Identification and Cloning of *unc-119*, a Gene Expressed in the *Caenorhabditis elegans* Nervous System

Morris Maduro and David Pilgrim

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada

Manuscript received May 1, 1995
Accepted for publication August 11, 1995

ABSTRACT

A spontaneous mutation affecting locomotion of the nematode *Caenorhabditis elegans* has been mapped to a new gene, *unc-119*. Phenotypic characterization of the mutants suggests the defect does not lie in the musculature and that the animals also have defects in feeding behavior and chemosensation. *unc-119* has been physically mapped relative to a previously identified chromosomal break in linkage group III, and DNA clones covering the region can rescue the mutant phenotype in transgenic animals. Three more alleles at the locus, with identical phenotypes, have been induced and characterized, all of which are putative null alleles. The predicted UNC-119 protein has no significant similarity to other known proteins. Expression of an *unc-119/lacZ* fusion in transgenic animals is seen in many neurons, suggesting that the *unc-119* mutant phenotype is due to a defect in the nervous system.

As foreseen by BRENNER over 20 years ago (BRENNER 1974), the free-living nematode *Caenorhabditis elegans* is an ideal system in which to study the development of a complete nervous system. Development in *C. elegans* follows a nearly invariant lineage, and the locations and patterns of connectivity of neurons are similar in every animal. Of the approximately 1000 somatic nuclei in the adult hermaphrodite, 37% comprise the nervous system (CHALFIE and WHITE 1988), including 302 neurons. Serial-section electron micrograph reconstructions have allowed the exact placement of each neuron cell body and axonal projections (WHITE *et al.* 1986). *C. elegans* has two independent nervous systems: feeding is controlled by the pharyngeal nervous system (ALBERTSON and THOMSON 1976), while movement and behavior are controlled by the somatic nervous system (WHITE *et al.* 1986). Since *C. elegans* is capable of self-fertilization, animals do not need to move in order to reproduce. These properties, coupled with the ease of *C. elegans* genetic manipulation (BRENNER 1974), have led to the identification of over 100 genes involved in the development of the muscle and nervous systems (WOOD 1988).

Over 30 different genes required for normal muscle position, structure or function have been identified (ANDERSON 1989), many of which confer an "Unc" mutant phenotype (for *unc*oordinated). Mutations in genes affecting the nervous system often have a more subtle Unc phenotype, affecting only a small subset of behaviors, such as response to gentle touch, sensitivity to volatile attractants, contractile ability of certain sets of body muscles and constitutivity of (normally) regulated behavior such as feeding or defecation. To date, several of these have been cloned and characterized, including kinesin homologues (HALL and HEDGECOCK 1991), clathrin-associated proteins (LEE *et al.* 1994), and cell adhesion and membrane proteins involved in nerve axon guidance and pathfinding (HEDGECOCK *et al.* 1990; ISHII *et al.* 1992; LEUNG-HAGESTEIJN *et al.* 1992; ROGALSKI *et al.* 1993). In some cases, human homologues have recently been identified for gene products previously known only in the *C. elegans* nervous system (HOSONO *et al.* 1992; HATA *et al.* 1993).

In this article, we report the identification of a new gene, *unc-119*, whose defect is likely neuronal based on both indirect and direct evidence. Animals homozygous for putative null alleles display many abnormalities in neuron-mediated behaviors; examination of muscle shows normal ultrastructure; and expression of a reporter gene fused to *unc-119* shows expression in different types of neurons. It is likely, therefore, that the *unc-119* gene product is required for the establishment or function of the nervous system. The predicted UNC-119 protein shows no significant similarity to other known proteins except for another *C. elegans* gene, of unknown function, suggesting that there may be a family of similar proteins involved in the nervous system.

MATERIALS AND METHODS

Strains and genetics: Growth, maintenance and manipulation of strains was performed as described (WOOD 1988). All strains were obtained from the stock collection of the MRC Laboratory of Molecular Biology, Cambridge, UK (with the help of J. HODGKIN), with the exception of *dpy-28(y1) III* and *daf-11(m47ts) V*, which were obtained from the Caenorhabditis Genetics Center; RW7097 [*mut-6(st702) unc-22(st192st527) IV*], which was a gift from B. WILLIAMS; and DA438 [*bli-4(e937) I; rol-6(e187) II; daf-2(e1368) vab-7(e1562) III; unc-31(e928) IV;*

Corresponding author: David Pilgrim, Department of Biological Sciences, CW405, Biological Sciences Building, Edmonton, Alberta, Canada T6G 2E9. E-mail: dave_pilgrim@biology.ualberta.ca

dpy-11(e224) V; lon-2(e678) X], which was a gift from L. AVERY (AVERY 1993).

Generation of *unc-119* alleles: The initial *unc-119* mutation, *e2498*, arose spontaneously in the progeny of a hybrid between two *C. elegans* strains, Bristol (N2) and Bergerac (BO) (PILGRIM 1993). *unc-119(ed3)* and *unc-119(ed4)* were isolated in the F_1 generation of 1500 *tDf2* +/+ *dpy-18(e499)* hermaphrodites mutagenized in 100 mM EMS (Sigma). *unc-119(ed9)* was isolated in a similar screen. The revertant DP41 [*unc-119(e2498ed5)*] arose spontaneously from a strain carrying both *e2498* and *mut-6(st702)*.

Mapping of *unc-119*: *unc-119* was initially mapped to chromosome *III* using the mapping strain DA438. Crosses with *daf-7, unc-64, dpy-1* and *lon-1* placed *unc-119* on the right half of chromosome *III*, between *lon-1* and *unc-64*. *unc-119* complements other Unc genes in this region including *unc-25, unc-32, unc-47, unc-49, unc-71* and *unc-81*.

(1) *unc-119* maps very close to *vab-7*. Dpy non-Vab and Vab non-Dpy self-progeny were selected from a hermaphrodite of genotype *vab-7(e1562) dpy-18(e499)* +/+ + *unc-119*. These recombinant progeny fell into the following classes: *vab-7 dpy-18/+ dpy-18* (0 animals); *vab-7 dpy-18* +/+ *dpy-18 unc-119* (31 animals); *vab-7 dpy- 18/vab-7* + (35 animals); and *vab-7 dpy-18* +/*vab-7* + *unc-119* (0 animals). Sma non-Vab self-progeny were selected from a hermaphrodite of genotype *sma-2(e502) vab-7(e1562)* +/+ + *unc-119(e2498)*. The progeny consisted of *sma-2 vab-7* +/*sma-2* + *unc-119* (14 animals) and *sma-2 vab-7/sma-2* + (0 animals).

(2) *unc-119* is deleted by *tDf2*. *unc-119(e2498)* heterozygous males were crossed to hermaphrodites of genotype *tDf2* +/ + *dpy-18(e499)*. Unc non-Dpy progeny were seen in the F_1 brood.

(3) *unc-119* maps to the break between *eDf2* and *eDp6*, as neither rearrangement complements *unc-119*. *unc-119(e2498) dpy- 18(e499)* heterozygous males were crossed to hermaphrodites of strain CB1517 [*eDf2; eDp6*]. Wild-type progeny were recovered in the F_1, but none carried the *unc-119 dpy-18* chromosome, as judged by progeny testing, suggesting that neither *eDf2* nor *eDp6* complement *unc-119*. Unc-non-Dpy animals were recovered among the F_1 outcross progeny, and shown to carry *eDf2; eDp6* and the *unc-119 dpy-18* chromosome by progeny testing. The chromosomal break that led to the production of *eDf2* and *eDp6* maps just to the left of *vab-7* (HODGKIN 1980), which places *unc-119* at this position.

Construction of *unc-119(e2498); daf-7(e1372ts)* and *unc-119(e2498); daf-11(m47ts)* double mutants: Heterozygous *unc*/+ males were crossed to adult *daf* hermaphrodites and placed at 25° after mating. F_1 animals were selfed at 25° and dauer progeny (scored by morphology) were picked from the F_2 brood to recover and self-fertilize at 15°. Unc animals were then picked from the F_3 brood. Verification of the double mutant genotype was made by crossing to wild type and checking the F_2 segregation proportions.

Phenotypic analysis: Pharyngeal pumping, egg retention and locomotion were scored as in SÉGALAT *et al*. (1995), with modifications. Locomotion was scored as complete sinusoidal waves per minute in liquid by adding 20 μl M9 buffer to a plate of worms and observing the motion under a dissecting microscope. To score egg retention, well-fed adult hermaphrodites were observed using differential interference contrast (DIC) optics. Pharyngeal pumping was scored by observing contractions of the terminal bulb of the pharynx for 1 min and then transferring the same animals to an unseeded plate. The animals were rescored after 5 min and then returned to a seeded plate to verify that pumping resumed to "on food" levels. For the transgenic strains *unc-119(e2498); edEx31* [pDP#MM016], *unc-119(ed3); edEx32* [pDP#MM051], and *unc-119(ed4); edEx33* [pDP#MM019], the array is extrachromosomal, with some meiotic and mitotic loss, and animals with the best visible locomotion were chosen for scoring of all traits, to assure at least partial inheritance of the transgenic array. At least 10 animals were scored for each trait.

Ability to form dauer larvae was initially measured by determining resistance to SDS in starved cultures at 25° (CASSADA and RUSSELL 1975). Plates on which animals had depleted the supply of bacteria were left for 4 days. At least 5000 worms per plate were collected, incubated in 2% SDS (in M9) for 2 hr at 25°, rinsed and dropped onto an unseeded plate. The ability to form dauers was scored as positive if surviving animals were seen. In all such cases, there were at least 50 survivors per original plate, and they had an appearance and behavior characteristic of dauer larvae. When fed bacteria, most of these animals recovered to form adults (at 15° in the case of *unc-119; daf-7*). Plates of strains that were unable to form SDS-resistant larvae also lacked animals with characteristic dauer morphology.

Molecular analysis: Genomic DNA was prepared as described (PILGRIM 1993). The λZAP cDNA library was a gift from B. BARSTEAD (BARSTEAD and WATERSTON 1989). Screening of the library and *in vivo* excision was performed as described by the manufacturer (Stratagene). Cosmids and yeast artificial chromosomes (YACs) from the physical map of *C. elegans* were obtained from A. COULSON. A clone containing the *C. elegans* transposable element Tc1 was a gift from L. HARRIS. The yeast strain N123 was obtained from R. C. VON BORSTEL.

Gel electrophoresis and Southern blotting was performed as described (PILGRIM 1993). All cloning, unless otherwise stated, was performed in pBluescript II KS− (Stratagene) grown in *Escherichia coli* XL1-B. Manipulation of DNA was performed as described (SAMBROOK *et al.* 1989). All sequencing was done on single-stranded templates as described in the USB Sequenase 2.0 kit (United States Biochemicals).

The *lacZ* fusion construct was made using the vector pPD22.04 (FIRE *et al.* 1990). A 3.6 -kbp *Hin*dIII-*Pst*I fragment was purified from pDP#MM016 (see Figure 7) and ligated to similarly digested pPD22.04 to produce the *unc-119*::*lacZ* fusion plasmid. The fusion is predicted to contain the first 101 amino acids of UNC-119.

PCR was performed using the primers MMA1 (5′-AGTCGGCCTTATTGTGCATTAC-3′) and MMA2 (5′-AAATTGCATGCCAGCACCGGTC-3′), on ~100 ng of genomic DNA of alleles *ed3, ed4, ed9* and *e2498ed5* in a 25 μl volume on a Stratagene Robocycler 40 using the following conditions: one cycle (95°, 60 sec; 61°, 90 sec; 73°, 180 sec) and 29 cycles (93°, 75 sec; 61°, 75 sec; 73°, 135 sec). PCR products were purified, digested with *Nhe*I and *Sst*I, and subcloned into pBluescript II SK− and KS− (Stratagene) that had been digested with *Xba*I and *Sst*I (all enzymes BRL). Single strands were packaged prior to sequencing. Two independent PCR products were subcloned and sequenced in each case.

RACE (Rapid Amplification of cDNA Ends; FROHMAN *et al.* 1988) was performed using a 5′ RACE System kit (BRL) according to the manufacturer's instructions. The primer used for first strand synthesis was MMA1 (sequence shown above) and the nested primer used was MMA5 (5′-GGAGCATAGGAATTCTTGAGTGATTCC-3′; the underlined base indicates a single mismatch that introduces an *Eco*RI site). The initial RACE product was reamplified using DHA16 (5′-TACTAGGCCACGCGTCGACTAGTA-3′; this primer contains part of the sequence found in the UAP primer supplied with the kit) and MMA5. The single 300-bp product was purified, digested with *Eco*RI (BRL) and *Sal*I (Pharmacia), and cloned into similarly digested pBluescript II SK− for sequencing. Multiple clones of two independent products were sequenced.

Worm and yeast plugs were prepared and handled as described (BIRREN and LAI 1993). Restriction digestions were performed using *Not*I (BRL) after treatment of plugs with 100

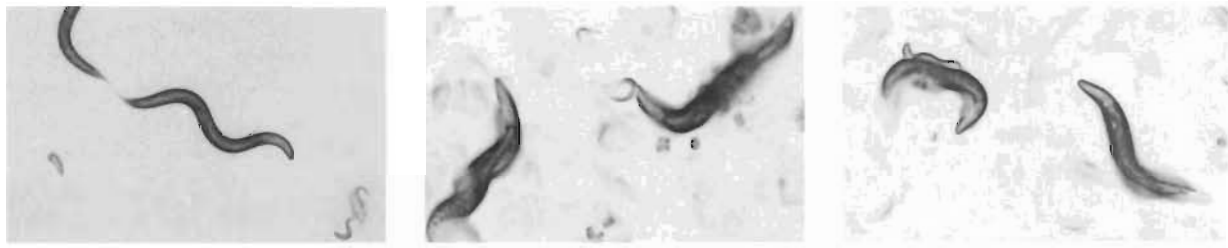

FIGURE 1.—Photographs of wild-type (N2), *unc-119(e2498)* and *eDf2; eDp6* animals. The leftmost panel shows an L4 animal displaying the characteristic sinusoidal wave during motion. The other panels show the similarity in *unc-119(e2498)* and *eDf2; eDp6* adults. The mutant animals are severely uncoordinated and tend to curl. Mutants also show a slight "dumpy" morphology. All photographs are at the same magnification; the L4 animal in the leftmost panel is approximately 1 mm long.

mM PMSF (Sigma). Pulsed-field gels were electrophoresed in 1% agarose (BRL) in a Hex-A-Field CHEF gel apparatus (BRL) for 16 hr at 150 V with a switching time of 60 sec. Gels were run in 0.5× TBE running buffer cooled by circulation through a glass coil immersed in a cooling bath set to 8°. YAC DNA was purified by running 1% low gel temperature agarose gels (BRL) and cutting out the correct band after staining in 200 ml of 0.5 μg/ml ethidium bromide in 0.5× TBE for 30 min at room temperature and visualizing bands under 254-nm light using a transilluminator. Agarose was removed using GELase (Cedarlane Laboratories) as per the instructions accompanying the enzyme.

Microscopy and *C. elegans* transformation: Generation of transgenic animals by microinjection of DNA into worm gonads was done as described (MELLO *et al.* 1991), using a Zeiss Axiovert microscope equipped with DIC optics. The plasmid pRF4, which contains the dominant mutation *rol-6(su1006)*, was used as a transformation control. Animals transgenic for the *unc-119::lacZ* fusion were fixed and stained for β-galactosidase activity as described (FIRE *et al.* 1990).

Electron microscopy was performed on a Philips EM201 transmission electron microscope. Embedding was performed with Epon after fixation with glutaraldehyde and osmium tetroxide as described (WOOD 1988). One hundred nanometer sections were cut using a Reichert OMU2 ultramicrotome using glass knives prepared on an LKB Knifemaker II. Sections were collected on 300-mesh copper grids.

RESULTS

Identification and mutant phenotype of *unc-119*: The *e2498* mutation arose spontaneously as a strong uncoordinated animal among the progeny of a cross between two nematode strains, Bristol (N2) and Bergerac (BO) during a search for strain polymorphisms on chromosome *III* (PILGRIM 1993). Interstrain crosses of this type are known to increase mobility of the transposable element Tc1 (MORI *et al.* 1990). The Unc phenotype was completely recessive, visible from hatching and remained strong after backcrossing 10 times to N2. Older *e2498* animals are slightly dumpier in shape than wild type, and while they can forage with their heads and appear to respond to touch, mutants usually tend to remain in one position on the plate, often forming a coil or limp "C' shape (Figure 1). When locomotion is assayed by scoring sinusoidal waves per minute in liquid (see MATERIALS AND METHODS), mutants cannot complete more than 10 body bends per minute, as compared with wild type, which can complete more than 100 (Figure 2). When exposed to levamisole (an acetylcholine agonist), both wild-type animals and homozygous *e2498* hypercontract, suggesting that acetylcholine receptors in *e2498* are normal (LEWIS *et al.* 1980). This also suggests that the defect in *unc-119* mutants lies in

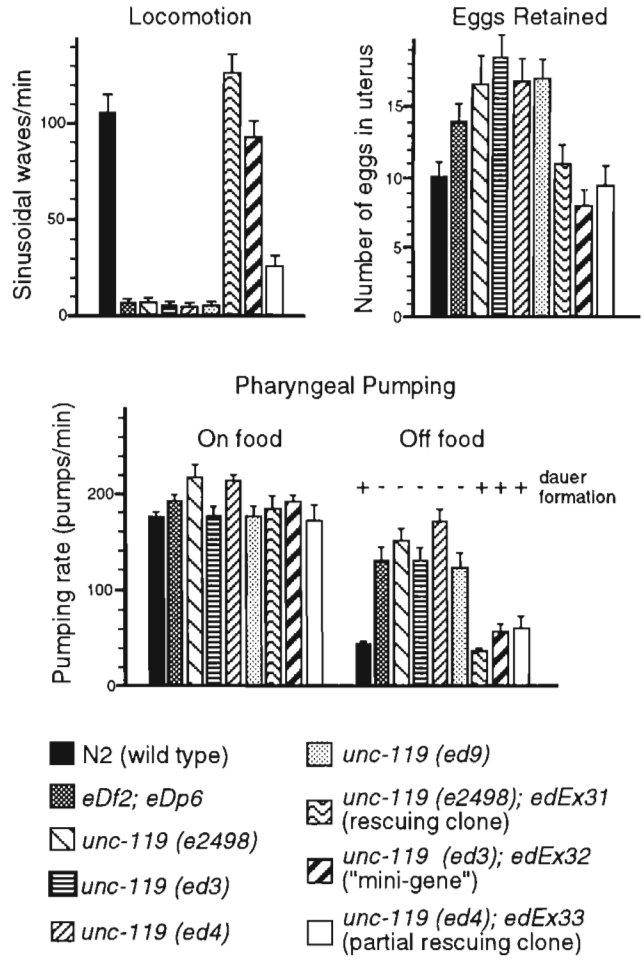

FIGURE 2.—Quantitation of defects in wild-type, *unc-119* homozygous mutants and homozygous *unc-119* animals carrying the putative *unc-119* wild-type gene (or portions thereof) on transgenic arrays. In the third histogram, dauer-forming ability is indicated above the "off food" bars as either + or −. Bars show the standard error of the mean (SEM). Data collection is described in MATERIALS AND METHODS.

the nervous system rather than the muscle system. Males homozygous for *e2498* are similarly paralyzed, but show normal bursa morphology and spicule movement under DIC optics. Hermaphrodites are still capable of self-fertilization and give reduced brood sizes about 50% of wild type, and the development time from embryo to adult is not affected.

Further evidence as to a role in the nervous system comes from analysis of neuron-mediated traits as shown in Figure 2. Under overcrowded conditions, or in the presence of dauer pheromone, wild-type nematodes can enter the dauer state, a developmentally arrested and morphologically distinct alternative third larval stage (CASSADA and RUSSELL 1975). *unc-119* mutants cannot form such larvae as assayed by testing starved plates for SDS-resistance (see MATERIALS AND METHODS). We tested for the persistence of this dauer-forming defect (Daf-d phenotype) in combination with *daf-7(e1372ts)* and *daf-11(m47ts)*, two mutations that cause constitutive dauer formation at 25° (a Daf-c phenotype; RIDDLE *et al.* 1981). *unc-119* is able to suppress the Daf-c phenotype of *daf-11* as *unc-119(e2498); daf-11(m47ts)* animals are Daf-d at 25°. However, *unc-119(e2498); daf-7(e1372ts)* animals are Daf-c at 25°, suggesting that the Daf-d phenotype of *unc-119* mutants does not result from a physiological inability to enter dauer.

Other behaviors are similarly affected. When shifted from a bacterial lawn to an unseeded plate, wild-type animals will dramatically slow the rate of pharyngeal pumping (AVERY and HORVITZ 1990). In contrast, while the rate of pumping is similar to wild type in the presence of food, *e2498* animals continue to pump their pharynxes off food at a moderately high rate, albeit somewhat reduced. Wild-type animals also cease egg laying in the absence of food (AVERY and HORVITZ 1990) and on average retain a consistent number of eggs (SÉGALAT *et al.* 1995). *unc-119* mutants tend to retain more embryos than wild type, and when they do lay eggs, they can do so in the absence of food, likely owing to increased pressure inside the uterus. Constitutive pharyngeal pumping, an egg laying defect and an apparent inability to enter dauer all suggest that *unc-119* has a role in the nervous system.

To rule out defects in musculature, *unc-119(e2498)* adults were fixed and sectioned for transmission electron microscope analysis of body wall muscle structure. Muscle ultrastructure, placement of sarcomeres and attachment to the nematode body wall appear normal. Mutant animals were also examined under polarized light, which can reveal defects in musculature (EPSTEIN and THOMSON 1974), as well as with indirect immunofluorescence with an anti-vinculin antibody, which stains the thin filaments. In all muscle characteristics examined, no differences were seen compared to wild-type controls (data not shown).

Mapping of *unc-119*: *e2498* was mapped to chromosome *III* using the multiply marked mapping strain DA438 and positioned with respect to several known

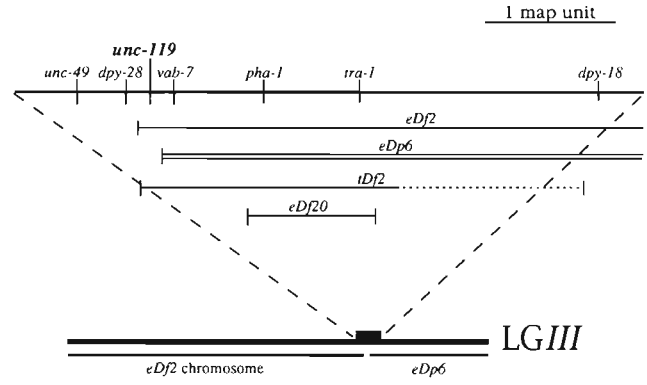

FIGURE 3.—Genetic map of a portion of chromosome *III* (right end), expanded to show the *unc-49* to *dpy-18* interval (EDGLEY and RIDDLE 1993). A subset of the genetic markers in the region is shown. Chromosomal rearrangements are indicated below the genetic map, deficiencies as single lines, and duplications as double lines. The left and right breakpoints of *eDf20* have been physically mapped relative to *pha-1* and *tra-1* (HODGKIN 1993; GRANATO *et al.* 1994). As is discussed in the text, the portion of chromosome *III* retained in *eDf2* is shown as "*eDf2* chromosome."

markers in the area (see MATERIALS AND METHODS). The new mutation is between *sma-2* and *dpy-18*, and is almost inseparable from *vab-7*. *e2498* is not complemented by either deficiency *eDf2* or *tDf2* (SCHNABEL and SCHNABEL 1990) and is not contained in the free duplication *eDp6*, although all other known genes in the area are complemented by either the *eDf2* chromosome or the *eDp6* duplication. This places *unc-119* just to the left of *vab-7*, and to the right of *dpy-28* (Figure 3). Since there were no known *unc* genes at this position, the mutation was assigned to a new gene, *unc-119*. J. HODGKIN noticed that the phenotype of *unc-119* homozygotes was similar to that of strain CB1517, which is homozygous for *eDf2* and *eDp6*. This suggests that *unc-119* is at least partially deleted in CB1517. *eDf2* and *eDp6* are chromosome *III*-derived aberrations obtained concomitantly after acetaldehyde mutagenesis (HODGKIN 1980). Presumably the mutagenesis created a chromosomal break at the site, which healed to allow *eDf2* and *eDp6* to segregate independently from one another. Since *C. elegans* chromosomes are mitotically holocentric (ALBERTSON and THOMSON 1982), both products of such a rearrangement can be maintained. *eDf2* and *eDp6* can be seen as bivalents using fluorescent *in situ* hybridization (ALBERTSON 1993). CB1517 animals are viable and otherwise healthy, although they do show reduced brood sizes due to meiotic loss of *eDp6*. Since the phenotypes of *e2498* and *eDf2; eDp6* are almost indistinguishable (see Figures 1 and 2), *unc-119* may be the only gene affected in these animals.

Three new alleles of *unc-119* (*ed3*, *ed4* and *ed9*) were recovered in the F_1 progeny of EMS-mutagenized animals heterozygous for *tDf2*. These new alleles arose at a forward mutation frequency of 2×10^{-5}. This figure is approximately 10-fold lower than the expected value for an average-sized gene in *C. elegans* (BRENNER 1974),

suggesting that either the gene is small or that few alleles are viable or have a visible phenotype. The new alleles are completely recessive, fail to complement *e2498*, *eDf2* or *eDp6* and are phenotypically similar to *e2498* (see Figure 2). Homozygotes are indistinguishable from hemizygotes, when *unc-119* is placed opposite the deficiency *tDf2*, suggesting the alleles may be null.

Cloning of *unc-119*: Since *unc-119(e2498)* was derived from a dysgenic cross, it seemed possible that it was due to the insertion of a transposable element into a single gene. *mut-6(st702) IV*, a presumed transposase source that causes high rates of mobilization of the transposable element Tc1 (MORI *et al.* 1990), was introduced into a strain carrying *e2498*, in an attempt to recover revertants due to transposon excision. Homozygous *unc-119(e2498); mut-6(st702)* animals were cultured for several generations, and wild-type animals were found on a single plate. These wild-type animals segregated wild-type and *unc-119* progeny, and the suppressor phenotype was mapped to the *unc-119* gene (data not shown). The putative revertant, *unc-119(e2498ed5)*, when homozygous, is indistinguishable from wild type and does not resegregate the *unc-119* phenotype, suggesting that reversion was intragenic, and may have been due to precise excision of a transposon insertion. Although "transposon tagging" has been used to isolate other Unc genes in *C. elegans* (MOERMAN *et al.* 1986), we were unable to detect an allele-specific polymorphism on Southern blots of *unc-119(e2498)* genomic DNA probed with Tc1 or any other transposable element, due to the background of other Tc1-hybridizing bands (data not shown).

The genome of *C. elegans* has been fractionated into overlapping YAC and cosmid clones, and a physical map of the genome has been assembled (COULSON *et al.* 1986, 1988, 1991). From the physical map, four YACs and several cosmid clones were selected for further analysis by their proximity to the cloned loci *tra-1* and *pha-1* (HODGKIN 1993; GRANATO *et al.* 1994). Since it was assumed that *unc-119* is disrupted or absent from both the *eDf2* deficiency and the *eDp6* duplication, and the breakpoints of the two rearrangements lie just to the left of *vab-7*, mapping the breakpoints of the two rearrangements should define the sequences necessary for *unc-119* function. T. BARNES suggested to us that Southern blots prepared from genomic DNA of strains carrying varying numbers of the duplications and deficiencies would reveal, by dosage, whether or not a clone was to the left or right of *unc-119* (see Figure 4). As judged by dosage on such blots, cosmids K02B6 and T27G1 contain DNA to the left of *unc-119* and T19F2, W06D6, W09D6, M02H11 and W03F10 lie to the right. None of these cosmids apparently contain DNA from both *eDf2* and *eDp6*.

The YAC clones were used for long-range restriction mapping of the genomic region. All the YACs overlap with a 300-kbp *Not*I fragment in genomic DNA from the wild-type N2 strain, as determined by cross-hybridization of the cosmid clones to *Not*I-digested YAC and worm DNA (Figure 5). By correlating the size of the cross-hybridizing *Not*I fragments in the YACs with the size of the YACs themselves, these clones were positioned to within 10 kbp. The DNA from a strain homozygous for both *eDf2* and *eDp6* contains an *eDf2*-specific *Not*I fragment on Southern blots that is larger than the N2 fragment, demonstrating that extra DNA, perhaps telomeric, has been added beyond the breakpoint. The size of the *eDp6*-specific *Not*I fragment implies that if the left end of the fragment is the actual end of *eDp6* itself, very little DNA has been added.

Based on the restriction map evidence of the YAC clones tested, only Y60D9 contains DNA from both *eDf2* and *eDp6*. Fluorescent *in situ* hybridization (FISH) has shown that the YAC clone Y39A1 cross-hybridizes to the meiotically visible *eDf2* bivalent and the *eDp6* chromosome (ALBERTSON 1993), implying that this YAC clone must contain *unc-119* and suggesting that its right endpoint is in between the leftmost *eDp6*-hybridizing cosmids including T19F2 (since all of the *eDp6*-containing cosmids fail to cross-hybridize Y39A1) and the breakpoint itself. Plasmid subclone libraries were constructed from Y39A1 and Y60D9 DNA after enrichment for DNA from these YACs by pulsed-field gel purification. Clones were tested for worm specificity by cross-hybridization against "spot blots" of genomic DNA from the *S. cerevisiae* strain N123 (which contains no YACs), Y42D3 (a YAC from the left of chromosome *III*), the other YACs shown in Figure 5 and wild-type *C. elegans* genomic DNA. Of the first 20 insert-containing clones picked from the library, four were found to be worm-specific and contained in Y39A1 and Y60D9 alone (data not shown). One subclone, pDP#MM008, was used to probe Southern blots of genomic digests of N2, BO, *unc-119(e2498)*, CB1517 [*eDf2; eDp6*] and the revertant *unc-119(e2498ed5)* (see Figure 6). This clone detects a 4.5-kbp *Sst*I fragment in N2 and BO genomic DNA (these are the parent strains of the original *e2498* mutation) and a larger, 6.1-kbp fragment in *e2498*. The revertant allele *e2498ed5* contains the normal 4.5-kbp band, consistent with pDP#MM008 being an *unc-119* specific clone, and that *unc-119(e2498)* resulted from the insertion of a 1.6-kbp fragment, presumably the transposable element Tc1 (EMMONS *et al.* 1983; LIAO *et al.* 1983). The larger pDP#MM008 cross-hybridizing fragment from *unc-119(e2498)* was subcloned and shown to contain Tc1 by its ability to hybridize to a Tc1-specific probe, by a partial sequence of the insertion site, and liberation of the 1.6-kbp insertion by *Eco*RV, which digests in the Tc1 terminal repeats (ROSENZWEIG *et al.* 1983). This Tc1 element contained a *Hin*dIII restriction site, which is unusual in the Bristol strain, but has been seen in Tc1 elements isolated from the Bergerac strain of *C. elegans* (ROSE *et al.* 1985), consistent with the isolation of *e2498* from a Bristol/Bergerac hybrid. Furthermore, the pDP#MM008 clone faintly cross-hybridizes to a much larger 9-kbp *Sst*I fragment from CB1517, suggesting that

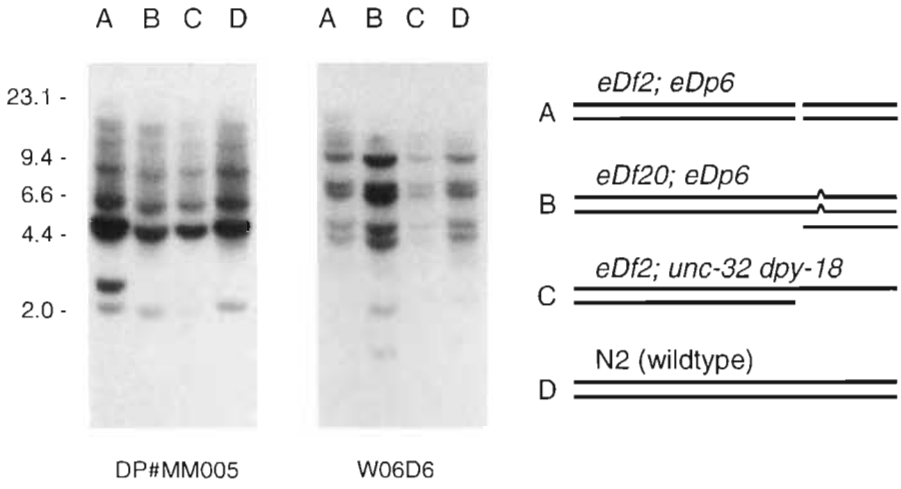

FIGURE 4.—Southern analysis of *Hin*dIII digests of strains containing varying copy numbers of rearrangements affecting the right end of chromosome *III*, and representation of chromosome *III* for each of the strains used. The same Southern blot was probed with two different clones, pDP#MM005, which is contained on the *eDf2* chromosome, and W06D6, which is contained on *eDp6*. In the right hand photograph, much more intense cross-hybridization is seen in lane B than in lane C due to the presence of more *eDp6* DNA. Approximate sizes (in kbp) are shown on the left.

pDP#MM008 contains DNA that is largely absent in this strain, and that pDP#MM008 identifies the site where breakage occurred to generate *eDf2*. At this point, we became aware of the placement of a new cosmid, M142, on the physical map in the region between T27G1 and T19F2; this cosmid contains the entire *vab-7* gene (J. AHRINGER, personal communication). M142 was found to contain sequences that hybridize to pDP#MM008 and was used to extend overlapping clones to the right. The breakpoints of *eDf2* and *eDp6* were placed on the physical map by cross-hybridization to the subclones in the region, and it was determined that strain CB1517 is missing approximately 8 kbp of DNA that is present in N2 (see Figure 7).

In order to verify that this region also contained the *unc-119* gene, cosmid M142 DNA was injected into the gonads of *unc-119(e2498)* hermaphrodites. This technique allows a small proportion of the progeny to inherit the transgenic DNA (FIRE 1986; MELLO *et al.* 1991). The cosmid was injected along with plasmid pRF4, which contains the dominant *su1006* allele of the collagen gene *rol-6*, which allows transformants to be scored by their characteristic rolling phenotype (MELLO *et al.* 1991). Rescue of the mutant phenotype was obtained with M142 and smaller subclones, which defined a minimum rescuing region of 5.5 kbp (see Figure 7). While rescue was initially only scored as restoration of wild-type movement and body shape, transmitting lines were also found to be rescued for all other traits tested (see Figure 2). One of the smaller subclones, pDP#MM019, was able to restore partial movement to homozygous *e2498* animals, such that the *rol-6* rolling phenotype was much clearer than in *unc-119* mutants transgenic for pRF4 alone, but the animals were still partially defective in movement, and slightly dumpy, although they could form dauer larvae. This suggests that partial *unc-119* function is present in this subclone. A smaller subclone and a clone from outside the *unc-119* region (not shown in the figure) failed to rescue. Clones which were able to rescue *e2498* were also able to rescue the *ed3*, *ed4* and *ed9* alleles. When the *unc-119*-rescuing subclones were injected into animals of strain CB1517 [*eDf2; eDp6*], they were able to restore normal movement, consistent with the model that *unc-119* is the only gene affected by this chromosomal aberration.

***unc-119* cDNAs:** The partial-rescuing clone pDP-

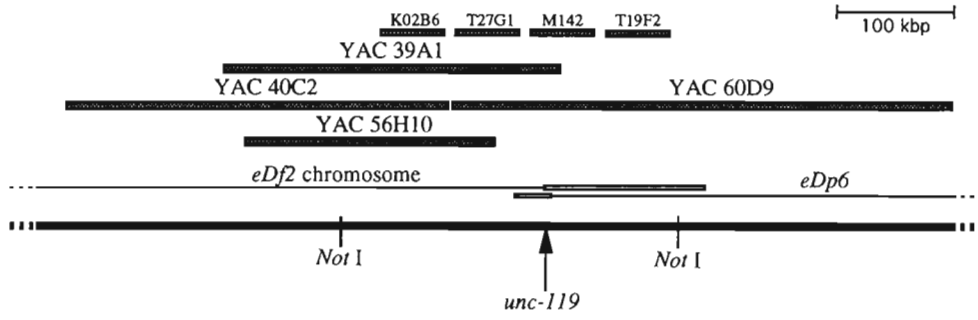

FIGURE 5.—Physical map of the *unc-119* region, showing yeast artificial chromosome (YAC), and cosmid clones. The physical map has the same alignment as the genetic map in Figure 3. *Not*I restriction enzyme recognition sites are indicated. The alignment was determined by YAC:cosmid cross hybridization, as well as long-range restriction mapping. The open boxes on the end of *eDf2* and *eDp6* indicate the presence of DNA, suggested by the size of the *Not*I fragment on pulsed-field gels, which fails to cross-hybridize with the cosmid clones directly above. YAC clone 39A1 has been previously shown to hybridize to both *eDf2* and *eDp6* DNA (ALBERTSON 1993). The boxes below the cosmid names do not indicate their size, but are meant solely to show the region of cross-hybridization. The cosmids W06D6, W03F10, M02H11 and W09D6, which map to the right of *unc-119* and only cross-hybridize to Y60D9 (see text) are not shown on this figure since they were not tested for cross-hybridization to the 300-kbp *Not*I fragment.

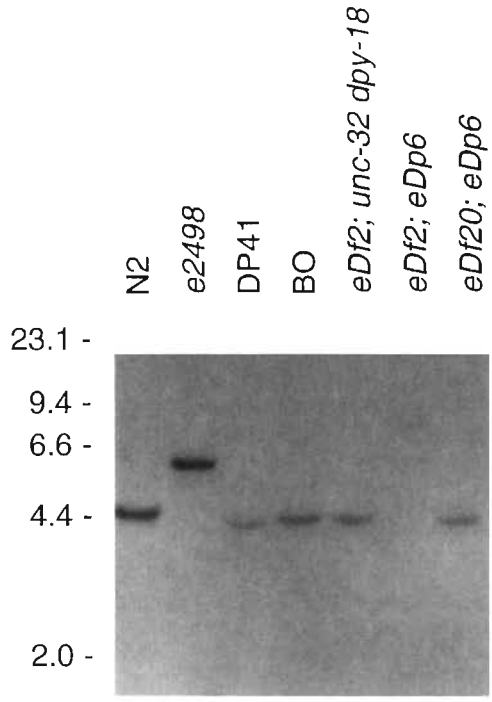

FIGURE 6.—Southern blots of *Ssl* genomic digests of N2, BO, *unc-119(e2498)*, CB1517 [*eDf2; eDp6*] and the revertant DP41, probed with pDP#MM008. The common band in N2, DP41, BO, *eDf2; unc-32 dpy-18* and *eDf20; eDp6* is approximately 4.5 kbp in size. The larger band in *e2498* is due to the insertion of the 1.6-kbp Tc1 transposable element. There is a larger, faint band in the *eDf2; eDp6* lane, consistent with a rearrangement of DNA in pDP#MM008, and the absence of DNA from this clone.

#MM019 was used to screen a mixed-stage cDNA library. Three positive clones were obtained, all of which had identical 3′ ends. The longest cDNA appears to be nearly complete, since it extends in the 5′ direction beyond the end of the minimal rescuing region, and beyond the largest open reading frame (ORF). The size of the predicted message is 1000 b. The intron/exon arrangement of the largest cDNA is shown in Figure 7, and the sequence of the cDNA and genomic DNA is given in Figure 8. The first ATG in the cDNA does not appear to be used; it precedes both a 33-bp region that is 85% A-T rich, and the sequence 5′-TAGTTAGTTAA-3′, which encodes stops in all three frames. The sequence of genomic DNA spanning the rescuing region, and extending upstream of the 5′ end of the longest cDNA, was determined in parallel. Some of the genomic sequences were also determined, and communicated, by the *C. elegans* Genome Sequencing consortium (WILSON *et al.* 1994). There is no further potential coding information in the 650 bp 5′ to the first base of the longest cDNA. The predicted transcript contains five exons spanning 3.6 kbp of genomic DNA. The sequences of 5′ RACE products (see MATERIALS AND METHODS) contain one more base than the longest cDNA, suggesting that the longest cDNA is full-length, and that the transcript is not trans-spliced to SL1 or SL2 (KRAUSE and HIRSH 1987; HUANG and HIRSH 1989).

The sequences of the cDNA clones predict a single open reading frame (ORF) in the rescuing region. This ORF predicts that *unc-119* encodes a 219 amino acid protein. Search of the protein databases using the

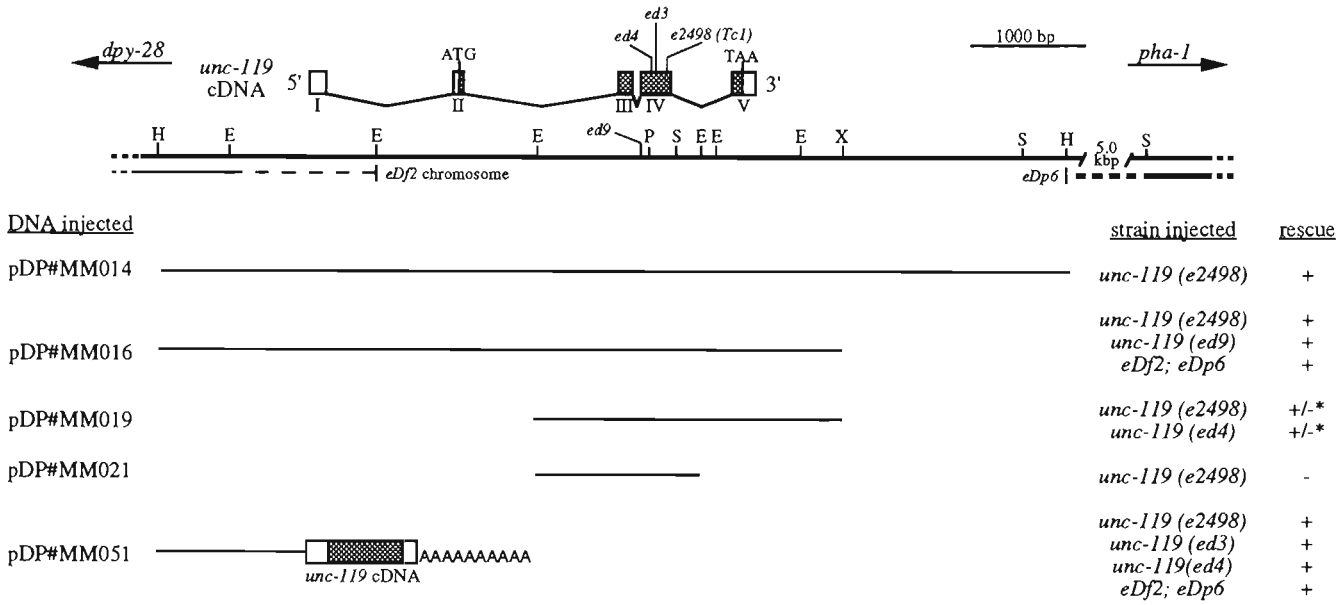

FIGURE 7.—Short-range physical map showing the genomic DNA in the *unc-119* region. The cDNA is shown above the genomic restriction map (the ORF is shown as shaded), while the limits of the breakpoints of the *eDf2* chromosome and *eDp6* are shown below. The rescuing ability of various clones from the region is shown. +, complete rescue of the mutant phenotype; +/-, partial rescue; and −, failure to rescue (see Figure 2). The pDP#MM051 clone is a "minigene" constructed by ligating the upstream genomic DNA to the full-length cDNA at an *Ase*I site in exon I (see text). The sites of the Tc1 insertion in *e2498* and the nonsense mutations in *ed3* and *ed4* are shown in the cDNA. The site of the change in *ed9* is shown above the genomic DNA. The fusion to the *lacZ* vector pPD22.04 was made at the *Pst*I site (see text). H, *Hin*dIII; E, *Eco*RI; S, *Ssl*; P, *Pst*I; X, *Xba*I. *Eco*RI and *Pst*I sites to the right of the *Xba*I site have not been determined and so are not shown.

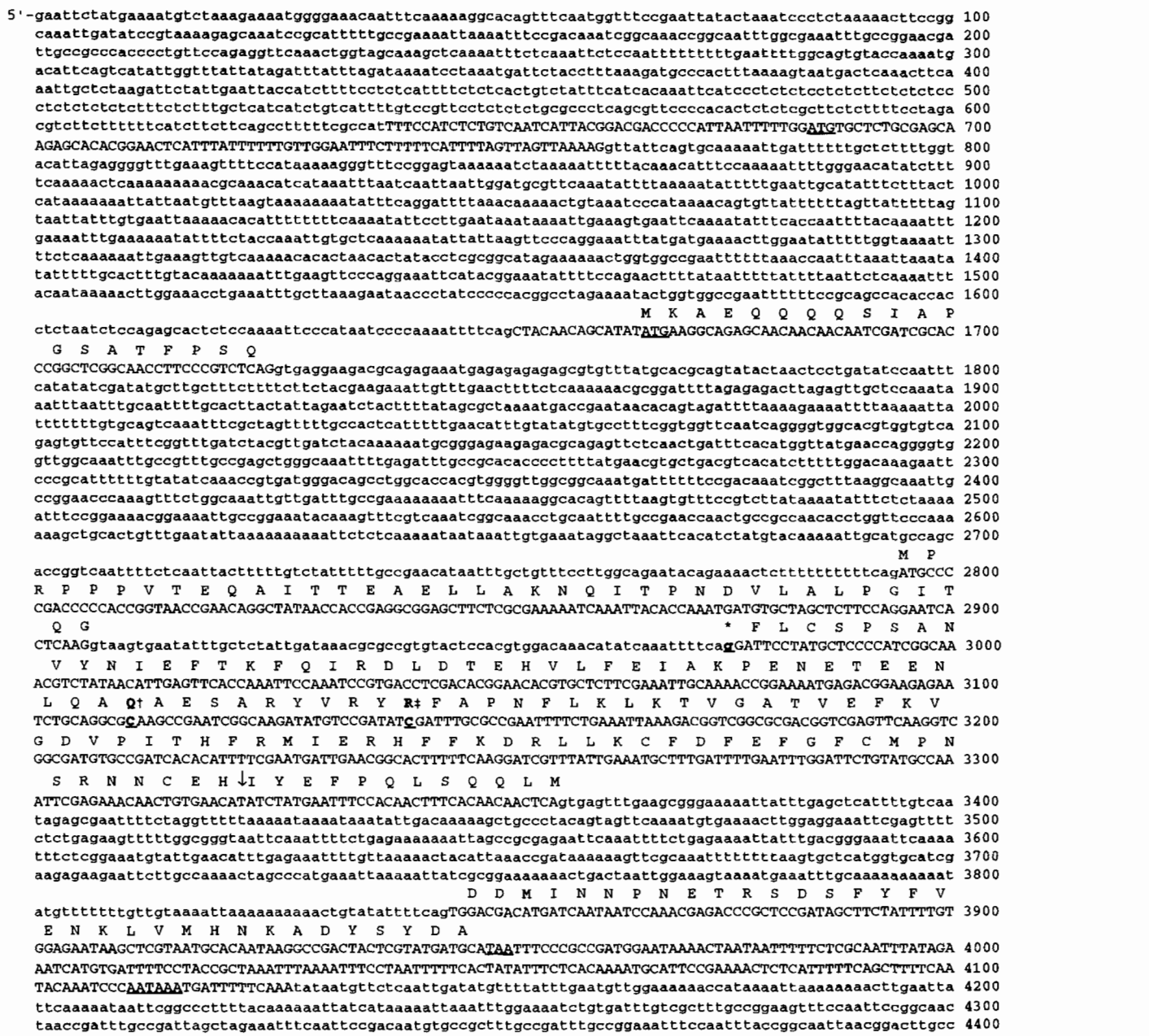

FIGURE 8.—Nucleotide sequence and predicted amino acid sequence of the *unc-119* locus. cDNA sequences are shown in capital letters, and the predicted amino acid sequence is denoted by single-letter abbreviations above the cDNA sequence. The first (unused) and second ATG are underlined, as are the stop codon and the putative polyadenylation site. The sequence has been submitted into GenBank (Accession No. U32854). The sites of the mutations are indicated as follows: *, *ed9* [splice acceptor sequence 5′-. . .AG-3′ → 5′-. . .AA-3′]; †, *ed4* [CAA(gln)→ TAA(ochre)]; ‡, *ed3* [CGA(arg)→TGA(opal)]; ↓, *e2498* [Tc1(Hin) insertion 5′-. . .GAACA(Tc1)TATCT. . .-3′].

BLAST protocol (ALTSCHUL *et al.* 1990) detected no significant similarity to other proteins in the databases, other than to C27H5.1, an unidentified ORF in *C. elegans* predicted by the genome sequencing project (GenBank Accession No. U14635; WILSON *et al.* 1994). A 92 amino acid region shows 26% identity and 51% similarity between C27H5.1 and UNC-119. The other nematode homologue maps to chromosome *II*, to the right of the cloned genes *tra-2* and *unc-104*. Although there are several mutations which map to the general location of this ORF, no mutant phenotype has been specifically correlated to the predicted gene.

It was not unexpected to find a cDNA that extended beyond pDP#MM019, which only contains exons III to V, since phenotypic rescue was only partial with this clone. This rescue, which occurs without the presumed *unc-119* promoter, may be possible through low-level constitutive expression of the genes in the extrachromosomal DNA. Transgenic DNA frequently undergoes extensive rearrangement and is present in many copies

as a single array (MELLO *et al.* 1991). There is an in-frame ATG at the start of exon III, which may permit translational initiation of a truncated form of UNC-119 to produce a polypeptide containing the carboxy terminal 196 of 219 amino acids.

In order to verify that *unc-119* had been cloned, oligonucleotide primers were designed that would allow PCR amplification of the majority of the coding region (see MATERIALS AND METHODS). Genomic DNA from *ed3*, *ed4*, *ed9* and the revertant allele *e2498ed5* was PCR amplified and the products were subcloned. This allowed sequencing of 25 bp of exon III and all of exon IV. Both of the EMS-induced alleles *ed3* and *ed4* contain single C to T transitions in exon IV that create in-frame stop codons. The allele *ed9* contains a G to A transition in the intron just preceding exon IV, which changes the splice acceptor sequence from 5′-TTTCAG-3′ to 5′-TTTCAA-3′. Since intron splice acceptors in *C. elegans* are invariant for the last two bases of this sequence (WOOD 1988), this change is expected to disrupt normal processing of the primary transcript. The revertant *e2498ed5* appears to be the result of a perfect excision event of Tc1, as no base changes from wild type were found. Since we did not check the sequences of *ed3*, *ed4* and *ed9* upstream of the last 25 bp of exon III, we cannot rule out additional base changes in the upstream regions in these mutants.

To test whether the cDNA sequences were sufficient to rescue the phenotype, the longest cDNA clone was fused at the *Ase*I site in the first exon with a 1.2-kbp genomic DNA fragment containing the upstream region to the *Hin*dIII site (Figure 7). Arrays carrying this "mini-gene" (pDP#MM015) were able to restore all aspects of the mutant phenotype in the F_1 progeny when injected into strains homozygous for each of the mutant alleles of *unc-119* (rescue of *ed3* is shown in Figure 2), consistent with the requirement of only one mRNA from this region for *unc-119* function.

Expression of *unc-119*: In order to determine the pattern of expression of *unc-119*, a fragment containing the 5′ half of the *unc-119* coding region and 1 kbp of DNA upstream of the 5′ end of the longest cDNA was fused in-frame with the *lacZ* gene encoding *E. coli* β-galactosidase, using the *C. elegans lacZ* expression vector pPD22.04 (FIRE *et al.* 1990). The portion of *unc-119* in the fusion construct does not encode enough of the protein to rescue, since the mutations in *ed3* and *ed4* are further toward the carboxy-terminus than the fusion junction. The *unc-119::lacZ* fusion construct was transformed into wild-type animals along with the dominant *rol-6* marker. Animals from mixed-stage plates of a strain with approximately 50% meiotic transmission (as determined by the presence of the "Rol" phenotype in the progeny) were fixed and stained with both DAPI and X-gal.

The fusion enables staining of many neurons in larval and adult animals (see Figure 9). The transgene is present as an extrachromosomal array, and is sometimes lost during mitosis, resulting in a number of animals

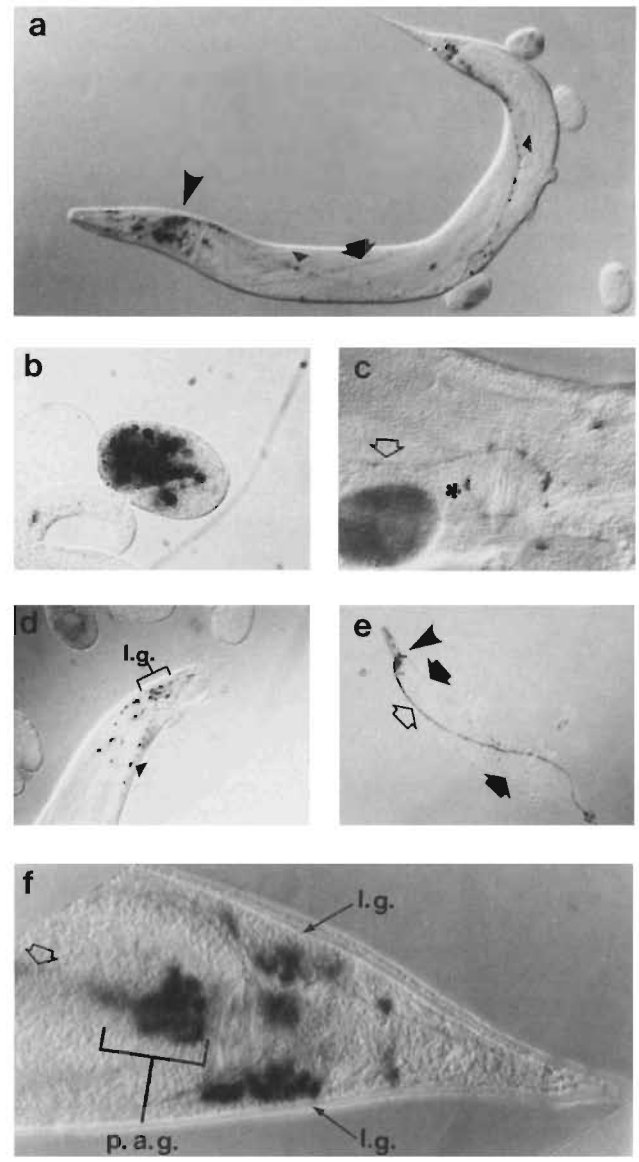

FIGURE 9.—X-gal staining of fixed *unc-119::lacZ* transgenic animals. (a) Adult hermaphrodite, showing ventral nerve cord cell bodies, the dorsal nerve cord and the nerve ring. Anterior to the nerve ring, staining can be seen in the pharynx. (b) Comma stage embryo. (c) Ventral nerve cord near the hermaphrodite vulva. An asterisk (*) indicates a VC neuron cell body, above and to the right of a developing embryo. (d) Adult male tail showing cell bodies in the posterior part of the lumbar ganglion. (e) Adult hermaphrodite, showing staining in the dorsal and ventral nerve cords and the nerve ring. (f) Magnification of posterior part of animal in e; a ventral view showing the pre-anal and lumbar ganglia. All animals result from a strain carrying the transgene extrachromosomally and may be mosaic for the transgene due to mitotic loss. ⇦, ventral nerve cord; ⇨, dorsal nerve cord; ▲, cell bodies in ventral nerve cord; ▲, nerve ring; l.g., lumbar ganglion; p.a.g., pre-anal ganglion.

that are mosaic for the transgene, and stain in only a subset of cells. Although the *unc-119::lacZ* fusion contains a nuclear-localizing signal (NLS; see FIRE *et al.* 1990), several hours after fixation and treatment with X-gal, staining begins to appear in axons and process bundles. Staining is seen in embryos with as few as 60

cells based on the counting of DAPI-stained nuclei. By the time development has reached "comma" stage, about 400 min after fertilization, staining appears localized largely to the anterior portion of the embryo (Figure 9b). After hatching, and continuing through larval and adult development, staining is visible in both the dorsal and ventral nerve cords and the nerve ring (Figure 9, a and e). Cell bodies of neurons which innervate the vulva can be seen in Figure 9c. Differences in staining can be seen near the tail between males and hermaphrodites, consistent with expression in lumbar ganglial cells (Figure 9d), where particular neurons develop to innervate the tail rays in the male (SULSTON *et al.* 1980). In the hermaphrodite tail, staining is seen that strongly resembles the positions of neuronal cell bodies in the pre-anal and lumbar ganglia (Figure 9f; SIDDIQUI and CULOTTI 1991). There is also some evidence of expression outside of the nervous system; in Figure 9a, staining can be seen anterior of the anterior pharyngeal bulb, where no neuronal cell bodies are found (WHITE *et al.* 1986).

DISCUSSION

We have identified a new gene, *unc-119*, which appears to be involved in neuron function or development in *C. elegans*. The DNA encoding UNC-119 has been identified on several sets of evidence. First, homozygous *unc-119* animals carrying the presumptive coding region (either an intact genomic DNA fragment, or a promoter:cDNA chimera) as a transgene are phenotypically normal. Second, strains which demonstrate the *unc-119* mutant phenotype contain sequence changes that are predicted to cause loss-of-function mutations in the predicted protein (*ed3* and *ed4*) or impede the production of a normal message (*e2498* and *ed9*). We have also shown that *unc-119* is nearly completely deleted in *eDf2; eDp6* and is likely the only gene affected. Therefore, an intact coding region is necessary, and sufficient, for UNC-119 function.

The observation of partial phenotypic rescue by a truncated *unc-119* gene suggests that specific promoter or expression sequences that may lie upstream of the gene are not essential. It is possible that rescue is accomplished by low level constitutive expression from many tandem copies. Partial rescue of the *unc-51* mutant phenotype by transgenic DNA containing only minimal upstream sequences has been reported (OGURA *et al.* 1993), but in that case the coding region was supposedly intact. The protein sequence predicted from the pDP#MM019 subclone is missing the first in-frame ATG, suggesting that either this ATG is not used for initiation *in vivo*, or the first 20 amino acids of UNC-119 are not essential for function, and that partial rescue with pDP#MM019 is due to lower amounts of expression.

The *unc-119* mutant phenotype is most apparent in its lack of movement, as mutants lack coordinated locomotion from hatching through adulthood. There is indirect evidence that this defect results from a disruption in neuron placement or function. Examination of muscle by EM, polarized-light microscopy and antibodies to thin filaments show no structural defects, and since mutants can hypercontract after exposure to levamisole, at least partial muscle function remains.

More compelling evidence of a role for UNC-119 in the nervous system comes from the defective ability of homozygous *unc-119* animals to respond to chemical signals in their environment. Although *unc-119* animals can move, albeit slowly, they do not appear to recognize a change in the abundance of food, as their egg laying, locomotory and pharyngeal responses do not change appropriately. This is best reflected in the inability of *unc-119* animals to form dauer larvae, similar to what is seen in *daf-10* mutants, which are dauer-defective (Daf-d) due to a chemosensory abnormality (ALBERT *et al.* 1981). This is the opposite phenotype of dauer-constitutive (Daf-c) mutants such as *daf-7* and *daf-11*, which inappropriately enter dauer in the presence of food (RIDDLE *et al.* 1981). The observation that *unc-119* can suppress the Daf-c phenotype of *daf-11* but not *daf-7* is consistent with the placement of *unc-119* in the pathway for dauer formation, at a position downstream of *daf-11* but independent of *daf-7*, at the same step as *daf-10* and other genes implicated in chemosensation (THOMAS *et al.* 1993). This suggests that mutants have an inability to recognize the absence of food, which would also explain the maintenance of pharyngeal pumping on removal from the bacterial lawn. Since a defect in chemosensation alone is insufficient to explain the mobility defect in *unc-119* animals (ALBERT *et al.* 1981), lack of UNC-119 may affect other aspects of the nervous system. *unc-31*, for example, while having a locomotory defect, also shows constitutive pharyngeal pumping and defects in egg-laying and dauer larvae response, although those defects are different than those seen for *unc-119* (AVERY *et al.* 1993).

Evidence for a direct role in neuron development or function, however, comes from the *in vivo* expression pattern of a reporter gene construct. The 5′ end of *unc-119* fused to the *E. coli lacZ* gene allows the X-gal staining of many neurons. This includes those whose function is not locomotory, such as those innervating the male tail and hermaphrodite vulva. The apparent neuronal defects seen in *unc-119* mutants are consistent with the expression patterns as seen with the *lacZ* fusions. This consistency is both temporal (gene expression beginning in the embryo, and movement defects are seen in L1 larvae immediately after hatching) and spatial (effects on dauer formation and movement consistent with expression in the sensory and motor nervous systems).

If UNC-119 is expressed in a large number of neurons, why is the phenotype of *unc-119* null mutants relatively mild? Perhaps UNC-119 is part of a larger gene family, with redundant function. The similarity of *unc-*

119 to a predicted ORF detected elsewhere in the genome by the genome sequencing project of *C. elegans* supports the idea that other genes may contribute to the same function. However, there is also precedent for a weak phenotype for mutations in well-conserved neuronal proteins. Synaptotagmin is a conserved transmembrane protein expressed in the *C. elegans* nervous system as an abundant protein which appears to be localized in synaptic vesicles (NONET *et al.* 1993). *C. elegans* synaptotagmin is over 70% similar to vertebrate synaptotagmin, and unlike in vertebrates, there appears to be only one gene in nematodes (*snt-1*). However, *snt-1* null mutants are viable, although slow growing, and uncoordinated (NONET *et al.* 1993). More recently, SÉGALAT *et al.* (1995) and MENDEL *et al.* (1995) report the phenotype of worms carrying loss-of-function mutations in the $G\alpha_o$ subunit of the heterotrimeric G-protein. Although this protein is apparently expressed in every neuron in *C. elegans*, in addition to other, non-neuronal cells, and has been thought to play an important role in neuronal development, loss-of-function mutants display a surprisingly subtle phenotype.

A specific role in neuronal development or function is not yet obvious, as the subcellular localization of UNC-119 has not been determined, and a non-nematode counterpart has yet to be identified. Since some Unc genes first cloned in *C. elegans* turn out to have important homologues in other systems, it is possible that *unc-119* may have a fundamentally conserved function in higher organisms.

The expression of UNC-119 may also have use as a general marker for the *C. elegans* nervous system. There have been several reagents reported which allow observation of all (HEKIMI 1990) or a subset of the nervous system in *C. elegans* (HEDGECOCK *et al.* 1985; HOPE 1991; SIDDIQUI and CULOTTI 1991; HAMELIN *et al.* 1992; MCINTIRE *et al.* 1993; MILLER *et al.* 1993; NONET *et al.* 1993; CHEN and LIM 1994), yet only *unc-51* has been reported to allow observation of the nervous system in the developing embryo (OGURA *et al.* 1994). It is possible that *unc-119*::reporter fusions may allow the specific visualization of the process of nervous system development in *C. elegans*, and hence facilitate the recovery and characterization of mutations affecting morphology, number, placement or migration of neurons.

We thank R. BHATNAGAR for technical assistance with electron microscopy and ultramicrotomy; M. GILBERT and D. MOERMAN for help with antibody staining and for donating the anti-vinculin antibody; A. FIRE for donating the *lacZ* vectors; B. BARSTEAD for the λZAP cDNA library; J. HODGKIN for strains and advice and T. BARNES for the dosage-blotting idea. Some strains used in this work were obtained from the Caenorhabditis Genetics Center, which is supported by a contract between the National Institutes of Health National Center for Research Resources and the University of Minnesota. Some of the genomic sequences in the *unc-119* region were kindly communicated prior to general release by the *C. elegans* Genome Sequencing consortium. This work was supported by grants from the Natural Sciences and Engineering Research Council and the Alberta Heritage Fund for Medical Research. The initial identification and characterization of *unc-119* by D.P. took place at the MRC Laboratory of Molecular Biology, Cambridge, UK, where he was supported by a Postdoctoral Research Fellowship from the Human Frontiers Science Program.

LITERATURE CITED

ALBERT, P. S., S. J. BROWN and D. L. RIDDLE, 1981 Sensory control of dauer larvae formation in *Caenorhabditis elegans*. J. Comp. Neurol. **198:** 435–451.

ALBERTSON, D., 1993 Mapping chromosomal rearrangement breakpoints to the physical map of *Caenorhabditis elegans* by fluorescent *in situ* hybridization. Genetics **134:** 211–219.

ALBERTSON, D. G., and J. N. THOMSON, 1976 The pharynx of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **275:** 299–325.

ALBERTSON, D. G., and J. N. THOMSON, 1982 The kinetochores of *Caenorhabditis elegans*. Chromosoma **86:** 409–428.

ALTSCHUL, S. F., W. GISH, W. MILLER, E. W. MYERS and D. J. LIPMAN, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

ANDERSON, P., 1989 Molecular genetics of nematode muscle. Annu. Rev. Genet. **23:** 507–255.

AVERY, L. 1993 The genetics of feeding in *Caenorhabditis elegans*. Genetics **133:** 897–917.

AVERY, L., and H. R. HORVITZ, 1990 Effects of starvation and neuroactive drugs on feeding in *Caenorhabditis elegans*. J. Exp. Zool. **253:** 263–270.

AVERY, L., C. I. BARGMANN and H. R. HORVITZ, 1993 The *Caenorhabditis elegans unc-31* gene affects multiple nervous system-controlled functions. Genetics **134:** 455–464.

BARSTEAD, B., and B. WATERSTON, 1989 The basal component of the nematode dense-body is vinculin. J. Biol. Chem. **264:** 10177–10185.

BIRREN, B., and E. LAI, 1993 *Pulsed Field Gel Electrophoresis: A Practical Guide*. Academic Press Inc., San Diego, CA.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CASSADA, R. C., and R. L. RUSSELL, 1975 The dauer larvae, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. **46:** 326–342.

CHALFIE, M., and J. WHITE, 1988 The nervous system, pp. 337–391 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

CHEN, W. and L. LIM, 1994 The *Caenorhabditis elegans* small GTP-binding protein RhoA is enriched in the nerve ring and sensory neurons during larval development. J. Biol. Chem. **269:** 32394–32404.

COULSON, A., J. SULSTON, S. BRENNER and J. KARN, 1986 Toward a physical map of the genome of the nematode *C. elegans* Proc. Natl. Acad. Sci. USA **83:** 7821–7825.

COULSON, A., R. WATERSTON, J. KIFF, J. SULSTON and Y. KOHARA, 1988 Genome linking with yeast artificial chromosomes. Nature **335:** 184–186.

COULSON, A., Y. KOZONO, B. LUTTERBACH, R. SHOWNKEEN, J. SULSTON *et al.*, 1991 YACs and the *C. elegans* genome. Bioessays **13:** 413–417.

EDGLEY, M. L., and D. L. RIDDLE, 1993 The nematode *Caenorhabditis elegans*, pp. 3.281–3.318 in *Genetic Maps: Locus Maps of Complex Genomes*, Ed. 6, No. 3, *Lower Eukaryotes*, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory Press, Plainview, NY.

EMMONS, S. W., L. YESNER, K.-S. RUAN and D. KATZENBERG, 1983 Evidence for a transposon in *Caenorhabditis elegans*. Cell **32:** 55–65.

EPSTEIN, H. F., and J. N. THOMSON, 1974 Temperature-sensitive mutation affecting myofilament assembly in *Caenorhabditis elegans*. Nature **250:** 579–580.

FIRE, A. 1986 Integrative transformation of *Caenorhabditis elegans*. EMBO J. **5:** 2673–2680.

FIRE, A., S. W. HARRISON and D. DIXON, 1990 A modular set of *lacZ* fusion vectors for studying gene expression in *Caenorhabditis elegans*. Gene **93:** 189–198.

FROHMAN, M. A., M. K. DUSH and G. R. MARTIN, 1988 Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA **85:** 8998–9002.

GRANATO, M., H. SCHNABEL and R. SCHNABEL, 1994 Genesis of an organ: molecular analysis of the *pha-1* gene. Development **120:** 3005–3017.

HALL, D. H., and E. M. HEDGECOCK, 1991 Kinesin-related gene *unc-104* is required for axonal transport of synaptic vesicles in *C. elegans*. Cell **65:** 837–847.

HATA, Y., C. A. SLAUGHTER and T. C. SÜDHOF, 1993 Synaptic vesicle fusion complex contains *unc-18* homolog bound to syntaxin. Nature **366:** 347–351.

HEDGECOCK, E. M., J. G. CULOTTI, J. N. THOMSON and L. A. PERKINS, 1985 Axonal guidance mutants of *Caenorhabditis elegans* identified by filling sensory neurons with fluorescein dyes. Dev. Biol. **111:** 158–170.

HEDGECOCK, E. M., J. G. CULOTTI and D. H. HALL, 1990 The *unc-5*, *unc-6*, and *unc-40* genes guide circumferential migrations of pioneer axons and mesodermal cells on the epidermis in *C. elegans*. Neuron **2:** 61–85.

HEKIMI, S., 1990 A neuron-specific antigen in *C. elegans* allows visualization of the entire nervous system. Neuron **4:** 855–865.

HODGKIN, J. A., 1980 More sex determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HODGKIN, J. 1993 Molecular cloning and duplication of the nematode sex-determining gene *tra-1*. Genetics **133:** 543–560.

HOPE, I. A., 1994 'Promoter trapping' in *Caenorhabditis elegans*. Development **113:** 399–408.

HOSONO, R., S. HEKIMI, Y, KAMIYA, T. SASSA, S. MURAKAMI *et al.*, 1992 The *unc-18* gene encodes a novel protein affecting the kinetics of acetylcholine metabolism in the nematode *Caenorhabditis elegans*. J. Neurol. **58:** 1517–1525.

HUANG, X.-Y., and D. HIRSH, 1989 A second trans-spliced RNA leader sequence in the nematode *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **86:** 8640–8644.

ISHII, N., W. G. WADSWORTH, B. D. STERN, J. G. CULOTTI and E. M. HEDGECOCK, 1992 UNC-6, a laminin-related protein, guides cell and pioneer axon migrations in *C. elegans*. Neuron **9:** 873–881.

KRAUSE, M., and D. HIRSH, 1987 A trans-spliced leader sequence on actin mRNA in *C. elegans*. Cell **49:** 753–761.

LEE, J., G. D. JONGEWARD and P. W. STERNBERG, 1994 *unc-101*, a gene required for many aspects of *Caenorhabditis elegans* development and behavior, encodes a clathrin-associated protein. Genes Dev. **8:** 60–73.

LEUNG-HAGESTEIJN, C., A. M. SPENCE, B. D. STERN, Y. ZHOU, M.-W. SU *et al.*, 1992 *UNC-5*, a transmembrane protein with immunoglobulin and thrombospondin type-1 domains, guides cell and pioneer axon migrations in *C. elegans*. Cell **71:** 289–299.

LEWIS, J. A., C.-H. WU, J. H. LEVINE and H. BERG, 1980 Levamisole-resistant mutants of the nematode *Caenorhabditis elegans* appear to lack pharmacological acetylcholine receptors. Neuroscience **5:** 967–989.

LIAO, L.W., B. ROSENZWEIG and D. HIRSH, 1983 Analysis of a transposable element in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **80:** 3585–3589.

MACLEOD, A. R., J. KARN, and S. BRENNER, 1981 Molecular analysis of the *unc-54* myosin heavy-chain gene of *Caenorhabditis elegans*. Nature **291:** 386–390.

MCINTIRE, S. L., E. JORGENSEN and H. R. HORVITZ, 1993 Genes required for GABA function in *Caenorhabditis elegans*. Nature **364:** 334–337.

MELLO, C. C., J. M. KRAMER, D. STINCHCOMB and V. AMBROS, 1991 Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. **10:** 3959–3970.

MENDEL, J. E., H. C. KORSWAGEN, K. S. LIU, Y. M. HADJU-CRONIN, M. I. SIMON *et al.*, 1995 Participation of the protein G_o in multiple aspects of behavior in *C. elegans*. Science **267:** 1652–1655.

MILLER, D. M., C. J. NIEMEYER and P. CHITKARA, 1993 Dominant *unc-37* mutations suppress the movement defect of a homeodomain mutation in *unc-4*, a neural specificity gene in *Caenorhabditis elegans*. Genetics **135:** 741–753.

MOERMAN, D. G., G. M. BENIAN and R. H. WATERSTON, 1986 Molecular cloning of the muscle gene *unc-22* in *Caenorhabditis elegans* by Tc1 transposon tagging. Proc. Natl. Acad. Sci. USA **83:** 2579–2583.

MORI, I., D. G. MOERMAN and R. H. WATERSTON, 1990 Interstrain crosses enhance excision of Tc1 transposable elements in *Caenorhabditis elegans*. Mol. Gen. Genet. **220:** 251–255.

NONET, M. L., K. GRUNDAHL, K., B. J. MEYER and J. B. RAND, 1993. Synaptic function is impaired but not eliminated in *C. elegans* mutants lacking synaptotagmin. Cell **73:** 1291–1305.

OGURA, K., C. WICKY, L. MAGNENAT, H. TOBLER, I. MORI *et al.*, 1994 *Caenorhabditis elegans unc-51* gene required for axonal elongation encodes a novel serine/threonine kinase. Genes Dev. **8:** 2389–2400.

PILGRIM, D., 1993 The genetic and RFLP characterization of the left end of linkage group *III* in *Caenorhabditis elegans*. Genome **36:** 712–724.

RIDDLE, D. L., M. M. SWANSON and P. S. ALBERT, 1981 Interacting genes in nematode dauer larva formation. Nature **290:** 668–671.

ROGALSKI, T. M., B. D. WILLIAMS, G. P. MULLEN and D. G. MOERMAN, 1993 Products of the *unc-52* gene in *Caenorhabditis elegans* are homologous to the core protein of the mammalian basement membrane heparin sulfate proteoglycan. Genes Dev. **7:** 1471–1484.

ROSE, A. M., L. J. HARRIS, N. R. MAWJI and W. J. MORRIS, 1985 Tc1(Hin): a form of the transposable element Tc1 in *Caenorhabditis elegans*. Can. J. Biochem. Cell Biol. **63:** 752–756.

ROSENZWEIG, B., L. W. LIAO and D. HIRSH, 1983 Sequence of the *C. elegans* transposable element Tc1. Nucleic Acids Res. **11:** 4201–4209.

SAMBROOK, J., E. F. FRITSCH and T. MANIATIS, 1989 *Molecular Cloning, A Laboratory Manual*, Ed. 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SCHNABEL, H., and R. SCHNABEL., 1990 An organ-specific differentiation gene, *pha-1*, from *Caenorhabditis elegans*. Science **250:** 686–688.

SÉGALAT, L., D. A. ELKES and J. M. KAPLAN, 1995 Modulation of serotonin-controlled behaviors by G_o in *Caenorhabditis elegans*. Science **267:** 1648–1651.

SIDDIQUI, S. S., and J. G. CULOTTI, 1991 Examination of neurons in wild-type and mutants of *Caenorhabditis elegans* using antibodies to horseradish peroxidase. J. Neurogenet. **7:** 193–211.

SULSTON, J., D. G. ALBERTSON and J. N. THOMSON, 1980 The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. **8:** 542–576.

THOMAS, J. H., D. A. BIRNBY and J. J. VOWELS, 1993 Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics **134:** 1105– 1117.

WHITE, J. G., E. SOUTHGATE, J. N. THOMSON and S. BRENNER, 1986 The structure of the nervous system of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. B Biol. Sci. **314:** 1–340.

WILSON, R., R. AINSCOUGH, K. ANDERSON, C. BAYNES, M. BERKS *et al.*, 1994 2.2 Mb of contiguous nucleotide sequence from chromosome *III* of *C. elegans*. Nature **368:** 32–38.

WOOD, W. B. (Editor), 1988 *The Nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Communicating editor: R. K. HERMAN