *e1416* DNA.

A. The 6.3 kb *Eco* RI fragment from the cosmid AA1 used as probe. Lane A, N2; lane B, *e1416*. B. The 6.0 kb *Eco* RI fragment from the cosmid AA1 used as probe. Lane C, N2; lane D, *e1416*.

Figure 4

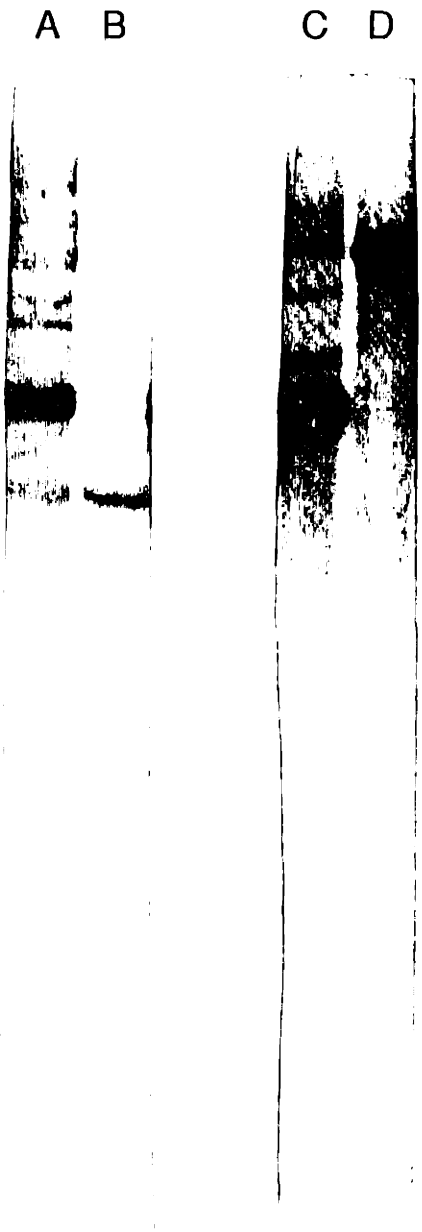
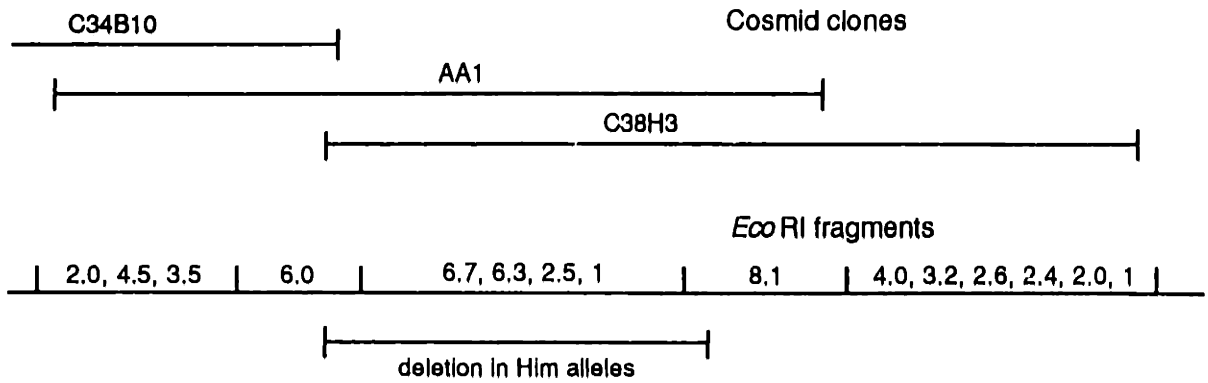


Figure 5

Map of Chromosomal DNA around *unc-86*.

The sizes of *Eco* RI fragments are shown on the main line, with unordered fragments grouped. The extent of three cosmid clones is shown above the line and the extent of the deletion in *unc-86* Him alleles is shown below.

Figure 5


C34B10
Cosmid clones
AA1
C38H3
Eco RI fragments
2.0, 4.5, 3.5
6.0
6.7, 6.3, 2.5, 1
8.1
4.0, 3.2, 2.6, 2.4, 2.0, 1
deletion in Him alleles

Sites for a number of restriction enzymes were mapped in the 6.0 kb fragment and some of these sites were used to make subclones by cutting inside and outside the insert and religating the plasmid (figure 8). Knowlege of this restriction map and use of some of these subclones as probes allowed us to map the sites of the mutations in a representative Him allele, *e1416*, and the other alleles with restriction fragment length polymorphisms.

The deletion in the Him alleles removes the right end of the 6.0 kb fragment, including the entire 1.4 kb *Hind* III to *Eco* RI fragment at the right end (figure 9 lane L). In figure 9 lane E, the lower band is almost as intense as the upper band, indicating that the lower band has considerable homology with the probe, and thus that the deletion does not extend close to the *Eco* RV site. Figure 5 shows a map of about 55 kb around *unc-86*, showing the endpoints of several cosmid clones and the Him deletion.

Other polymorphic alleles

18 *unc-86* alleles were analyzed by Southern blots probed with AA1 (figure 6) and the 6.0 kb *Eco* RI fragment (figure 7). Besides the Him alleles, two, *n946* and *n1351*, showed detectable size differences; both of these size differences were in the 6.0 kb fragment. Southern blots of *u371* and *rh1029* were probed with the 6.0 kb fragment and also showed size differences (figure 12).

The size difference in *n946* results from a deletion of approximately 200 bases (e.g. figure 10 lane M), removing the *Sac* I site (figure 9 lane O) and the Xba I site (data not shown).

Figure 6

Gross DNA arrangement in *unc-86* alleles. Southern blot of *Eco* RI-cut DNA from 19 *unc-86* alleles, probed with the cosmid AA1.

A, *e1416*; B, *n306*; C, *n412*; D, *n843*; E, *n844*; F, *n845*; G, *n846*; H, *n847*; I, *n848*; J, *n946*; K, *n992*; L, *n993*; M, *n994*; N, *n1041*; O, *n1042*; P, *u21*; Q, *u44*; R, *u68*; S, *n1351*; T, N2. *n994* and *n1041* DNA ran anomolously as a result of salt contamination of the samples; nevertheless, it can be determined that there is no gross rearrangement in these strains.

Figure 6

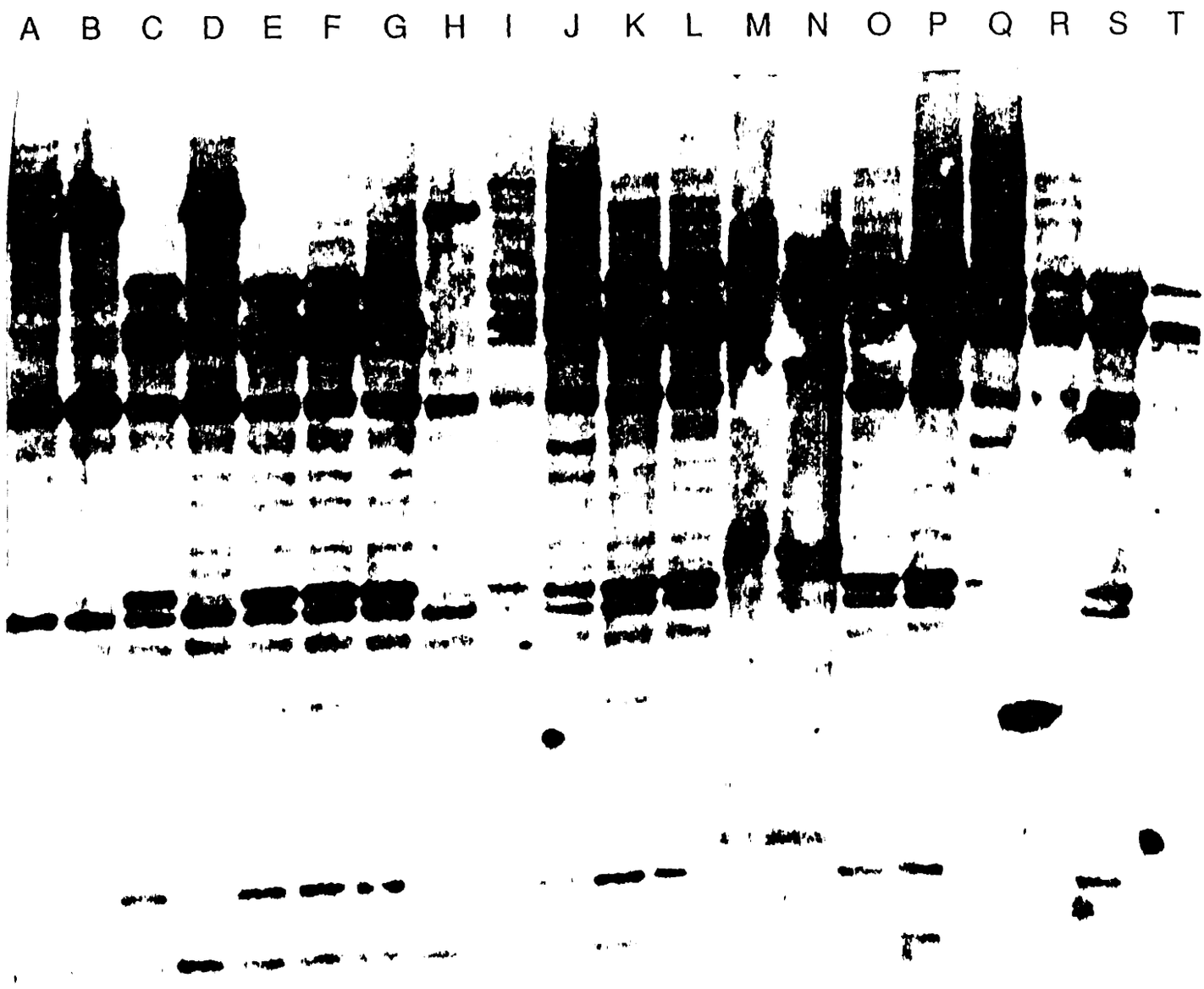


Figure 7

Gross DNA arrangement in *unc-86* alleles. The same Southern blot as in figure 6, reprobed with the 6.0 kb *Eco* RI fragment from cosmid AA1.

A, *e1416*; B, *n306*; C, *n412*; D, *n843*; E, *n844*; F, *n845*; G, *n846*; H, *n847*; I, *n848*; J, *n946*; K, *n992*; L, *n993*; M, *n994*; N, *n1041*; O, *n1042*; P, *u21*; Q, *u44*; R, *u68*; S, *n1351*; T, N2.

Figure 7

A B C D E F G H I J K L M N O P Q R S T

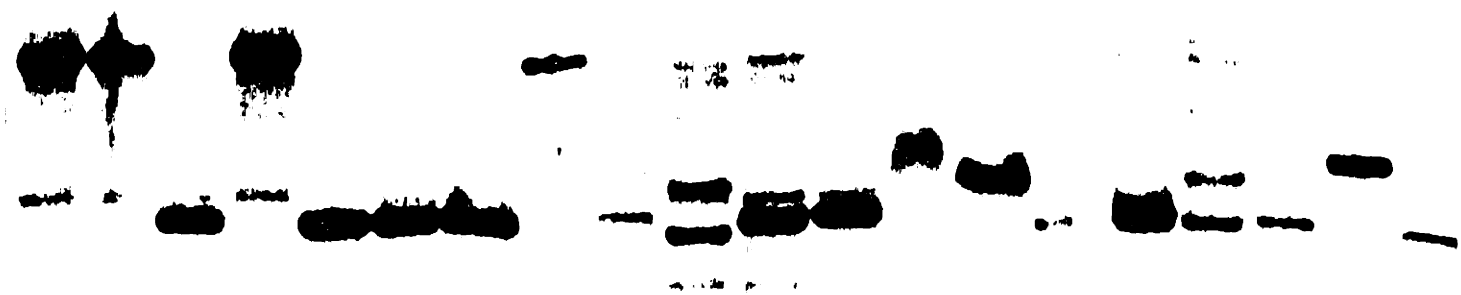


Figure 8

Restriction map of the 6.0 kb *Eco* RI fragment that is rearranged in *unc-86* alleles, showing the sites of those rearrangements. Right and left directions are consistent with the genetic map and are the same as those refered to in the text. The lines above the restriction map indicate those fragments which have been subcloned. Fragments were subcloned by cutting a plasmid containing the fragment cloned into the *Eco* RI site of the pUC-related vector Bluescribe M13+ (Strategene, Inc.) in one of the two orientations with an enzyme that cleaves in the insert, and if necessary also with an enzyme that cleaves in the polylinker and leaves complementary termini, religating, and transforming *E. coli*.

Figure 8

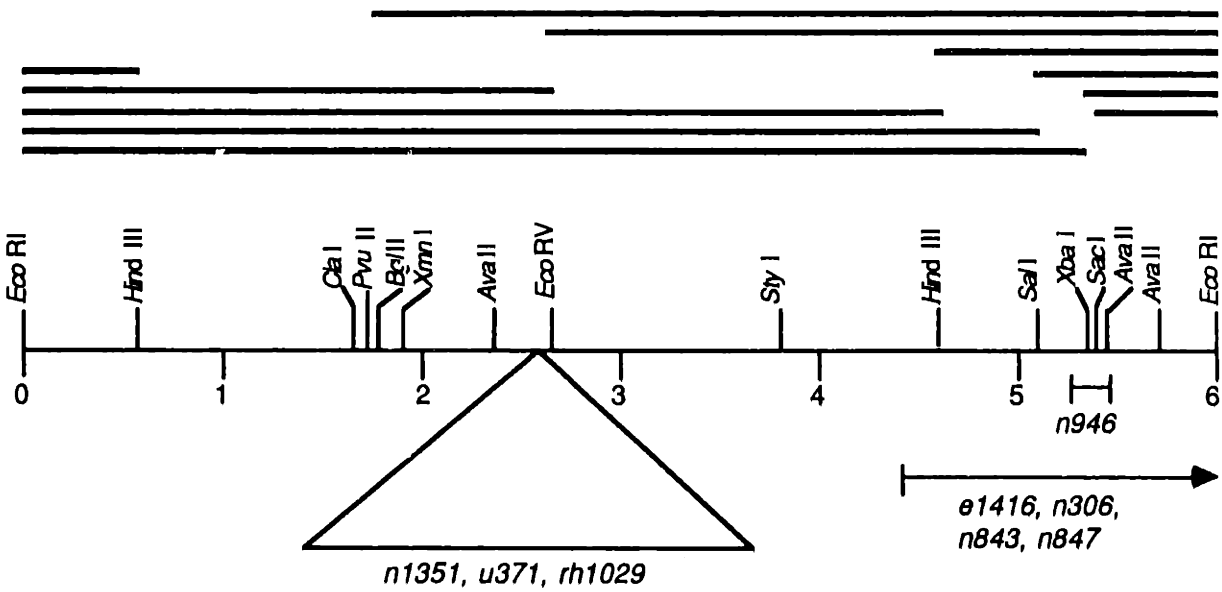
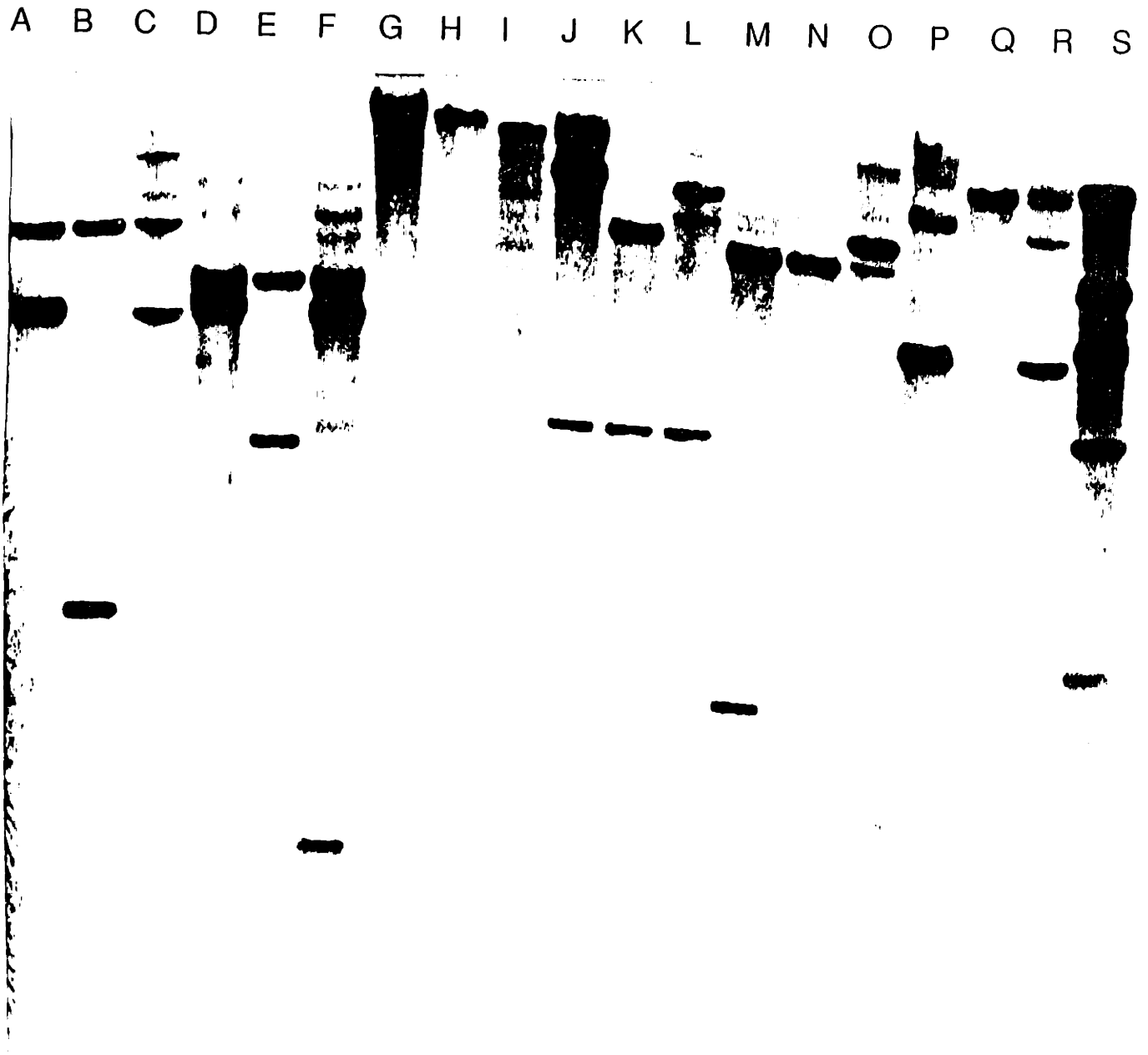

Eco RI
Hind III
Cla I
Pvu II
BclI
Xmn I
Ava II
Eco RV
Sty I
Hind III
Sal I
Xba I
Sac I
Ava II
Ava II
Eco RI
0
1
2
3
4
5
6
n946
e1416, n306,
n843, n847
n1351, u371, rh1029

Figure 9

Restriction fragment size differences in *unc-86(e1416)* and *unc-86(n946)*. Southern blot of DNA from N2 and mutants.

Lanes A-C, *Bgl* II; D-F, *Eco* RV; G-I, *Kpn* I; J-L, *Pst* I; M-O, *Sac* I; P-R, *Sal* I. Lanes A, D, G, J, M and P, N2; B, E, H, K, N and Q, *e1416*; C, F, I, L, O and R, *n946*. Lane S, λ *Hind* III size markers.

Figure 9



n1351, *u371*, and *rh1029* were derived from Mutator strains, which are known to give insertion mutations (see chapter 2). Each has an insertion into the 6.0 kb fragment. Genomic DNA from strains carrying each allele was cut with restriction enzymes, Southern blotted and probed with the entire 6.0 kb fragment and the left *Eco* RI to *Eco* RV fragment (figures 10 through 12).

Table 1 lists the sizes and restriction sites of each insertion and those of Tc1 for comparison. It is apparent that none of these insertions is Tc1 and that the insertion in *n1351* is different from those in *u371* and *rh1029*. *u371* and *rh1029* have the same spectrum of sites but with different placement; all of the data are consistent with the hypothesis that they are insertions of similar elements in opposite orientations.

All three alleles have insertions right of the *Bgl* II site and left of the *Eco* RV site. In Southern blots of DNA cut with an enzyme that cuts within the insert and probed with the left *Eco* RI to *Eco* RV fragment, the expected band consisting of the right end of the insert and the flanking *unc-86* DNA is missing or difficult to find (e.g. figure 12 lanes J, P and Q). This indicates that the insertion site in all three alleles is very close to the *Eco* RV site (100 base pairs or less for *u371* and *rh1029* and 200 base pairs or less for *n1351*).

The search for a transcript

We were interested to know if *unc-86* is transcribed, and, if so, what is the structure of that transcript and where and when it is expressed. Since *unc-86* is only known to be required for the correct development of a small number cells in the hermaphrodite, and may be

Figure 10

Restriction fragment size differences in *unc-86* alleles. Southern blot probed with the 6.0 kb *Eco* RI fragment from cosmid AA1.

Lanes A-E cut with *Eco* RI; lanes F-J cut with *Eco* RV; lanes K-O cut with *Hind* III. Lanes A, F and K, N2; B, G and L, *unc-86(e1416)*; C, H and M, *unc-86(n946)*; D, I and N, *unc-86(n1351)*; E, J and O, *unc-86(u371)*.

Figure 10

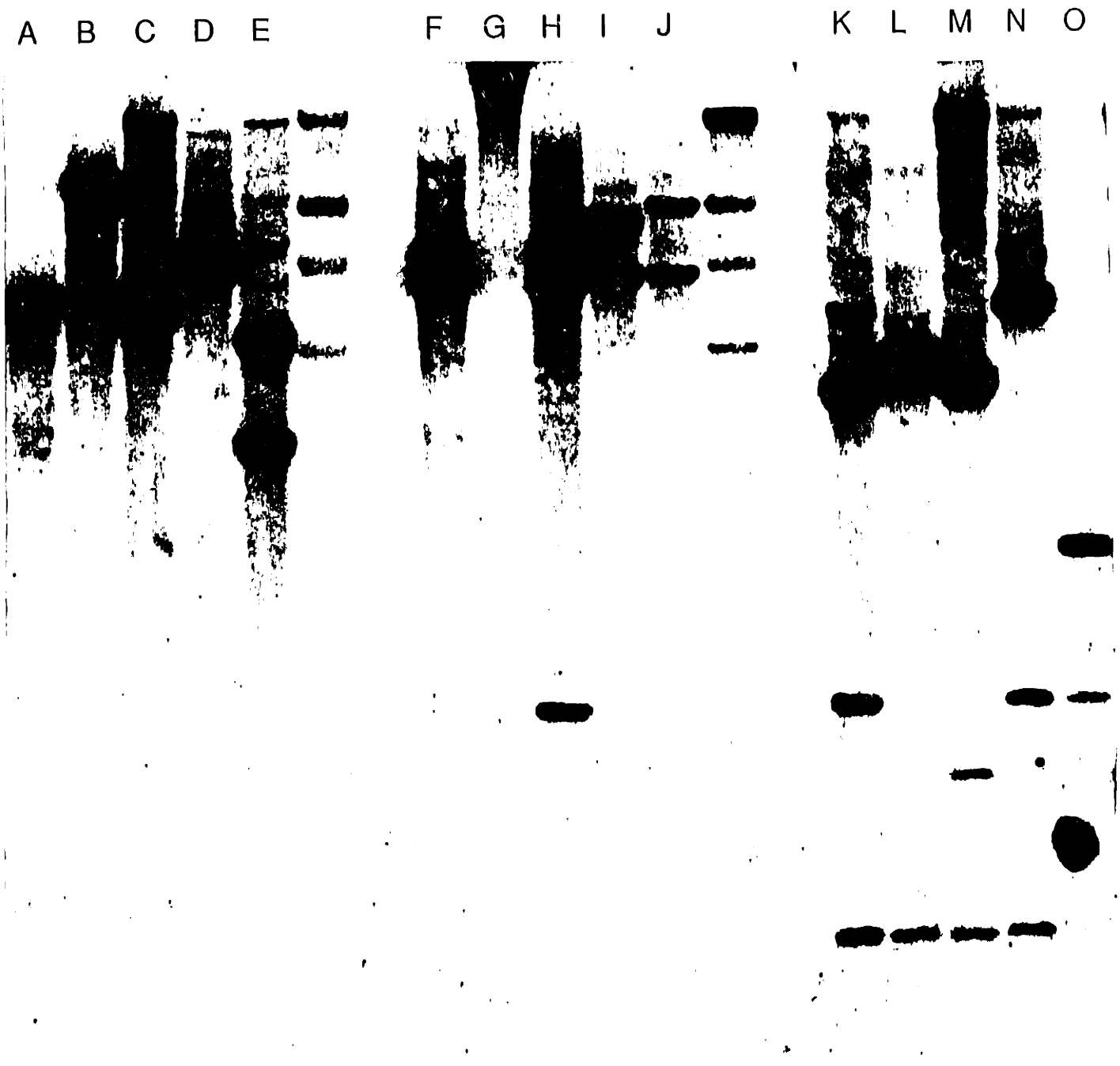


Figure 11

Restriction fragment size differences in *unc-86* alleles. The same Southern blot as in figure 10, reprobed with the left *Eco* RI to *Eco* RV subclone (see figure 8).

Lanes A-E cut with *Eco* RI; lanes F-J cut with *Eco* RV; lanes K-O cut with *Hind* III. Lanes A, F and K, N2; B, G and L, *unc-86(e1416)*; C, H and M, *unc-86(n946)*; D, I and N, *unc-86(n1351)*; E, J and O, *unc-86(u371)*.

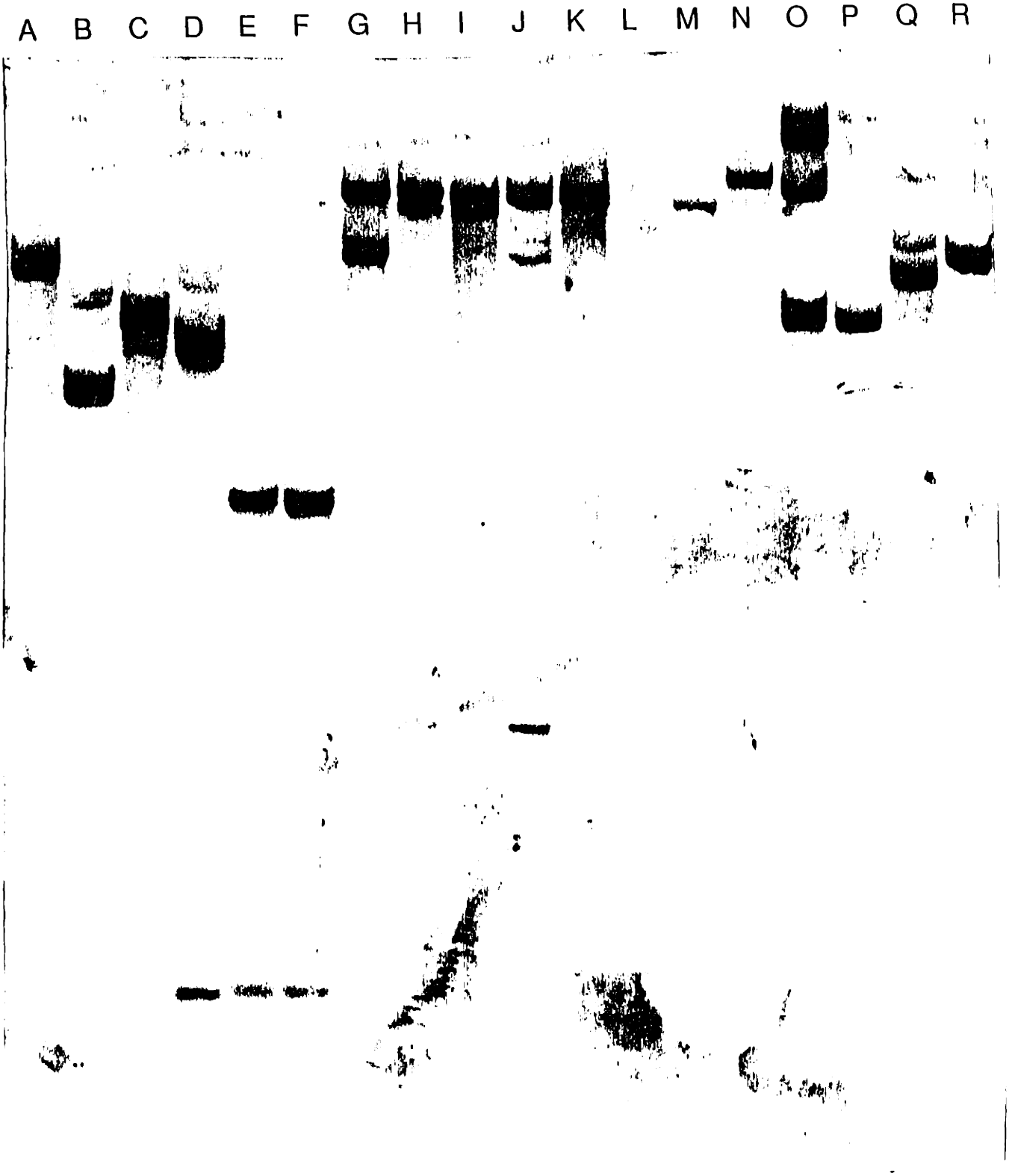
Figure 11

Figure 12

Restriction fragment size differences in *unc-86* alleles. Southern blot probed with the left *Eco* RI to *Eco* RV subclone (see figure 8).

Lanes A-C cut with *Eco* RI; lanes D-F cut with *Hind* III; lanes G-J cut with *Bgl* II; lanes K-N cut with *Sac* I; lanes O-R cut with *Bcl* I. Lanes A, D, G, K, and O, N2; B, E, H, L and P, *unc-86(u371)*; C, F, I, M and Q, *unc-86(rh1029)*; J, N and R, *unc-86(n1351)*.

Figure 12



required only for a short time in those cells, we expected that the transcript might be difficult to find.

We began by probing a Northern blot, gift of Larry Casson and Barbara Meyer, of N2 adult and mixed-stage total RNA, with the 6.0 kb *Eco* RI fragment labelled to high specific activity (figure 13). The only band that appears on a 3.5 day exposure corresponds to the position of DNA contamination in the RNA samples.

To increase sensitivity we turned to RNase protection experiments (Zinn, DiMaio, and Maniatis, 1983). For best sensitivity high specific activity probes are needed; to make sure that these are mostly full length, probes longer than 1 kb should not be attempted.

Since we could not probe the entire 6 kb fragment in both directions in pilot experiments (much less the region deleted in Him mutations), we attempted to guess where the functional sequences of *unc-86* were. We used two criteria, both of which gave the same answer. The first criterion is location of mutations—*n946* is located in the rightmost 1 kb of the 6.0 kb fragment and the three insertion mutations are located just left of the *Eco* RV site. The second is distribution of restriction sites—*C. elegans* introns average 74% AT (T. Blumenthal, personal communication) while exons are less AT rich, so GC rich restriction sites are most likely to be found in the coding regions. There are two segments of the 6.0 kb fragment that have several GC rich restriction sites—one in the rightmost 1 kb and one in the 1 kb left of the *Eco* RV site (see figure 8).

We made four RNA probes (LA, LB, RA and RB in figure 14). Figure 15 shows the probe protected by 0 and 50 μg of total N2 RNA. The only hint of a specific band is in lane 0; however, the probe for this lane

Figure 13

Northern blot of total *C. elegans* RNA probed with the 6.0 kb Eco RI fragment from cosmid AA1. The bands visible near the top are at the position of DNA contamination of the RNA samples (L. Casson and B. Meyer, personal communication).

A, a strain carrying *mnDp10*; B, N2 mixed stages; C, a strain carrying *mnDp10*; D, N2 mixed stages; E, N2 adults.

Figure 13

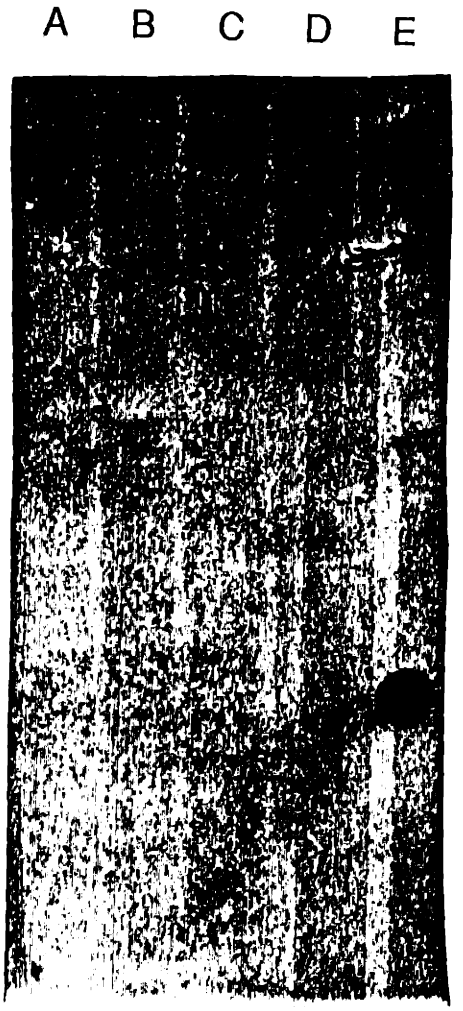


Figure 14

Templates for RNA probes used in RNase protection experiments. LA, *Eco* RV to *Bgl* II; RA, *Eco* RI to *Sal* I; LB, *Bgl* II to *Eco* RV; RB, *Hind* III to *Ava* II; HSalB, *Hind* III to *Sal* I; HSacB, *Hind* III to *Sac* I; HRB, *Hind* III to *Eco* RI.

Figure 14

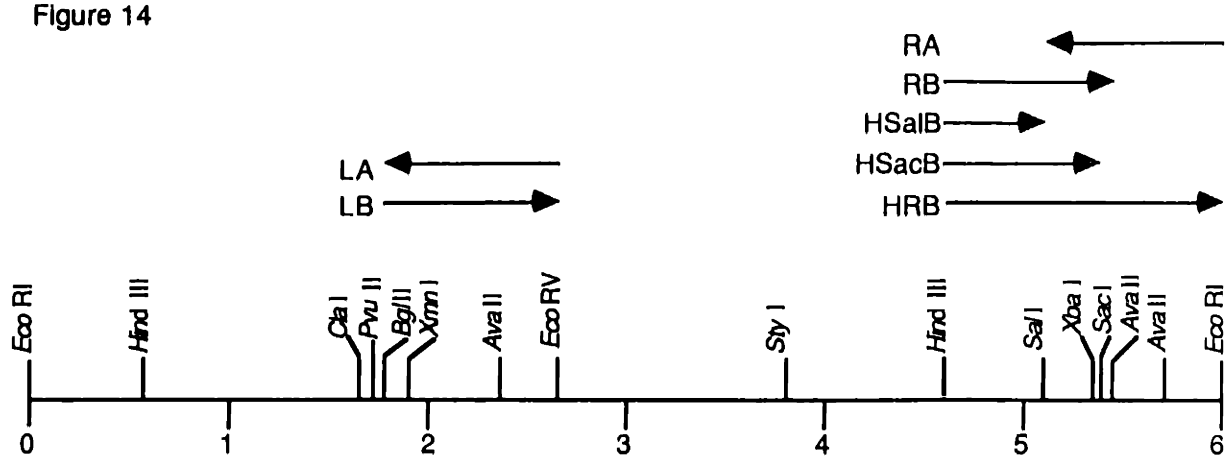

RA
RB
HSalB
HSacB
HRB
LA
LB
Eco RI
Hind III
Cla I
Pvu II
Bgl II
Xmn I
Ava II
Eco RV
Sty I
Hind III
Sal I
Xba I
Sac I
Ava II
Ava II
Eco RI
0
1
2
3
4
5
6

Figure 15

RNase protection by N2 total RNA. Probes are shown in figure 15. Lanes B-F, probes; lanes G-J, no *C. elegans* RNA added; lanes K-O, 50 μg of N2 total RNA added. A, pBR322 *Hinf* I digest; C, G and L, probe LA; D, H and M, probe RA; E, I and N, probe LB; F, J and O, probe RB.

Figure 15

A B C D E F G H I J K L M N O

was made from a plasmid that had been incompletely cleaved and thus a fraction of the probe had extended into vector sequences (lane F).

Three more probes were made, overlapping the probe that had resulted in a signal in the above experiment (figure 14). Each was hybridized with 50 μg of total RNA from N2, *n946*, and *e1416*. No protection is seen (figure 16). The background in lanes M-P is often seen with probes made from plasmids cut with enzymes that leave a 3' overhang (S. Kim, personal communication) and probably results from aberrant probe synthesis that makes self-complementary RNA probe.

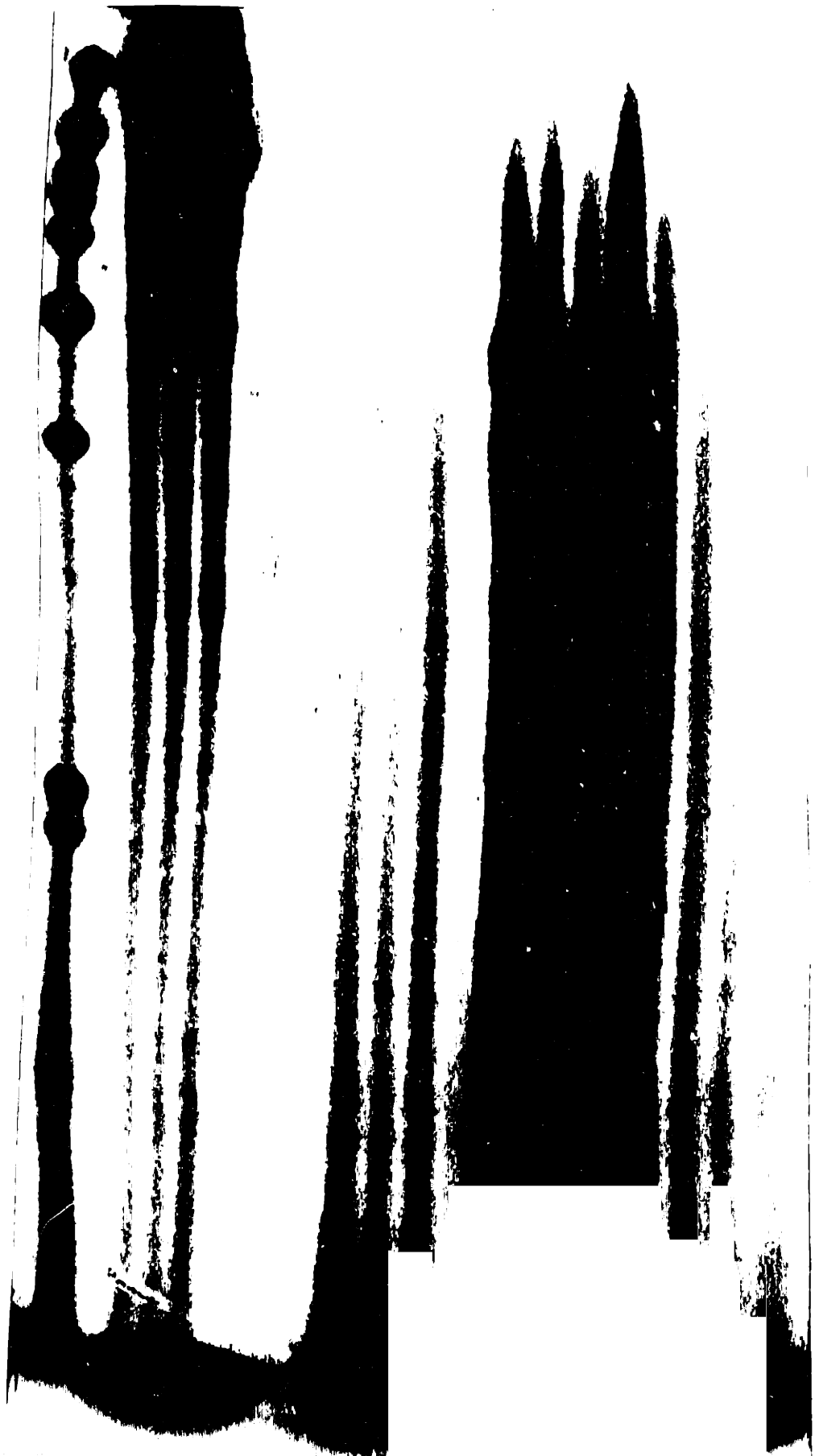
Figure 16

RNase protection by N2, *unc-86(n946)*, and *unc-86(e1416)* total RNA. For description of probes, see figure 15.

A, pBR322 *Hinf* I digest. Lanes B and F-H, actin control; E and I-L, probe HSalB; D and M-P, probe HSacB; C and Q-T, probe HRB. Lanes B-E untreated probe; I, M and Q, no added RNA; F, J, N and R, 50 μg of N2 RNA; G, K, O and S, 50 μg of *n946* RNA; H, L, P and T, 50 μg of *e1416* RNA.

Figure 16

A B C D E F G H I J K L M N O P Q R S T



Discussion

Null phenotype

When we began this work the null phenotype of *unc-86* was not known. Four alleles existed, two each of two classes, Him and non-Him. Either class could have been null, or null alleles could have been different from either class, for instance lethal or sterile. We were interested in the null phenotype because in order to understand what a gene does in the wild type it is important to know all of the processes that are affected by the gene. In particular we were interested to know if all of the effects of a null mutation in *unc-86* were homeotic.

We isolated a deficiency, *nDf20* that deletes *unc-86* and at least one gene on each side of it. Another deficiency, *nDf16*, also deletes *unc-86* and at least one gene on each side, but when we started we thought for two reasons that it might not. First, *unc-86* had been mapped incorrectly, so it appeared that the genes deleted by *nDf16* were not all contiguous. Second, *nDf16* had been isolated on the basis of failure to complement *unc-86(e1507)*, and therefore might have been selected for reduction but not elimination of *unc-86* activity. For these reasons we considered it possible that *nDf16* was a deletion ending near *unc-86* and reducing *unc-86* activity by position effect (Sturtevant, 1925).

unc-86 alleles of both classes, when heterozygous with either deficiency, resulted in viable animals whose phenotypes are not significantly more severe than homozygotes of either allele. This result is important for two reasons. First, it suggests that the null phenotype is not greatly more severe than the phenotype of either class

of allele. However, other evidence (chapter 1 figure 3B) suggests that there may be sharp thresholds in the relation between amount of *unc-86* activity and the resulting phenotype; therefore the lack of phenotypic change resulting from a presumed twofold reduction in function is inconclusive. Second, it establishes that a null allele in *trans* to an allele of either class is viable; therefore a null allele could be found in a complementation screen.

Complementation screens using EMS as a mutagen were performed and mutants were found at a rate of one per 3000 mutagenized chromosomes. This rate is similar to that found by Brenner (1974) and Greenwald and Horvitz (1980) for the production of null alleles in average genes by an EMS mutagenesis, suggesting that these are null alleles. All five alleles found in the complementation screen result in viable animals when homozygous, suggesting that null alleles are not lethal or sterile. However, three of the five new alleles were non-Him and two were Him, leaving unresolved if either is the null phenotype.

Twenty-one additional *unc-86* alleles were isolated by M. F., C. Desai, M. Chalfie and coworkers, N. Tsung, S. Kim, and E. Hedgecock during the time this work was in progress (see Materials and Methods, table 1). All were isolated as homozygotes; none results in a Him phenotype. Five of these alleles are weak, having some Unc-86 phenotypes but not others (chapter 1 table 3).

One interesting feature of these weak alleles is that their phenotypes are generally not variable, at least as scored with a disecting microscope. In almost all cases all animals of a genotype (and at a given temperature, for the two temperature-sensitive alleles) had the same Mec and Egl phenotype. This phenomenon could be caused by

sharp thresholds in the requirement for *unc-86* function, as mentioned above. Furthermore, the two phenotypes are differentially affected by reduction in *unc-86* function: all animals that are Egl are also Mec, but it is possible for animals to be Mec but not Egl. Of the 11 alleles isolated by M. Chalfie and coworkers on the basis of the Mec phenotype, 3 do not result in an Egl phenotype; of the nine alleles isolated by various people on the basis of the Egl phenotype (see Materials and Methods), all result in an Mec phenotype.

On the basis of the above results we advanced two hypotheses to account for the data. The first is that the null phenotype of *unc-86* is lethal or sterile, the existing alleles form a graded series of increasing reduction of function with the Him alleles being the most severe class, and that the apparent bunching of alleles into phenotypic classes results from thresholds in the requirement for *unc-86* function. The second hypothesis is that the phenotype of the non-Him alleles is the null phenotype and the Him alleles are multigenic mutations affecting at least one other nearby gene.

The first hypothesis is appealing because it provides a convenient explanation for the Him alleles. The second hypothesis is more consistent with the complementation screen and deficiency data, but makes a specific prediction about the molecular biology of the Him alleles, a prediction whose *a priori* probability was hard to evaluate. The second hypothesis is additionally appealing because it means that the Him phenotype, which is probably not homeotic, is not a null phenotype of *unc-86*.

The data in chapter three indicate that all four *unc-86* Him alleles are deletions of about 18 kb, while some non-Him alleles have

smaller rearrangements. Thus the Him alleles are extremely likely to be null alleles of *unc-86*. None of the rearrangements in non-Him alleles map to the rightmost 15 kb of the region deleted by the Him alleles. Therefore it is entirely possible that those deletions affect at least one other gene besides *unc-86*. The rearrangements in some non-Him alleles suggest that these may also be null alleles. Thus the first hypothesis is almost certainly wrong and the second probably right.

Are all phenotypes of *unc-86* homeotic?

The lineage alterations previously described for *unc-86* are clearly homeotic (Chalfie, Horvitz, and Sulston, 1981; see introduction). Two defects are not obviously homeotic: Egl and Him.

Egl phenotype

The Egl phenotype results from the failure of the HSN neuron to differentiate. Sulston has observed another *unc-86* lineage, that leading to the production of the ALM and BDU neurons, in which the cell division pattern is not altered, yet no functional neurons are produced (Introduction, figure 2). Such changes in cell fate would be homeotic if they are transformations to a different fate; if, however, they are caused by the lack of some product that these neurons need in order to function, then the changes are not homeotic.

We investigated the nature of the defect in HSN maturation by determination of the temperature-sensitive period of that defect in *unc-86(n848ts)*. Exposure to the permissive temperature (15°) for any of several intervals during the period from the early fourth larval stage to the early adult stage is sufficient for the Egl^+ phenotype.

The HSNs in *n848* animals kept at the restrictive temperature (25°) until the third larval stage are capable of differentiating correctly if the animals are shifted to 15°, implying that *unc-86* function is not required by these cells before that point. The HSNs in *n848* animals kept at 15° until the fourth larval stage differentiate into functional neurons even if the animals are shifted to 25°, implying that *unc-86* function is no longer required after that point (unless *unc-86* product synthesized at 15° can function later at 25°). The straightforward explanation for these observations is that in wild-type animals *unc-86* provides a function required for HSN maturation that acts during the late third or early fourth larval stage.

The *unc-86* function necessary for HSN maturation need not, *a priori*, act within the HSN neuron. All non-gonadal cell lineages that have divisions occurring any time near the tsp for HSN maturation, including those of the sex muscles (the synaptic targets of the HSNs), have been examined in *unc-86* animals and found to be wild-type (Sulston and Horvitz, 1981). Therefore the HSN maturation defect can not be caused by a defect in those lineages. The HSN defect is unlikely to be caused by a defect in either the gonad or the sex muscles because *unc-86* prevents production of serotonin by the HSNs, and interaction between the HSNs and the gonad and sex muscles is not necessary for production of serotonin by the HSNs: animals in which the gonad or sex muscles are altered in ways preventing them from interacting with the HSNs have HSNs that contain serotonin (C. Desai, personal communication). Thus it seems likely that *unc-86* mutations cause a defect that is not related to a change in cell division patterns.

Are the HSN neurons transformed to some other cell type? In many cases (*e.g.* Introduction, figure 1) *unc-86* mutations result in a transformation in the fate of a cell to the fate of that cell's mother. *unc-86* appears to act on the HSN at a time when there are no cell divisions, so that sort of transformation is impossible. The failure of a newly divided cell to become different from its mother may be analogous (in the absence of cell divisions) to the failure of a cell of one type to change to another type. If the analogy holds, then the HSN is unable to change from the normal third larval stage state to the differentiated adult state. In support of this possibility is the observation that HSNs in *n848* animals grown at 25° are still capable of becoming functional if the animals are shifted to 15° as young adults, more than 24 hours after the normal time of *unc-86* function. Thus the absence of *unc-86* function prevents the HSN from maturing and also keeps it in a state that is capable of maturing given *unc-86* function.

Suppressors

Three weak suppressors of *unc-86* representing two complementation groups were identified. The combination of one suppressor from each group partially suppresses a null allele of *unc-86* (Chapter 1, table 7). In particular, functional ALM (touch-receptor) neurons can be made. Thus, these suppressors can bypass the requirement for *unc-86*, and so can not work only by increasing the level of *unc-86* gene activity.

Him phenotype

The effect of the *unc-86* Him alleles on meiotic disjunction could be due to 1) an indirect effect of a homeotic transformation (through, for example, stress) 2) a direct effect of lack of the *unc-86* gene

product or 3) a direct effect of the lack of a product of a nearby gene affected by a multigenic mutation. None of the genetic experiments in this thesis can eliminate either hypothesis 2 or 3, but the interactions between *unc-86* alleles and *him-5* and *him-8* help to distinguish them from hypothesis 1. The basis for this distinction is the difference between direct and indirect effects on a process.

For the sake of argument imagine a process for which there are two or more partly redundant pathways. An indirect effect on the process reduces the efficiency of each pathway, while a direct effect effects only one of the pathways. If two mutations affecting the same process are combined in one animal, the process is expected to be at least as strongly affected as in either single mutant. If one effect is direct and the other is indirect, then the effects should be roughly additive: since the mutation with the indirect effect only reduces the efficiency of the redundant pathway by a fraction, the efficiency of the whole process is also reduced by a fraction. If, however, the effect of each mutation on the process is direct, the effect of both together is unpredictable. If both are in the same pathway then the effect of both together may be no more severe than either alone; if they are in different redundant pathways then together they may result in a much more severe phenotype than either alone.

A non-Him allele of *unc-86*, as expected, has no appreciable effect on the Him phenotype of either *him-5* or *him-8*. All three of the Him alleles tested, however behave in an unexpected way. They have no appreciable effect on the Him phenotype of *him-8*, but very strongly enhance the Him phenotype of *him-5* (Chapter 1, table 6). These results

suggest that the *unc-86* Him alleles have a direct effect on meiotic chromosome segregation.

Disruption of meiotic chromosome segregation seems quite unrelated the other effects of *unc-86* alleles. This lack of relatedness adds weight to the hypothesis, proposed above, that the Him alleles affect another gene in addition to *unc-86*.

Conclusion

All evidence is consistent with, and the weight of the evidence supports, the conclusion that all effects of a null mutation in *unc-86* are homeotic. Therefore we believe that *unc-86* is primarily involved in the specification of cell fate, and is a worthwhile object of future study, particularly molecular cloning.

Cloning and gene structure.

The power of genetics lies in its ability to identify genes involved in particular processes. The power of molecular biology lies in its ability to analyze the structure and action of genes. Complete analysis of *unc-86* and other genes identified by genetics therefore requires that the genes be molecularly cloned. Two approaches were used to the general problem of the cloning of *C. elegans* genes defined only by mutations.

Both approaches made use of transposable elements, and in particular the element Tc1. The first idea was to make use of a naturally-occurring strain of *C. elegans* that has a very high number of copies of Tc1 scattered over its chromosomes. Each of these insertions creates a restriction fragment length polymorphism for which there is a hybridization probe. The polymorphisms can be mapped and cloned and

used as the starting point of a chromosomal walk. The second idea was to use mutator strains made by P. Anderson, in which he has shown that Tc1 is transpositionally active, to tag genes with a transposon insertion. That insertion can then be mapped and cloned.

Transposon tagging

Transposon tagging did not work, possibly because of the site specificity of Tc1 insertion. Of the three mutator-derived spontaneous *unc-86* alleles examined, all had insertions of sequences that are not Tc1. These may well be other transposons that are active in mutator strains. J. Yuan (personal communication) has used cloned *unc-86* sequences as a probe to molecularly clone the insertions in these three *unc-86* alleles.

Size of insert, restriction map, and hybridization between clones indicate that the insertions in *unc-86(u371)* and *unc-86(rh1029)* are of the same element, named Tc3, found by J. Collins and P. Anderson (personal communication) in some insertion alleles of *unc-22*. Thus the transposon tagging approach would have worked as soon as the Tc3 probe became available. Furthermore, if the insertion from *unc-86(n1351)* is also a transposable element, then there are two new elements available whose site specificity may differ from that of Tc1. So although transposon tagging did not work for *unc-86*, it may work for more other genes in the future partly because of our efforts.

Chromosome walking

Walking from linked polymorphisms yielded a clone of *unc-86*. This approach consists of three parts: mapping and cloning of Tc1 polymorphisms, walking, and identification of the gene.

on the basis of the Southern blot phenotype are rather small. Perhaps those chances could be improved by the isolation of mutator-induced and γ-ray-induced alleles.

A. Fire has recently reported integrative DNA transformation of *C. elegans* (Fire, 1986) and rescue of the *unc-54* mutant phenotype by transfromation with the wild-type copy of the gene (personal communication). Thus even if mutation sites can not be found, it may still be possible to identify a clone carrying the wild-type allele of a gene by its ability to rescue mutant alleles by DNA transformation.

We have investigated two methods for cloning *C. elegans* genes identified only by mutation, and found that both can work, although neither is certain to work for any particular gene. The chances of cloning any given gene are likely to be even better if both approaches are tried.

Gene structure

The fact that all four alleles have identical or virtually identical deletions indicates either that they are not independent or that they were produced by a mechanism that has some specificity, perhaps homologous recombination. Since *unc-86(e1416)*, one of the Him alleles, has been used as the canonical allele of *unc-86* (and therefore was the allele in most frequent use), it is possible that one or more of the other Him alleles had been mixed up with it. We do not think it is possible that all four were switched. As for homologous recombination, any homology between the fragments containing the endpoints of the deletion is either too short or too imperfect to show up on Southern blots. Eide and Anderson (1985b) have shown that 23% of

spontaneous mutations in *unc-54* are deletions but that the deletions are not associated with direct repeats.

unc-86(n946) is associated with a deletion of about 200 bases. Its location approximately 3 kb from the insertion sites in the mutator-derived alleles suggests that *unc-86* spans at least 3 kb.

All three mutator-derived alleles have insertions within a small region of about 100 base pairs, possibly at the same site. This suggests either that these two classes of elements have the same site specificity, or that few sites in *unc-86* are accessible in germ-line DNA. Tc1, which is responsible for 21 of 28 insertion mutations in mutator-derived *unc-22* alleles (P. Anderson, personal communication), is not found in any *unc-86* alleles. This suggests that no favored sites for Tc1 insertion in *unc-86* are accessible in germ-line DNA.

Transcript

Any transcript from the 6 kb fragment containing the non-Him mutations must be present at relatively low level; any transcript from the areas investigated using RNase protection experiments must be present at very low level. The Northern blot filter had previously been probed with a cloned myosin gene; the RNA abundance for *unc-86* is at least several hundred fold less (L. Casson and B. Meyer, personal communication). RNase protection experiments were performed which should have detected RNA if it were present at one part in 10^5 to 10^6. The sensitivity of the RNase protection experiments could be improved by a factor of at least 100 by using probe with higher specific activity, poly A-selected RNA, and RNA from staged animals.

The fact that transcript of *unc-86* is not present at more than trace levels vindicates the genetic approach to the identification of genes that control cell fate. No currently feasable biochemical or molecular experiment would have identified *unc-86* as being important in the specification of cell fate.

Appendix: Genetic Mapping of a Mutator Activity

Introduction

The *C. elegans* transposable element Tc1 (Emmons, Yesner and Katzenberg, 1983) is present in 30 copies in the Bristol strain used as the wild type for genetic studies, and in 500 or more copies in the Bergerac wild-type strain (see chapter 2). Eide and Anderson (1985a) and Greenwald (1985) have shown that a weak mutator activity in the Bergerac strain results from Tc1 transposition. Eide and Anderson (personal communication) have isolated EMS-induced mutator (*mut*) strains by selecting for increased frequency of reversion of the allele *unc-54(r323::Tc1)*. In addition to promoting excision, these strains have an increased frequency of spontaneous mutations, at least some of which are caused by insertion of Tc1 (J. Collins and P. Anderson, personal communication; J. Way and M. Chalfie, personal communication; S. Kim, personal communication).

We began working with mutator strains because we wanted to find insertion mutations in *unc-86*. TR679, the most active Mutator strain, has not been outcrossed since the EMS mutagenesis that generated it and has since accumulated many Tc1 insertions, presumably some of them to deleterious effect. TR679 animals appear unhealthy and produce very small broods (zero to about ten animals) at least partly because they produce many inviable zygotes. As a consequence TR679 animals do not accumulate enough late-stage eggs to allow one to score directly the Egl phenotype of *unc-86*. Our desire to make a healthier mutator strain

led us to the general problem of mapping a mutator activity from such a strain.

It would be useful to genetically map the mutator activity from a mutator strain for several reasons. It would allow one to move the mutator activity into a healthy background, a background with few Tc1 insertions in a region of interest, or a background containing particular mutations (*e.g.*, for reversion studies). It would also be the first step in the genetic analysis and the molecular cloning of the locus responsible for the mutator activity.

Results

Genetic mapping of a mutator activity

We began with TR674, generously provided by P. Anderson, which carries *mut-2(r459)* and *unc-54(r323::Tc1)* and is the parent strain of TR679, mentioned above. We crossed N2 males into this strain, crossed the wild-type (WT) male progeny into N2 hermaphrodites, and again crossed the WT male progeny into N2 hermaphrodites. We picked a large number of WT hermaphrodite progeny from the last cross (in which the presence of many males indicated that there was a high fraction of cross progeny) and looked for the segregation of Unc-54 animals. These thrice-backcrossed animals have an X chromosome that is completely derived from N2 and should have lost about 7/8 of the Tc1 insertions not linked to X or *unc-54*. They should also have roughly a 1/8 chance of carrying any *mut* mutation unlinked to *unc-54*.

We picked about 30 *unc-54* segregants from several heterozygous parents, placed them together on a single plate, and watched to see if any would revert to WT. After several generations a WT revertant was found and transferred to a new plate. Since we didn't know if *mut-2* was dominant or recessive, the possibility existed that this revertant was not homozygous for *mut-2*. Therefore we picked *unc-54* segregants from the first revertant, waited for them to produce a revertant, and picked *unc-54* segregants from this second revertant. These segregants had been through about ten generations since the original revertant and so were very likely to be homozygous; since all strains derived from these animals have mutator activity we believe that the animals were

homozygous. The *unc-54* strain was named MT2878; a WT revertant from MT2878 was named MT2879.

In all work in which we used reversion of *unc-54* as an assay, our operational definition of mutator activity was this: a strain is judged to have mutator activity if one or a small number of *unc-54(r323)* animals placed on a bacterial lawn on a 60 mm petri plate consistently produce a revertant before all of the bacteria have been consumed. This corresponds to a reversion rate of roughly 10^{-3} to 10^{-4} per animal (*i.e.*, revertants usually appear after about 10^3 animals have hatched on a plate but before 10^4 have hatched); it may be lower on a per zygote basis since many Unc-54 embryos do not hatch into viable larvae. A strain was judged to have no mutator activity if no revertant was found on two plates started as above.

Even if the mutator were recessive and the plate started from a heterozygous animal, then nearly half of the animals on the plate should be homozygous for the mutator within the five to ten generations required by the assay. Therefore the *unc-54* reversion assay can measure whether or not a single animal used to start it had at least one copy of the mutator locus.

Reversion of *unc-54(r323)* is caused by Tc1 excision (P. Anderson, personal communication). Derivatives of MT2878 also retain the ability to produce new mutations. A number of people in this and other laboratories have isolated spontaneous mutations from MT2878 derivatives; we have found mutations in *unc-8*, *unc*-35, *unc-49*, *unc-58*, *unc-63*, *dpy-19*, *lon-2*, and other unidentified genes (the *unc-8*, *unc-35*, and *unc-63* mutations were mapped and the complementation tests done by M. Stern).

Table 1

Phenotypes of *dpy-19* alleles and heterozygotes.

\+ Wild-type
\- Slight Dpy—difficult to tell from Wild-type
— Large Dpy
--- Medium Dpy
---- Strong Dpy, borderline viable
----- Strong Dpy, infertile
nd Not determined

dpy-19 animals are partially rescued if their hermaphrodite parent carried a + allele. The left half of the table lists the phenotype if the parent carried a + allele (homozygotes) or the weaker of the two alleles (heterozygotes). The right half lists the phenotype if the parent was homozygous for the stronger (or only) allele.

dpy-19 genotype	+ or weak Mat. effect			– Mat. effect		
	15°	20°	25°	15°	20°	25°
e1259	+	—	——	+	——	———
e1259/n1347	+	–	—	nd	nd	nd
e1259/n1347n1348	+	——	———	+	——	———
n1347	+	+/–	--	+/–	–/—	—
n1347/n1347n1348	+	——	——	+	——	nd
n1347n1348	+	——	———	+	——	———

A new assay for mutator activity

The *mut-2*-induced *dpy-19* mutation, *n1347*, proved particularly useful in further mapping experiments. The mutation causes a weak Dpy phenotype; in mutator strains weak Dpy animals occasionally produce progeny with a stronger Dpy phenotype. The strong Dpy phenotype is also caused by a recessive mutation in *dpy-19*—the strong *dpy* maps to LG III near *unc-86* and fails to complement *dpy-19(e1259)* (table 1). Phenotypes of *n1347*, a strong Dpy derivative, and heterozygotes are listed in table 1. The original mutation is linked to a new Tc1 insertion, which is missing in strong Dpy derivatives (figure 1). The simplest explanation is that there is a Tc1 insertion into *dpy-19* that causes a slight reduction in gene function, and function can be reduced further by imprecise excision. Tc1 usually excises imprecisely, leaving a small insertion behind (D. Eide and P. Anderson, personal communication).

dpy-19(n1347) has two main advantages over *unc-54(r323)* as an assay for mutator activity. First, strains carrying *n1347* are far healthier than strains carrying *r323*, so they are easier to handle and grow. Second, the mutation rate to strong Dpy is about 10^{-2} per gamete, so the assay can be done in a week with only a few animals instead of the three to four weeks required by the *unc-54* reversion assay. *n1347* enhancement was used as the assay in most further mapping experiments.

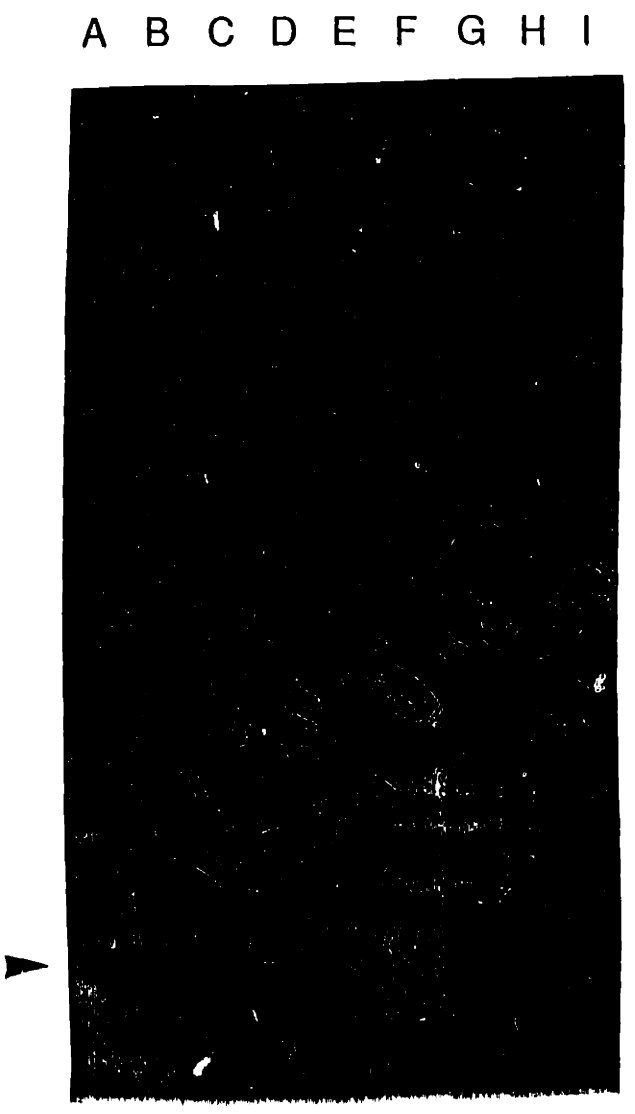
The *n1347* enhancement assays are performed by mating *dpy-19(e1259) unc-86(e1507)* males into *dpy-19(n1347)* hermaphrodites. Most of the cross progeny will be *n1347/e1259*, which are not very Dpy;

Figure 1

A Tcl insertion associated with *dpy-19(n1347)*. Southern blot of *Eco* RI-cut DNA from strains carrying *n1347*, strong Dpy derivatives of *n1347*, or *dpy-19(+)*, probed with Tcl. "*" refers to how many times a strain has been crossed into Bristol-derived strains, starting from TR674 or TR679; for instance, *10 means 10 times backcrossed.

A-E strains carrying *n1347*: A, *dpy-19(n1347) unc-49(n1324)* *10; B, *dpy-19(n1347) unc-49(n1324) dpy-18(e364)* *10; C, *dpy-19(n1347) unc-49(n1324n1345)* *10; D, *dpy-19(n1347)* *9; E, *dpy-19(n1347)* *10 (backcrossed independently from lane D); F-H three independent strong Dpy derivatives: F, *dpy-19(n1347n1348)* *10; G, *dpy-19(n1347)*VD2 *5; H, *dpy-19(n1347)*VD6 *8; I, N2.

Figure 1



if the hermaphrodites have mutator activity, about 1% of the cross progeny are *n1347*/e1259* (where *n1347** means a strong *dpy-19* allele derived from *n1347*), which are strongly Dpy. This protocol has two advantages over simply screening for strong Dpy segregants from an *n1347* strain: first, *n1347** mutations are easier to pick up as *n1347*/e1259* heterozygotes than as *n1347*/n1347*; this allows them to be found in the first generation after they are produced and so does not require that they be homozygous viable, and gives a better measure of the per-gamete frequency; second, since the strong Dpys are cross progeny and both male and hermaphrodite, it prevents one from being fooled by second site mutations and triplo-X hermaphrodites, either of which might produce a strong Dpy phenotype in an *n1347* background. The linked *unc-86* mutation used in this assay makes it possible to distinguish the new *n1347** allele from *e1259* and to isolate the new allele as a homozygote.

The mutator activity from MT2878 is presumed to be *mut-2*. It maps to LG I in two- and three-factor crosses (figure 2). The region of LG I balanced by *unc-13* is sufficient for mutator activity. We crossed the region of MT2879 balanced by *unc-13* into a Bristol background ten times, making a total of 13 backcrosses in all (figure 3).

This region has mutator activity in the *dpy-19* assay and produces occasional Uncs and Twitchers (probably alleles of *unc-22*). A strain containing this region ten times backcrossed into a Bristol background has about 15 extra copies of Tc1 on *Eco* RI genomic Southern blots (figure 4).

Figure 2

Map position of *mut-2(r459)*.

A. Map data for *mut-2*.

B. Genetic map of part of LG I showing the position of *mut-2*.

Figure 2

A

Heterozygote	Animals picked	Number *mut*	Results
mut-2(r459) + unc-54 / + unc-75(e950) +	20 Unc-75		
	4/20 seg. Unc-54	0/4	*(mut-2 unc-75) unc-54*
	Unc-54	3/4	*unc-75 mut-2* p<12.5%
mut-2(r459) / unc-13(e51)	>15 Unc-13^{+}	All	*unc-13 mut-2* p<3%
	1 Unc-13	0	

B

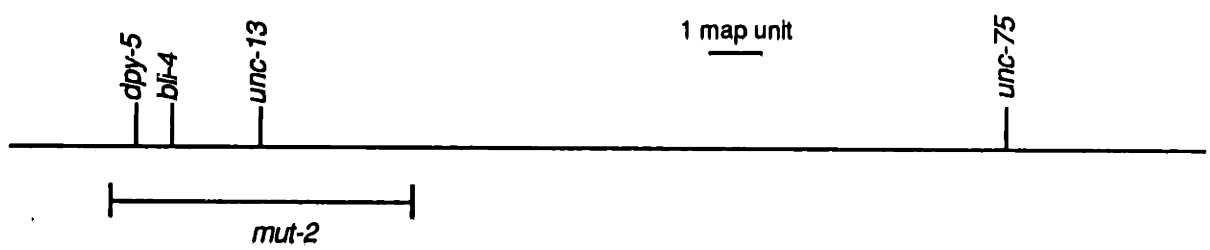


Figure 3

Scheme used to cross mut-2(r459) into a Bristol background.

Figure 3

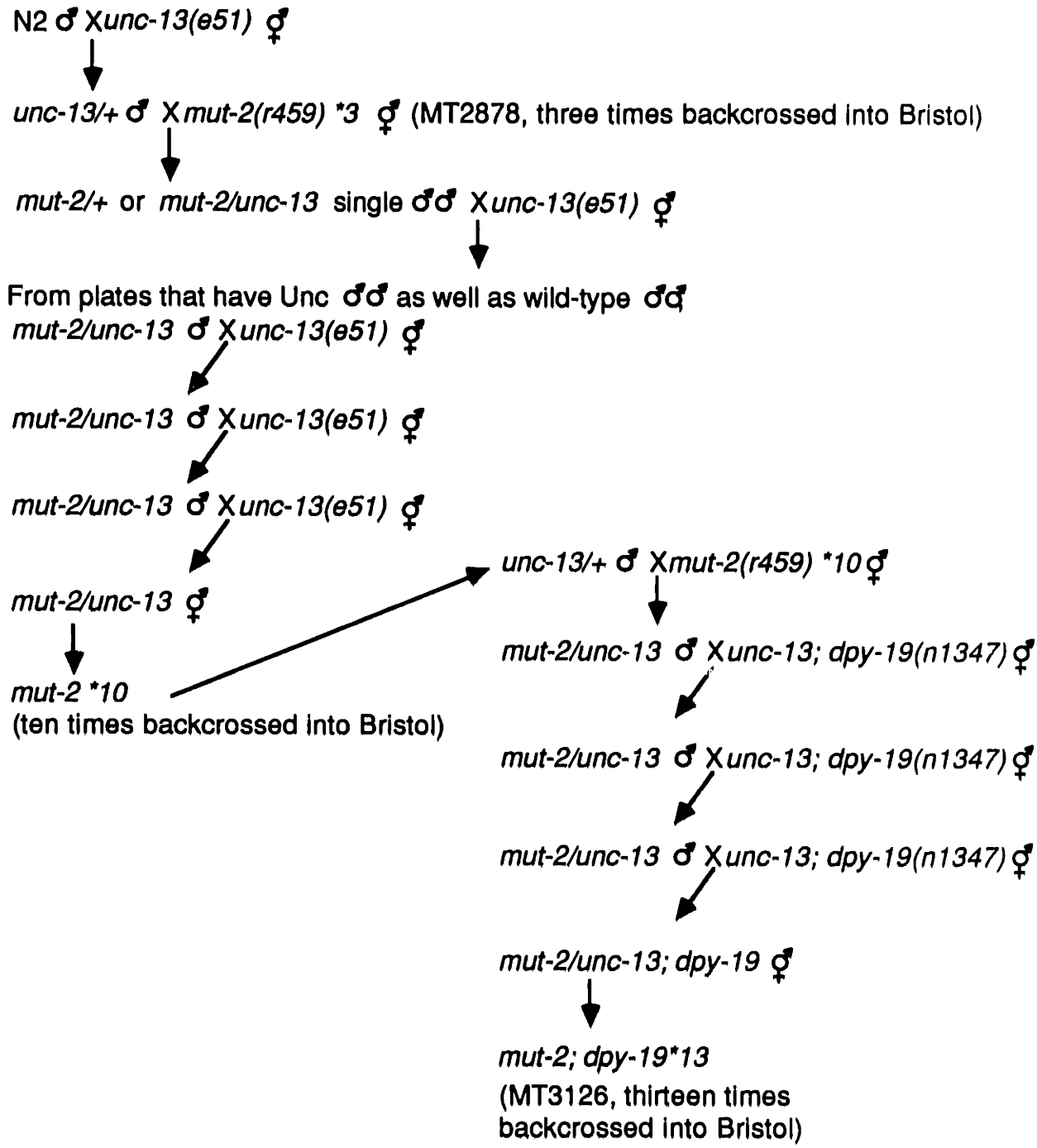

N2 ♂ X unc-13(e51) ♀
unc-13/+ ♂ X mut-2(r459) *3 ♀ (MT2878, three times backcrossed into Bristol)
mut-2/+ or mut-2/unc-13 single ♂♂ X unc-13(e51) ♀
From plates that have Unc ♂♂ as well as wild-type ♂♀
mut-2/unc-13 ♂ X unc-13(e51) ♀
mut-2/unc-13 ♂ X unc-13(e51) ♀
mut-2/unc-13 ♂ X unc-13(e51) ♀
mut-2/unc-13 ♀
mut-2 *10
(ten times backcrossed into Bristol)
unc-13/+ ♂ X mut-2(r459) *10 ♀
mut-2/unc-13 ♂ X unc-13; dpy-19(n1347) ♀
mut-2/unc-13 ♂ X unc-13; dpy-19(n1347) ♀
mut-2/unc-13 ♂ X unc-13; dpy-19(n1347) ♀
mut-2/unc-13; dpy-19 ♀
mut-2; dpy-19*13
(MT3126, thirteen times
backcrossed into Bristol)

Figure 4

Extra copies of Tc1 in a strain carrying *mut-2(r459)* crossed ten times into Bristol.

A, N2; B, *mut-2(r459)* crossed ten times into Bristol.

Figure 4

A B

Further mapping was attempted by constructing a *mut-2/bli-4(e937)unc-13(e51); dpy-19(n1347)* heterozygote and picking Bli and Unc recombinants. 2/2 Unc and 2/2 Bli recombinants carried lethal mutations on the recombinant chromosomes. This implies that two genetically separable compensating lethal mutations are present close to *mut-2* in this strain (see discussion).

Discussion

Starting with a mutator strain, TR679, made by P. Anderson, we mapped a mutator activity to a particular genetic location. This activity is not necessarily the only one in the starting strain, nor is it impossible that this activity is capable of transposing to a new chromosomal location; in fact, I. Mori, D. Moereman and R. Waterston have seen just such a phenomenon with *mut*-4 (personal communication).

The mapped mutator activity is probably sufficient to account for the observed elevation in the reversion rate of *unc-54(r323::Tc1)* in the starting strain. The forward mutation rate, at least for some genes, seems to be less in mutators crossed into mostly Bristol backgrounds than in those with more Bergerac-derived Tc1 insertions (P. Anderson, personal communication; J. Levin, personal communication). This could reflect a difference in the size of the pool of Tc1s that can serve as a source for transposition events.

While attempting finer mapping of the mutator activity we found an unusual genetic phenomenon. In the area where the mutator activity maps there are a genetically separable pair of compensating lethal mutations. That is, when a strain is constructed with the mutator chromosome in *trans* to a double mutation and recombinants are selected between the two *cis* mutations, the recombinant chromosomes carry lethal mutations. One possible explanation for this phenomenon is that the mutator chromosome carries nested inversions spanning the double mutation. A recombination between the markers would result in a chromosome with some sequences duplicated, some sequences deleted, or

both. Prokaryotic transposons are known to catalyze the formation of inversions (review, Kleckner, 1981).

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