Molecular and Genetic Analysis of *unc-7*, a *Caenorhabditis elegans* Gene Required for Coordinated Locomotion

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ABSTRACT

Mutations in the *Caenorhabditis elegans* gene *unc-7* confer an uncoordinated phenotype. Wild-type animals trace smooth, sinuous waves as they move; *unc-7* mutants make irregular bends or kinks along their bodies, particularly when they move forward. The *unc-7* locus has also been implicated in the nematode's response to volatile anesthetics. We have cloned *unc-7* by transposon tagging: an *unc-7* mutation was correlated with the insertion of the transposon Tc1, and reversion of the mutant phenotype was correlated with loss of the Tc1 element. We have physically mapped the region flanking the sites of Tc1 insertion and identified DNA rearrangements corresponding to eight additional *unc-7* alleles. Northern analysis indicates that a 2.7-kb *unc-7* message is present in all developmental stages but is most abundant in L1-L3 larvae. The 5′ end of the message contains a *trans*-spliced leader SL1. An 18-kb intron is located upstream of the predicted translational start site of the gene, and DNA breakpoints of four gamma-ray-induced alleles were located within this intron. We determined the sequence of a cDNA corresponding to the *unc-7* message. The message may encode a 60-kd protein whose amino acid sequence is unrelated to any other available protein sequence; a transmembrane location for the *unc-7* protein is predicted. We predict from our analysis of *unc-7* genetic mosaics that the *unc-7* gene product is not required in muscle cells for wild-type coordination but is probably required in motor neurons (although a hypodermal role has not been excluded). We speculate that *unc-7* may be involved in the function of neuronal ion channels.

THE nematode *Caenorhabditis elegans* was originally chosen as a model organism for systematic study with the aim of elucidating nervous system development and animal behavior (BRENNER 1974). The relative simplicity of *C. elegans*' cell number and organization along with the genetic tractability of the organism offered the possibility of relating components defined by mutation to their specific roles in the construction and function of the nervous system. Significant progress toward that goal has now been made. The complete wiring diagram of the hermaphrodite has been determined by reconstruction from serial section electron micrographs (WARD *et al.* 1975; WARE *et al.* 1975; ALBERTSON and THOMSON 1976; WHITE *et al.* 1976, 1986; HALL and RUSSELL 1991), and mutations defining genes involved in neuronal process outgrowth (HEDGECOCK *et al.* 1987; HEDGECOCK, CULOTTI and HALL 1990; MCINTIRE *et al.* 1992; Li, HERMAN and SHAW 1992), wiring specificity (J. WHITE *et al.*, unpublished results, cited by CHALFIE and WHITE 1988; MILLER *et al.* 1992; WHITE, SOUTHGATE and THOMSON 1992), synaptic transmission (LEWIS *et al.* 1987; JOHNSON *et al.* 1988; RAND 1989; HALL and HEDGECOCK 1991; OTSUKA *et al.* 1991), neuronal cell fate (FINNEY, RUVKUN and HORVITZ 1988; MILLER *et al.* 1992; WHITE, SOUTHGATE and THOMSON 1992), chemosensation (reviewed by CHALFIE and WHITE 1988), mechanosensation (WAY and CHALFIE 1988; CHALFIE and AU 1989; SAVAGE *et al.* 1989; CHALFIE and WOLINSKY 1990; DRISCOLL and CHALFIE 1991), and other properties of the nervous system have been described (for review, see CHALFIE and WHITE 1988).

Wild-type *C. elegans* moves with a sinusoidal motion, with the bending of the worm's body occurring in the dorsoventral plane. The mechanics of movement thus requires coordinating muscle contraction and relaxation on the dorsal and ventral sides of the worm. Animals mutant in *unc-7* are unable to propagate the smooth sinusoidal body bends normally seen in wild-type animals, but instead display sharp irregular bends, both when moving and when stationary. Animals with this phenotype are referred to as "kinkers," and in the case of *unc-7* mutants this uncoordination is most apparent when the animals try to move forward.

The *unc-7* gene product is also involved in the nematode's response to certain anesthetics (MORGAN, SEDENSKY and MENEELY 1990). Mutations in the genes *unc-79* or *unc-80* render the worms hypersensitive to volatile anesthetics such as halothane and chloroform (SEDENSKY and MENEELY 1987). Animals additionally mutant in the gene *unc-7* are no longer hypersensitive to these anesthetics. This suppression

TABLE 1

***unc-7* alleles**

Allele	Mutagen	Origin	Comments
e5	EMS	BRENNER (1974)	
e42	EMS	BRENNER (1974)	
e65	EMS	BRENNER (1974)	Weak phenotype
e133	EMS	BRENNER (1974)	
e139	EMS	BRENNER (1974)	
bx5	Spont., Bergerac BO	S. EMMONS	Tc1 insertion (this work)
wd2	Spont., TR679	D. MILLER	Tc1 insertion (this work)
wd6	Spont., TR679	D. MILLER	Tc1 insertion (this work)
wd7	Spont., TR679	D. MILLER	Complex rearrangement (this work)
mn347	Spont., RW7097	This work	Tc1 insertion
mn382	γ-ray	This work	Translocation
mn383	γ-ray	This work	6-kb deletion
mn384	γ-ray	This work	2-kb insertion; weak phenotype
mn408	EMS	This work	
mn409	EMS	This work	
mn410	EMS	This work	
mn413	γ-ray	This work	Translocation

of the *unc-79* or *unc-80* anesthetic-sensitivity phenotype can also be effected by mutations in some, but not all, other genes that confer a kinked phenotype (MORGAN, SEDENSKY and MENEELY 1990).

The precise function of the wild-type *unc-7* gene product and how its loss can affect coordinated locomotion and responses to certain anesthetics are unknown. We have undertaken a molecular and genetic study of the *unc-7* gene to begin to elucidate its role in the development and function of the *C. elegans* nervous system.

MATERIALS AND METHODS

Strains and general genetics: Cultivation and genetic manipulations of *C. elegans* followed standard procedures (BRENNER 1974). Alleles of *unc-7* that we have used are listed in Table 1. Names of other genes and alleles used were: linkage group (LG) *III*: *unc-93(e1500)*. LG*IV*: *dpy-13(e184)*, *mut-6(st702)*, *him-8(mn253)*. LG*V*: *dpy-11(e224)*. LG*X*: *lin-2(e1309)*, *unc-84(e1420)*, *unc-3(e151)*, *daf-6(e1377)*, *osm-1(p808)*, *sup-10(n983, mn219)*, *mnDf124* (a gamma-ray-induced recessive lethal that fails to complement both *daf-6* and *unc-7* and is missing *unc-7* sequences, data not shown). All of these mutations except for *mut-6*, which derives from RW7097 (a derivative of RW7096, MORI, MOERMAN and WATERSTON 1988), were generated in the Bristol strain N2 or descendants of N2 (BRENNER 1974); for gene descriptions, see WOOD (1988). A *sup-10(n983) osm-1* double mutant strain was provided by A. VILLENEUVE and B. MEYER. Phenotypic scoring of *daf-6* and *osm-1* was carried out by staining with fluorescein isothiocyanate (FITC) (HEDGECOCK *et al.* 1985). A genetic map of the *X*-linked genes used in the mosaic analysis is given in Figure 1.

Transposon-tagged alleles of *unc-7* were originally isolated as spontaneous mutations in the laboratories of S. EMMONS (*bx5*), D. MILLER (*wd2*, *wd6*, *wd7*), and this laboratory (*mn347*). The *bx5* mutation was identified after a heat-shock treatment (S. EMMONS, personal communication) of Bergerac strain BO (NIGON 1949; EMMONS *et al.* 1983); the three *wd* alleles were identified in strain TR679 (COLLINS, SAARI and ANDERSON 1987); and *mn347* was identified

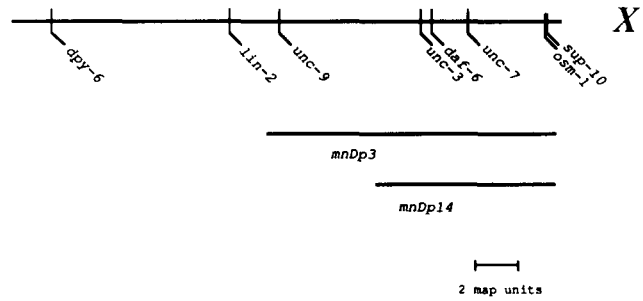

FIGURE 1.—Genetic map of the right end of the *X* chromosome including markers used for mosaic analysis.

in strain RW7097. The *unc-7* alleles *mn382*, *mn383*, *mn384* and *mn413* were obtained through noncomplementation screens by mating gamma-irradiated (HERMAN 1984) *him-8 IV; lin-2 X* males with *dpy-11 V; unc-84 unc-7(bx5) X* hermaphrodites. Resultant Unc-7 non-Dpy progeny were allowed to self-fertilize, and homozygous *lin-2 unc-7* hermaphrodites were then selected. Mutations distinct from *bx5* were unequivocally identified by noting the absence of the Tc1 insertion associated with the *unc-7(bx5)* allele. Mutations were assigned to *unc-7* by complementation tests against *unc-7(e5)* (BRENNER 1974); typically, *dpy-11; unc-7(e5)* hermaphrodites were mated with *unc-7?/0; mnDp3(X;f)* males, and non-Dpy hermaphrodite progeny were scored with respect to the Unc-7 phenotype.

The high Tc1 copy number of the original Bergerac-derived *unc-7(bx5)* isolate was reduced by crossing to N2 males, picking wild-type hermaphrodite cross-progeny, and picking their Unc-7 segregants. After 20 such outcrosses, the strain SP1158 was established. A *dpy-13 IV; unc-7(bx5) X* strain was established from an *unc-7(bx5)* line that had been outcrossed 10 times. These animals were mated to RW7097 (*mut-6 IV*) males; wild-type hermaphrodite progeny were picked, and their non-Dpy, Unc-7 progeny were picked. Those animals that failed to segregate Dpy progeny (and which were therefore presumed to be homozygous for *mut-6*) were used to establish lines that were screened for spontaneous reversion of the Unc-7 phenotype. Two independent revertants were obtained, *bx5 mn369* and *bx5 mn370*, and outcrossed at least five times to SP1158. After

the final outcross in each case, *unc-7(bx5)* and non-Unc-7 homozygous *unc-7(bx5 mn369)* or *unc-7(bx5 mn370)* siblings were isolated and used to establish lines to determine the presence or absence of the putative Tc1 insertion in *unc-7(bx5::Tc1)*.

Genomic analysis: *C. elegans* DNA was isolated as previously described (SULSTON and HODGKIN 1988). Cloning and sequencing vectors used were pUC118 and pUC119 (VIEIRA and MESSING 1987). Plasmid pRH1 carries a 7-kb *Eco*RI fragment, which includes the *unc-7(bx5::Tc1)* mutation, in pUC119. Tc1 sequences were removed by *Eco*RV digestion (ROSENZWEIG, LIAO and HIRSH 1983) to create pRH2, which was used to screen an N2 λEMBL4 genomic library provided by C. LINK. A hybridizing clone from this library, SP#2, was positioned on the *C. elegans* physical map by A. COULSON and J. SULSTON (COULSON *et al.* 1986, 1988), who provided cosmid clones (C11G10, F56B12 and F09B12) covering the *unc-7* region.

To screen cDNA libraries in λgt10 (mixed-stage, provided by S. KIM and R. HORVITZ) or λgt11 (embryonic, provided by I. SCHAUER and W. WOOD), a 2.3-kb *Xba*I fragment from pRH2 representing sequences flanking the *unc-7(bx5::Tc1)* insertion site was used as probe. The insert from a resultant hybridizing phage clone, SP#8, was then used for additional screening. The SP#8 probe hybridized to plaques at a frequency of approximately 1 in 60,000 for both cDNA libraries.

Sequencing of single-stranded templates employed the dideoxy chain-termination method (SANGER, NICKLEN and COULSON 1977), and Sequenase Version 2.0 (USB) was used for sequencing reactions. To generate nested sets of deletions for sequencing templates, controlled exonuclease III digestion was used (HENIKOFF 1987). Single-stranded templates were recovered with M13K07 as the helper phage (VIEIRA and MESSING 1987). Both strands of the SP#8 insert were sequenced, and one or both strands of most of the genomic sequence corresponding to SP#8 was also determined to verify the cDNA sequence.

Southern blots (SOUTHERN 1975) employed Zeta-Probe nylon filters (Bio-Rad), using alkaline or high-salt transfer solutions. Hybridization was carried out in 0.25 M sodium phosphate, pH 7.0, 0.25 M NaCl, 7% SDS, 1 mM EDTA, 150 μg/ml herring sperm DNA, 50% formamide at 42°. Filters were washed at 50° with 0.2× SSPE, 0.1% SDS. Nitrocellulose filters were used for plaque and colony lifts and were screened using 4× SSPE, 1% SDS, 200 μg/ml herring sperm DNA, 50% formamide at 42°. The nitrocellulose filters were washed in the same way as the nylon filters. Other recombinant DNA techniques followed standard protocols (MANIATIS, FRITSCH and SAMBROOK 1982).

Northern analysis: RNA was isolated from worms grown at 18–20° in liquid cultures (SULSTON and HODGKIN 1988). Packed worms or eggs were lysed by resuspending in cold 5 M guanidine-HCl, 0.2 M sodium acetate, 0.5% sodium sarkosyl, 1 mM EDTA, 0.5 M 2-mercaptoethanol and immediately pressed in a French press at 11,000 psi. The lysate was extracted repeatedly with phenol:chloroform:isoamyl alcohol (25:24:1); RNA was precipitated by adding one-half volume 95% ethanol overnight at −20°, and after centrifugation, samples were resuspended in water treated with diethyl pyrocarbonate. Poly(A)-enriched RNA was isolated by passing total RNA over oligo-dT (Pharmacia) columns (JACOBSON 1987).

Worms at specific developmental stages were obtained by harvesting gravid adults, purifying eggs by alkaline hypochlorite treatment (SULSTON and HODGKIN 1988), and allowing eggs to hatch overnight in S medium without *E. coli* (SULSTON and HODGKIN 1988). The resulting synchronized culture (first larval stage) was then added to S medium with *E. coli* and harvested as development proceeded through later stages. RNA was isolated from eggs, each of the four larval stages, and young adults (before eggs appeared).

RNA was electrophoresed in formaldehyde gels (0.8–1.0% agarose, 6.6% formaldehyde, 20 mM 3-(N-morpholino) propanesulfonic acid (MOPS), pH 7.0, 2.5 mM sodium acetate, 1 mM EDTA). Typically, 5–10 μg per lane of poly(A)-enriched RNA or 30 μg per lane total RNA were loaded in 50% formamide, 6.6% formaldehyde, 5% glycerol, 0.04% bromphenol blue, 250 mg per ml ethidium bromide, and 1X running buffer (20 mM MOPS, pH 7.0, 25 mM sodium acetate, 1 mM EDTA). RNA quantities were determined by spectrophotometry, and in the case of total RNA, relative concentrations among different samples were verified by comparing rRNA levels by northern blot hybridization. In the case of poly(A)-enriched RNA samples, we relied primarily on spectrophotometric readings and ethidium bromide staining to ensure that mRNA quantities in individual samples were comparable. Although actin probes have been used for standardizing RNA amounts from different developmental stages, we, and others (KRAUSE *et al.* 1989), find that the *act-1* gene does not exhibit a constant level of message during development. Gels were blotted to Zeta-Probe nylon membranes (Bio-Rad). Hybridization was carried out as described for Southern blots but at 50°, and washes were done at 55°.

To detect the *unc-7* transcript, a 1.7-kb *Eco*RI fragment from the cDNA clone SP#8 was used as the radioactively labeled probe. This fragment contains the entire coding sequence for the predicted protein product of *unc-7* and was cloned into pUC118 to create the plasmid pRH1.7c. The plasmid pCe7 (EMMONS, KLASS and HIRSH 1979) was used as probe to detect rRNA, and the plasmid pT7/T3–18–103 (kindly provided by M. KRAUSE) was used as a probe for transcripts from the *act-1* gene (KRAUSE *et al.* 1989).

RACE-PCR: The procedure for RACE analysis of the 5′ ends of the *unc-7* transcripts was based on FROHMAN (1990). AMV reverse transcriptase (USB) was used to synthesize cDNA from 1 μg of poly(A)-enriched RNA (mixed-stage or L1) from *C. elegans* strain Bristol, var. N2 (wild type), the *unc-7(mn383)* strain SP1379, or the *unc-7(mn384)* strain SP1380. The reactions were carried out at 52° for 1 hr, using as primer a 20-mer corresponding to nucleotide positions 290–309 of the cDNA sequence of the SP#8 insert (5′-GATTGTCGAGATGTCTGGCT-3′). The primers were removed after the reaction by using a Centricon 100 microconcentrator (Amicon). The cDNA products were tailed with dATP using terminal deoxynucleotidyl transferase (USB) and then used as templates for PCR amplification.

Primers used for PCR amplification of 5′ ends included a gene-specific 20-mer corresponding to the cDNA nucleotide positions 217 to 236 of the SP#8 insert (5′-GTTCCTAGTGTCTGGCTGTT-3′), an adapter primer (5′-GACTCGAGTCGACATCG-3′), and a hybrid dT_{17}-adapter primer (FROHMAN 1990). Primers were annealed at 55° for 1 min, and products were amplified for 40 cycles at 72° with 2 min extension times. Double-stranded products were melted at 93° for 1 min after each extension. A *Sal*I site internal to the gene-specific primer facilitated cloning products into pUC118.

RESULTS

Uncoordinated phenotype conferred by loss-of-function *unc-7* mutations: In Table 1 are listed *unc-7* alleles identified by others and by us. All alleles are

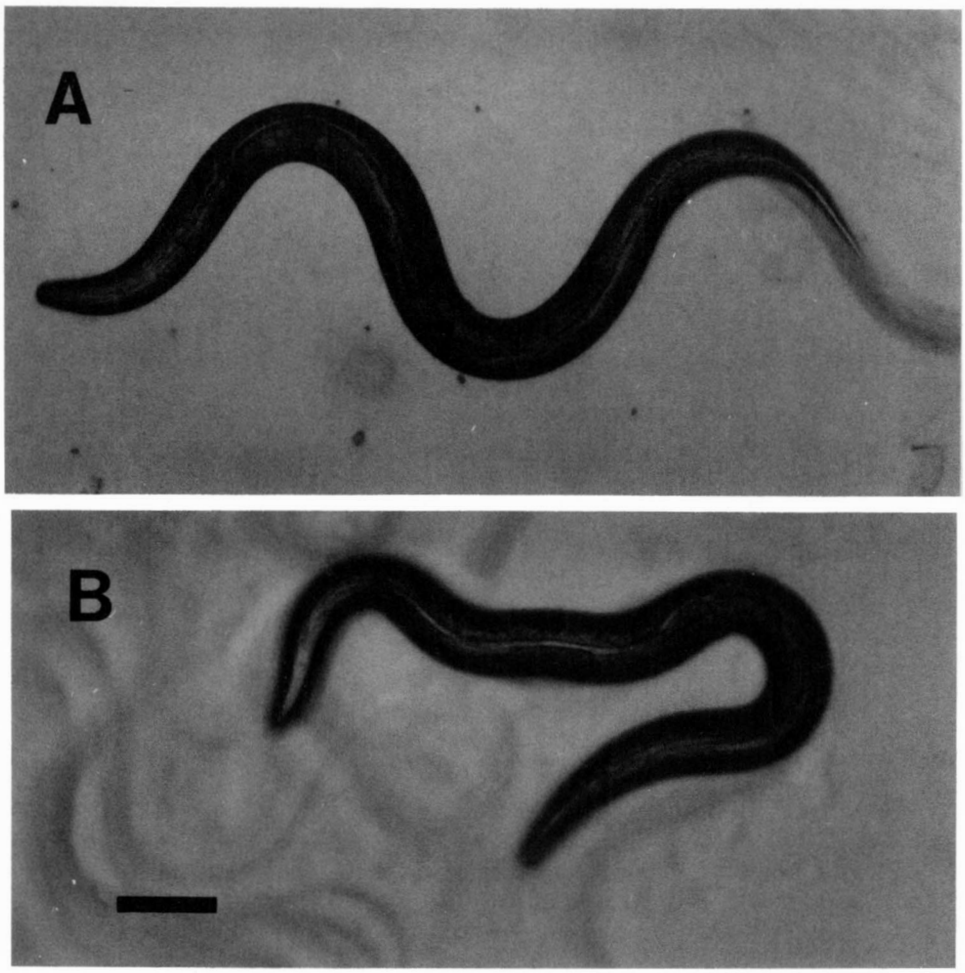

FIGURE 2.—Postures of wild-type N2 (A) and *unc-7(bx5)* (B) nematodes on an agar surface. Bar indicates 0.1 mm.

recessive to *unc-7(+)*. The homozygous mutants all display a similar uncoordination, both when moving and when stationary (Figure 2). Animals with this phenotype are referred to as "kinkers," and in the case of *unc-7* mutants, the uncoordination is more apparent for forward than for backward movement. Backward locomotion is also affected, but animals often move several body lengths backward before showing evidence of kinking. The uncoordinated phenotype of *unc-7* animals is apparent upon hatching and is most severe in the early larval stages. Later stage larvae and adults remain uncoordinated but are less severely affected.

Two of the 17 known *unc-7* alleles, *e65* and *mn384*, stand out as weaker alleles. The weaker phenotype is distinguished by worms that, after being prodded on the tail, can move forward a considerable distance before showing evidence of kinking. The remaining alleles produce worms that kink almost immediately when trying to move forward after being prodded. When the strong alleles *bx5* or *e5* were put opposite the deficiency *mnDf124*, the phenotype was indistinguishable from the homozygous *unc-7* mutants. In earlier work (MENEELY and HERMAN 1979), *unc-7(e139)* was put opposite four different deficiencies with no enhancement of the mutant phenotype. We therefore suggest that the strong alleles may represent the null phenotype.

No neuronal or body muscle cell abnormalities that might account for the uncoordinated locomotion have been detected at the level of light microscopy. When viewed with polarized light (WATERSTON 1988) the body muscles of all *unc-7* mutants that have been examined (*bx5*, *wd7* and *mn383*) displayed a wild-type pattern of birefringence. The nervous system of *unc-7(bx5)* animals was examined immunocytochemically using antibody to *unc-33* gene product, which is localized to neuronal processes (W. LI and J. SHAW, unpublished results); no abnormalities were detected (W. LI, personal communication).

Cloning *unc-7*: The *unc-7(bx5)* allele was originally identified (S. EMMONS, personal communication) in a spontaneous uncoordinated mutant from *C. elegans* strain Bergerac var. BO. This strain has been shown to exhibit a relatively high frequency of movement of the transposable element Tc1 (MOERMAN and WATERSTON 1984; GREENWALD 1985; EIDE and ANDERSON 1985), which suggested that the *bx5* mutation could

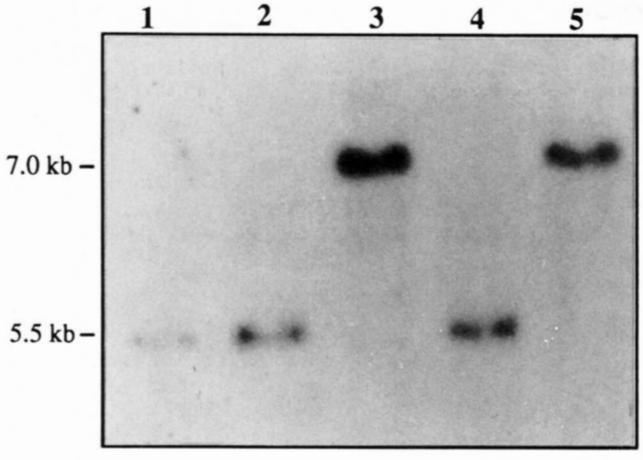

FIGURE 3.—Reversion of the *unc-7(bx5)* mutation corresponds to a deletion the size of the transposon Tc1. Sequences flanking the *bx5::Tc1* insertion site were hybridized to *Eco*RI-restricted DNA isolated from revertants and their corresponding mutant siblings. (1) Wild-type N2; (2) revertant *bx5 mn369*; (3) *bx5* sibling of *bx5 mn369*; (4) revertant *bx5 mn370*; (5) *bx5* sibling of *bx5 mn370*.

be the insertion of a Tc1 element into the *unc-7* gene. Identification of a Tc1 insertion associated with *bx5* followed the standard protocol of reducing the Tc1 copy number by outcrossing to the low copy number strain Bristol var. N2 (EMMONS *et al.* 1983). A candidate Tc1 element, present in all *unc-7(bx5)*-containing strains but absent in *unc-7(+)* animals, was identified as part of a 7-kb *Eco*RI fragment and cloned as pRH1. Confirmation that this element was responsible for the *unc-7(bx5)* mutation came first from the examination of revertants (Figure 3).

Two independent non-Unc-7 revertants, *bx5 mn369* and *bx5 mn370*, were isolated in a mutator background that promotes excision of Tc1 (see MATERIALS AND METHODS). The revertants were outcrossed to an *unc-7(bx5)* nonmutator line, and corresponding revertant and mutant siblings were saved after the final outcross. DNA from strains derived from these animals was probed with sequences flanking the candidate Tc1 element from the plasmid pRH2. This probe detected a 7-kb fragment in both *unc-7(bx5)* strains (Figure 3, lanes 3 and 5); but in the two revertants and in wild-type N2, the size of the hybridizing *Eco*R1 fragment was reduced by 1.6 kb, the size of Tc1 (lanes 1, 2, and 4). We conclude that a Tc1 element was lost in conjunction with the reversion events, and we designate the original mutation *unc-7(bx5::Tc1)*.

The insert in pRH2 was used to screen a genomic library (kindly provided by C. LINK), and a hybridizing clone, SP#2, was identified. This clone was placed on the *C. elegans* physical map by A. COULSON and J. SULSTON (COULSON *et al.* 1986, 1988), who provided us with cosmid clones encompassing the *unc-7* region. A partial restriction map of the sequences flanking *unc-7(bx5::Tc1)* was constructed from cosmid clones C11G10, F56B12, and F09B12 (Figure 4A).

Analysis of additional *unc-7* alleles: Because the identification of our cloned gene as *unc-7* relied on the single *unc-7(bx5::Tc1)* insertion, we searched for corroborative evidence by analyzing other *unc-7* alleles. Four alleles derived from mutator strains showed alterations in the same *Eco*RI fragment affected by the *bx5::Tc1* mutation. Three of these alleles, *wd2*, *wd6* and *mn347*, were found to have Tc1 insertions (indicated in Figure 4B). The fourth, *wd7*, is a complex rearrangement involving sequences to the right of the *bx5::Tc1* insertion site on our physical map. This rearrangement is not associated with any of the known transposable elements in *C. elegans*.

Further evidence that the cloned region contains *unc-7* was derived from the examination of homozygous viable *unc-7* alleles generated by gamma irradiation. Four alleles were obtained at a frequency of approximately one in 6,500 mutagenized haploid genomes. All four were shown to have DNA rearrangements. Two of these, *mn382* and *mn413*, appear to be translocations; animals heterozygous for either mutation are Him (high incidence of male self-progeny), a trait associated with translocations involving the X chromosome (HERMAN, KARI and HARTMAN 1982), and hybridization using a restriction fragment from N2 that extends through the X chromosome breakpoint is consistent with this interpretation. In both cases, a restriction fragment found in wild-type N2 is replaced by two novel restriction fragments (data not shown). Of the other two alleles, one, *mn383*, has a deletion of approximately 6 kb, and the other, *mn384* (a weak allele), has a 2-kb insertion (data not shown). The approximate locations of the DNA lesions or rearrangements in *unc-7* mutants are indicated in Figure 4A.

Identification of an *unc-7* transcript: Sequences flanking the *bx5::Tc1* site were used to screen two cDNA libraries, one in λgt10 (provided by S. KIM and R. HORVITZ) and the other in λgt11 (provided by I. SCHAUER and W. WOOD). A hybridizing clone with a 2.4-kb insert was isolated (SP#8), and this clone was shown by hybridization to derive from sequences represented in the cosmid clones spanning the *unc-7* region. Both strands of the insert from this clone were sequenced (see below), and at least one strand of most of the genomic sequence corresponding to SP#8 was sequenced to verify the cDNA sequence and to examine gene structure. Placement of the cDNA sequence on the restriction map of the region showed that this transcript spans a distance of at least 25 kb and includes an unusually large intron of at least 18 kb (Figure 4). Examination of the gamma-ray-induced alleles showed that their corresponding DNA rearrangements mapped to this large intron and appeared to affect only noncoding sequences. This observation raised the possibility that disruption of an-

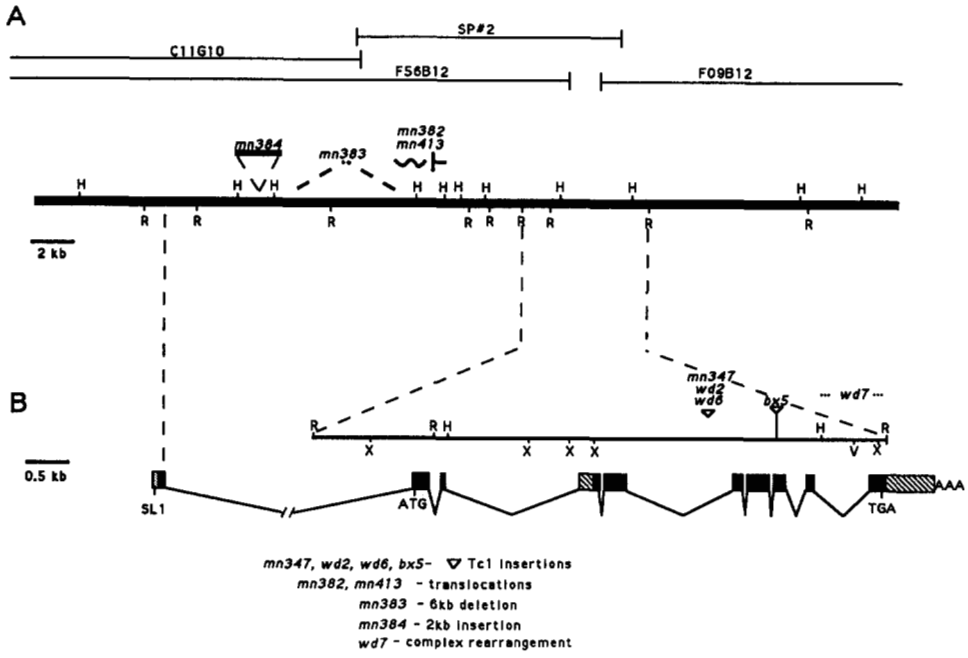

FIGURE 4.—Genomic map of the *unc-7* region. (A) The cosmid clones C11G10, F56B12, F09B12 and the phage clone SP#2 span the region. The approximate locations of the DNA rearrangements associated with the alleles *mn382*, *mn383*, *mn384* and *mn413* are indicated. The mutations *mn382* and *mn413* are putative translocations, *mn383* is a deletion, and *mn384* is an insertion. (B) Expanded region of the genome encoding most of the exons comprising the *unc-7* transcript. The transcript alignment is shown below the map. Intron/exon boundaries are indicated where known. The hatched boxes indicate cDNA sequence for which the corresponding genomic sequence has not been determined. The presence of the spliced leader sequence SL1 is inferred from RACE analysis. The location of Tc1 insertions associated with the mutant alleles *mn347*, *wd2* and *wd6* have been mapped to a 2.6-kb *Xba*I-*Hin*dIII fragment, and the location of *bx5::Tc1* has been determined directly by sequencing. H, *Hin*dIII; R, *Eco*RI; X, *Xba*I; V, *Eco*RV.

other transcript originating from the large intron might be responsible for the *unc-7* phenotype. Using as probes genomic sequences spanning the sites at which DNA rearrangements occurred in the gamma-ray-induced mutants, we failed to detect any other transcripts by northern analysis. However, because we cannot rule out the possibility that a transcript of very low abundance might originate from this region, we sought evidence that these mutations, as well as other *unc-7* mutations, affected the transcript represented by the cDNA insert in SP#8.

Northern analysis of the *unc-7* transcript: Using the entire coding sequence of the cDNA insert in SP#8 as a hybridization probe, northern analysis of *unc-7* mRNA from N2 showed the presence of a 2.7-kb transcript that could be detected in all developmental stages but was most abundant in the early larval stages L1–L3 and greatly decreased by the adult stage (Figure 5). This transcript was subsequently shown to be affected in some manner by all nine *unc-7* mutations examined.

All of the alleles associated with Tc1 insertions (*bx5*, *wd2*, *wd6* and *mn347*) produced transcripts increased in size by 1.6 kb compared with wild type (Figure 6A, lanes 1–5). These results suggested that all four Tc1 insertions lay within exons; the location of *bx5::Tc1* within an exon was confirmed directly by sequencing (indicated in Figures 3B and 7). The Tc1 insertion mutants also expressed a fairly abundant transcript of approximately wild-type size. The nature of this transcript is unknown; it may be due to somatic excision of Tc1 (EMMONS *et al.* 1983; EMMONS and YESNER 1984), or it could reflect the processing of the message at alternative splice sites as a result of Tc1 insertion (A. RUSHFORTH and P. ANDERSON, personal communication).

Qualitative analysis of transcripts for the gamma-ray-induced alleles *mn382*, *mn383*, *mn384* and *mn413* (all DNA rearrangements in the first intron) showed a common pattern: two major transcripts, one slightly larger than wild-type and one slightly smaller (Figure 6A, lanes 8–11). It therefore seems likely that sequences in the large intron are important for normal transcription. In the two translocation mutants we presume that the transcripts did not initiate at the usual site; and because all four alleles showed the same pattern of transcripts, we infer that transcription is initiated at an alternative site(s) in all of these mutants.

In *wd7* mutants, only a faint low molecular weight smear was detected using an *unc-7* probe (Figure 6A, lane 6). The integrity of *act-1* RNA (Figure 6B, lane 6) indicated that, in general, RNA in this sample was intact. Because the Tc1 mutants all showed some expression of transcripts of wild-type size and because

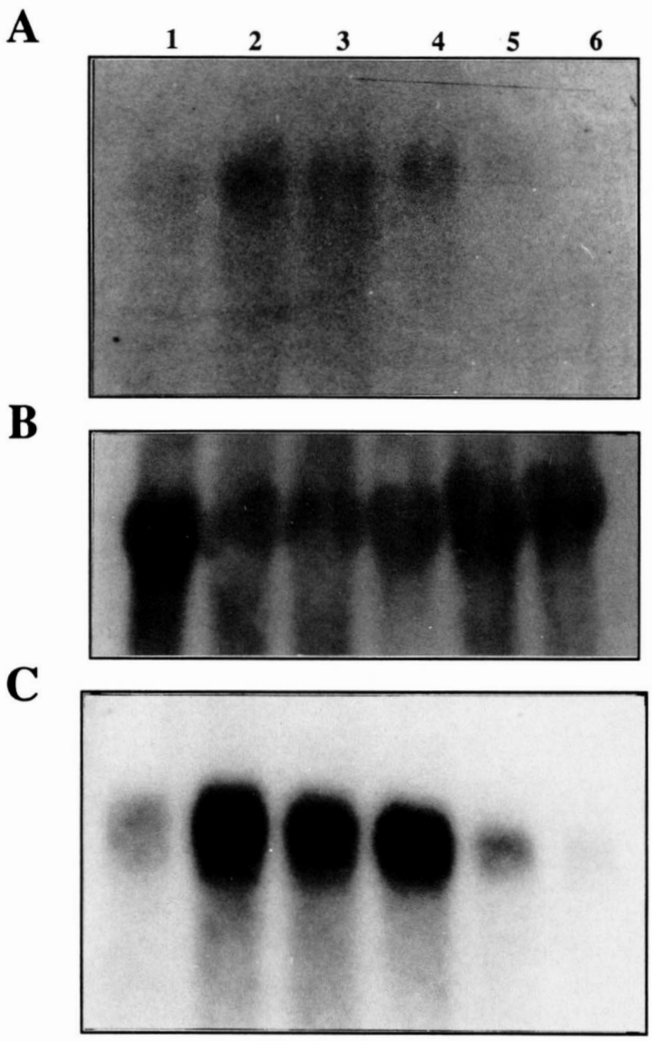

FIGURE 5.—Transcript levels of *unc-7* vary over the course of development. RNA was isolated from eggs (lane 1), the four larval stages L1 (lane 2), L2 (lane 3), L3 (lane 4), and L4 (lane 5), and young adults (lane 6). A northern blot containing total RNA, 30 μg per lane, was hybridized to *unc-7* coding sequences (A) or an rRNA probe (B, showing 18S signal). Poly(A)-enriched RNA, 10 μg per lane (C), was hybridized to the same *unc-7* probe as in A.

the gamma-ray-induced mutants showed significant levels of transcripts that are not predicted to alter protein coding sequences, the *wd7* allele may be the best candidate for a null allele.

Although a contribution to the Unc-7 phenotype by other transcripts which might originate from this region cannot be excluded, all of the present evidence indicates that disruption of the transcript represented by the cDNA insert in SP#8 is sufficient to confer an Unc-7 phenotype, and we henceforth refer to this transcript as the *unc-7* transcript.

Sequencing of the *unc-7* transcript: Analysis of the cDNA sequence revealed a long open reading frame that could encode a polypeptide 522 amino acid residues in length (Figure 7). Stop codons were present in all three reading frames upstream of the putative translational start site, suggesting that this cDNA clone was nearly full length. The first exon does not appear to encode any portion of the predicted protein; translation appears to initiate in the second exon, 3′ to the large 18-kb intron.

The predicted *unc-7* amino acid sequence does not reveal any compelling similarities to sequences currently available in data banks. A hydropathy profile (KYTE and DOOLITTLE 1982) does, however, suggest that there may be as many as four transmembrane domains, centered around amino acid positions 160, 230, 330 and 420 (Figure 8). No signal peptide is predicted. Sequencing of the SP#8 insert suggested that this cDNA clone was nearly full length, but comparison of the 5′ end to the genomic sequence revealed a disparity between sequences. The cDNA sequence and the genomic sequence are colinear except for 14 nucleotides at the 5′ end of the SP#8 insert (see legend, Figure 8). This divergence occurs within an exon predicted from the genomic sequence; the 3′ consensus splice site TTTCAG (EMMONS 1988) can be found in the genomic sequence 12 nucleotides upstream of the point of divergence. A 5′ cDNA probe was used to identify other cDNA clones, and four clones from two libraries were identified as independent. The sequences of the 5′ ends of all cDNA clones examined were identical to that in SP#8.

To resolve the disparity between cDNA and genomic sequence, we analyzed the 5′end of the *unc-7* message by anchored PCR amplification, or RACE (rapid amplification of cDNA ends) analysis (FROHMAN, DUSH and MARTIN 1988). A primer corresponding to SP#8 nucleotides 290–309 was used for cDNA synthesis, and a primer corresponding to nucleotides 217–236 was used for PCR amplification of the cDNA products. Amplified products were then cloned and sequenced.

The sequence obtained from wild-type *unc-7* RACE products showed the presence of the *trans*-spliced leader SL1 (KRAUSE and HIRSH 1987) along with the complete sequence of the exon predicted by genomic analysis (Figure 8). Shorter PCR clones appeared to be the products of incomplete cDNA synthesis and did not suggest any alternative initiation sites. The origin of the 14 nucleotides found at the 5′ ends of the cDNA clones is still undetermined but may be an artifact arising during library construction. No sequences similar to this stretch of 14 nucleotides were found in any of the products of RACE analysis.

Analysis of the 5′ end of mutant *unc-7* messages: The mutation *mn383* corresponds to a 6-kb deletion within the large intron in the *unc-7* gene, and, as already described, at least some *unc-7* transcripts from *mn383* animals appear to be slightly larger than wild type. To investigate whether this difference might be attributed to an alternative transcriptional initiation site, we examined the 5′ ends of clones obtained by

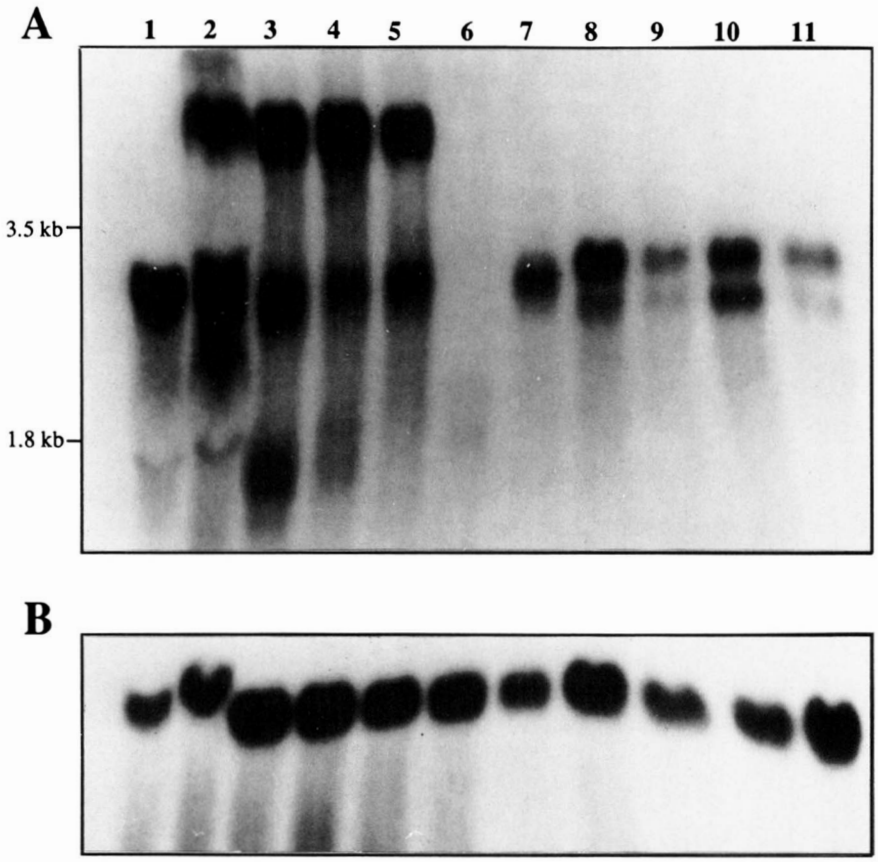

FIGURE 6.—Northern analysis of *unc-7* mutants. (A) Radioactively labeled coding sequences of the *unc-7* gene were hybridized to poly(A)-enriched RNA from nonsynchronized cultures of N2 (lanes 1 and 7); the Tc1 alleles *mn347* (lane 2), *bx5* (lane 3), *wd6* (lane 4) and *wd2* (lane 5); *wd7* (lane 6); and the gamma irradiation-induced alleles *mn382* (lane 8), *mn383* (lane 9), *mn384* (lane 10) and *mn413* (lane 11). Markers at 3.5 kb and 1.8 kb indicate the position of 28S and 18S rRNA species. (B) Same blot hybridized to *act-1* gene sequences.

applying the RACE procedure to mRNA from *mn383* mutants. Sequencing of individual cloned products showed that most of the transcripts represented in these clones appeared to initiate within the large intron of wild-type message just upstream of the first coding exon. These clones corresponded to PCR products of 431, 299, 263, 210 or 170 nucleotides in length and included 229, 97, 61, or 8 nucleotides of the intron (the sequence of the shortest product did not extend beyond the first coding exon), respectively. No sequences originating from the wild-type first (noncoding) exon were found in any PCR clones produced from *mn383* RNA. No products hybridizing to an *unc-7* probe were detected when genomic DNA was amplified using the same primers, or when reverse transcriptase was omitted; it is therefore unlikely that these RACE clones arose from amplification of contaminating genomic DNA. Instead, it is most likely that at least some transcripts initiate in the large intron as a result of the *mn383* deletion. It is unknown if these transcripts are unique to *mn383* mutant animals or whether similar transcripts are present at very low abundance in N2.

A similar RACE-PCR analysis was carried out using mRNA from *mn384* mutants. Products from this analysis indicated that transcripts were initiated within the large intron in these mutants also. The longest PCR products included sequences from the intron extending 166 nucleotides upstream of the wild-type second exon. In contrast to the results obtained in the analysis of *mn383* mutants, the longest products derived from *mn384* transcripts began with the *trans*-spliced leader SL1.

Mosaic analysis of *unc-7*: *A priori* the requirement for *unc-7(+)* function in promoting coordinated movement might be specific to body muscle cells or to certain neurons, or perhaps even to hypodermis if a wiring defect is responsible. The genetic location of *unc-7* lends itself readily to mosaic analysis, and a scheme paralleling that used for analysis of the *unc-3* gene was used (HERMAN 1984, 1987) to try to delimit the anatomical focus of *unc-7(+)* action.

We concentrated on the first embryonic cell division, at which P_0 divides to give AB and P_1 (Figure 9). All but one of the 95 body muscle cells derive from P_1, and all but two of the 282 body (nonpharyngeal) neurons derive from AB. Strains used for mosaic analysis carried a mutant *unc-7* gene on their *X* chromosomes and *unc-7(+)* on a free duplication or chromosome fragment. A free duplication is subject to rare spontaneous mitotic loss. Using other genetic markers carried by the free duplication, we have identified animals in which duplication loss occurred at P_1 or at AB (or AB.p, the posterior daughter of AB).

To identify $P_1(-)$ mosaics (animals in which the

```
GTTTAATTACCCAAGTTTGAG ACAATCTGGCCGACTTGGAGTAAACTCATTTGCGCTCGGGAAAGTTTGCAGGGCTAGTTGGATTGATC   68
GACGGATGT ATG CTC GGC TCC TCC AGC AAT CCT GAA CCG CCA CTC CTG TCG CGG ATA ATC GGA GTA CCT   137
          M  L  G  S  S  S  N  P  E  P  P  L  L  S  R  I  I  G  V  P   20
  CCA CCG CCG CCG CCA CGA GCA CCG ACC ACA GCT CTC GTC CTT CGG GTG CCC ACC GTC GAC AGT CCA   203
   P  P  P  P  P  R  A  P  T  T  A  L  V  L  R  V  P  T  V  D  S  P   42
  TCG AAA AAG AAA CAA CAG CCA GAC ACT AGG AAC AAA TAT CAG GAA ACT GCG CTC AGG GAT AAA AAA   269
   S  K  K  K  Q  Q  P  D  T  R  N  K  Y  Q  E  T  A  L  R  D  K  K   64
  ACT CGG ACA CCA CTT GAA AAA GCC AGA CAT CTC GAC AAT CTT CCC TCG TAT CAG GCC CAA AAA CTA   335
   T  R  T  P  L  E  K  A  R  H  L  D  N  L  P  S  Y  Q  A  Q  K  L   86
  TTA GAC GGT TCG CAC CAG CTT CGC ATC GAC TCC CAC CAC GTT GGT AGT GCA GGT CAC GGT GCA GGA   401
   L  D  G  S  H  Q  L  R  I  D  S  H  H  V  G  S  A  G  H  G  A  G   108
  CAA GGT CAC GGA CAC AAG AAA GAG TTC GGA CCT GCA ATG ATT CTC TAT TAT TTA GCT TCT GCT TTT   467
   Q  G  H  G  H  K  K  E  F  G  P  A  M  I  L  Y  Y  L  A  S  A  F   130
  CGG GCG CTC TAT CCG CGT CTA GAT GAT GAT TTT GTG GAC AAA CTT AAT TAT TAT TAT ACT ACT ACA   533
   R  A  L  Y  P  R  L  D  D  D  F  V  D  K  L  N  Y  Y  Y  T  T  T   152
  ATA TTA GCT TCC TTT GCA CTT CTT GTT TCC GCC AAG CAG TAT GTC GGT TTT CCC ATC CAA TGT TGG   599
   I  L  A  S  F  A  L  L  V  S  A  K  Q  Y  V  G  F  P  I  Q  C  W   174
  GTG CCC GCC ACA TTC ACA GAT GCC ATG GAG CAG TAC ACT GAG AAC TAC TGC TGG GTA CAG AAC ACA   665
   V  P  A  T  F  T  D  A  M  E  Q  Y  T  E  N  Y  C  W  V  Q  N  T   196
  TAC TGG GTT CCC ATG CAA GAG GAC ATT CCA CGT GAA ATT TAT TCG CGT AGA AAT CGA CAA ATT GGC   731
   Y  W  V  P  M  Q  E  D  I  P  R  E  I  Y  S  R  R  N  R  Q  I  G   218
  TAT TAC CAA TGG GTG CCG TTT ATT CTT GCT ATT GAG GCC TTG TTG TTC TAC GTT CCA TGC ATT CTC   797
   Y  Y  Q  W  V  P  F  I  L  A  I  E  A  L  L  F  Y  V  P  C  I  L   240
  TGG AGA GGA TTG TTG TAT TGG CAC AGT GGT ATC AAC CTA CAA GGG CTA GTC CAG ATG GCG TGT GAC   863
   W  R  G  L  L  Y  W  H  S  G  I  N  L  Q  G  L  V  Q  M  A  C  D   262
  GCA CGA CTG ATG GAT TCG GAA ATA AAG ACG AGA ACA GTC TAC ACA ATG GCC CGG CAC ATG CAA GAC   929
   A  R  L  M  D  S  E  I  K  T  R  T  V  Y  T  M  A  R  H  M  Q  D   284
  GAG GTT CAG CTC ACC AAT ATC GAC CGG CAA GGA CAC AGT CGG AGT TGT TTC TCG AAT TTG CAA CTT   995
   E  V  Q  L  T  N  I  D  R  Q  G  H  S  R  S  C  F  S  N  L  Q  L   306
  GGC GCA AAC TGT GGG AGA CAT TGT GGT TGC TAC GTC ACT ATG CTC TAC ATA GGA ATC AAG GTT CTC  1061
   G  A  N  C  G  R  H  C  G  C  Y  V  T  M  L  Y  I  G  I  K  V  L   328
  TAC TCA GCA AAT GTG TTG CTC CAG TTT TTC TTG CTG AAC CAT CTT CTT GGG TCT AAC GAC TTG GCT  1127
   Y  S  A  N  V  L  L  Q  F  F  L  L  N  H  L  L  G  S  N  D  L  A   350
  TAT GGA TTC TCT TTG TTG AAA GAC TTG ATG CAC GCG ATT GAG TGG GAA CAG ACT GGA ATG TTC CCC  1193
   Y  G  F  S  L  L  K  D  L  M  H  A  I  E  W  E  Q  T  G  M  F  P   372
                                                  bx5::Tc1
AGG GTC ACA CTT TGT GAT TTT GAG GTT CGA GTT CTG GGA AAC ATT CAT CGA CAT^V ACC GTT CAG TGC  1259
 R  V  T  L  C  D  F  E  V  R  V  L  G  N  I  H  R  H  T  V  Q  C   394
GTG TTA ATG ATC AAT ATG TTT AAT GAG AAA ATT TTC TTA TTC CTC TGG TTT TGG TTC TTA ACC TGT  1325
 V  L  M  I  N  M  F  N  E  K  I  F  L  F  L  W  F  W  F  L  T  C   416
GGA ATT GTT ACC GTA TGT AAC ACT ATG TAC TGG ATT CTC ATT ATG TTC ATT CCG TCT CAG GGA ATG  1393
 G  I  V  T  V  C  N  T  M  Y  W  I  L  I  M  F  I  P  S  Q  G  M   438
TCA TTT GTC CGC AAA TAC CTC CGT GTT CTA CCT GAC CAC CCT GCG AAA CCA ATA GCT GAT GAC GTC  1457
 S  F  V  R  K  Y  L  R  V  L  P  D  H  P  A  K  P  I  A  D  D  V   460
ACG CTG AGG AAG TTC ACT AAT AAC TTC CTC CGC AAA GAT GGA GTC TTC ATG TTG CGA ATG ATT TCC  1523
 T  L  R  K  F  T  N  N  F  L  R  K  D  G  V  F  M  L  R  M  I  S   482
ACA CAC GCC GGC GAA CTG ATG AGC AGT GAG CTC ATT CTT GCT CTC TGG CAG GAT TTC AAC AAC GTC  1589
 T  H  A  G  E  L  M  S  S  E  L  I  L  A  L  W  Q  D  F  N  N  V   504
GAC CGC AGT CCG ACG CAA TTT TGG GAT GCG GAA CAC GGT CAA GGG ACG ATA GAC TGA TCTTCCTCTA  1656
 D  R  S  P  T  Q  F  W  D  A  E  H  G  Q  G  T  I  D                522

GAACTATGTATATCACTTCTAATTCTTTCATCAGAGCTTCACCAAGACAAGAAATTCTGTGTGTGTGTGTCTTTTGTTTTGAATTC  1742
TAGTCTGTTTTATTTCAAATTGCGCTTTAACAAACTTGCTTTTCACAAACCGGTGCAGTGTCATCAGAAATCTCTTCAAAAATTTA  1828
AACTTCAGTATTAATAATCAAAATTCAGAAAACCTTTCCTTTTCCATTTCACCTCAAAACTTCTCTTGTTCTTTCAAAAGTTCATC  1914
ATCAAGATGTAGAGTAATTTTTTTTTAAATTTTCATCTCTCTTTCAAACTGACTCACAACAATTAAACTACCGGAATATATTTCAT  2000
ATTTTCAGTCTCTCAAAAAATTGTTGTGTGGATTACACGTGTCTTATGTTGTGACTTGCTACCTTAATTTCAGCTTCTCCCCCCTT  2086
TTGACTTTCCCTACGAACTCAATTGATCTGGATGAAAATCTCATTTCATCTTGCCATATTTCAAAATTAAATTTGCCAAAAATGTG  2172
TTATTCTAGTCCTTTCTTTATATCTGAAACCTGTTCAAGACTGTCTCTATGAACCATTTCCTTTTTTTTTTTTATATAGGAAATCT  2258
CTTATCCGTTTTTTTTTCTCTGTAACCACGTTTTTATACCTTCTTTTCTTTTCCAATAAACCAAATAATGGTAAAACGTCGACATT  2344
TATTTTTAGTGTGGAGACGAATAAAGTTTTAAATTATAAAAAAAAAAAAAA                                      2394
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FIGURE 7.—Sequence of the *unc-7* message and putative protein product as determined from the cDNA clone SP#8 and RACE-PCR analysis of *unc-7* transcripts. The 5′ end of the cDNA clone represented in SP#8 contained 14 nucleotides not found in the genomic sequence for *unc-7*. This sequence, CTGCGGACTTTGCG, was joined to the *unc-7* transcript sequence beginning at nucleotide position 12 (GACTTGG. . .), but no evidence of this sequence was found in any of the RACE-PCR products (see text). RACE-PCR analysis indicated that the *unc-7* message is *trans*-spliced with SL1, and this sequence is indicated with italics. Possible poly(A) addition signals are underlined. Position of the *bx5::Tc1* insertion is indicated. The sequence of the *unc-7* message will appear in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession no. Z19122.

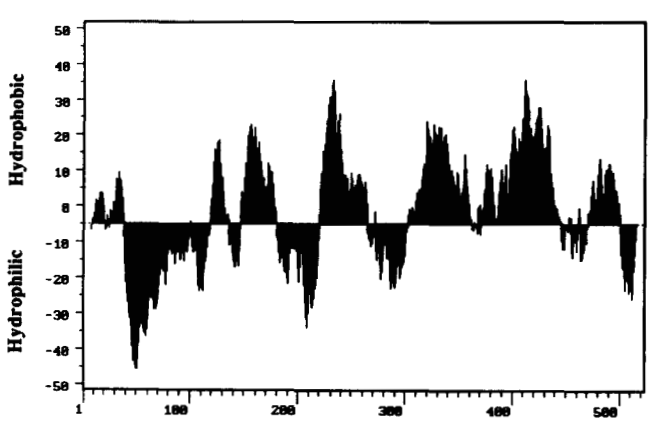

FIGURE 8.—KYTE and DOOLITTLE (1982) hydropathy profile of the predicted *unc-7* protein product using an interval of 15 amino acids. Potential transmembrane segments are centered around amino acid numbers 160, 230, 330 and 420.

duplication had been lost at P_1), we used a strain of genotype *unc-93 III; unc-3 unc-7(bx5) osm-1 sup-10(mn219) X; mnDp3(X;f)[unc-3(+) osm-1(+) sup-10(+)]*. The gain-of-function *unc-93* mutation confers a "rubberband" phenotype (GREENWALD and HORVITZ 1980). Rubberband animals have long thin bodies and assume abnormal postures when not moving; they move in a slow and uncoordinated manner, and they recoil and then quickly relax when touched on the head. They are also egg-laying defective. The loss-of-function mutation *sup-10(mn219)* is a recessive suppressor of *unc-93* (GREENWALD and HORVITZ 1980), *i.e.*, the rubberband phenes–uncoordination and egglaying defect–are suppressed when *sup-10(+)* is missing from body muscle cells and egglaying muscles as a consequence of duplication loss at P_1 (Figure 9). $P_1(-)$ mosaics also segregate only nullo-duplication progeny, because the germ line descends from P_1.

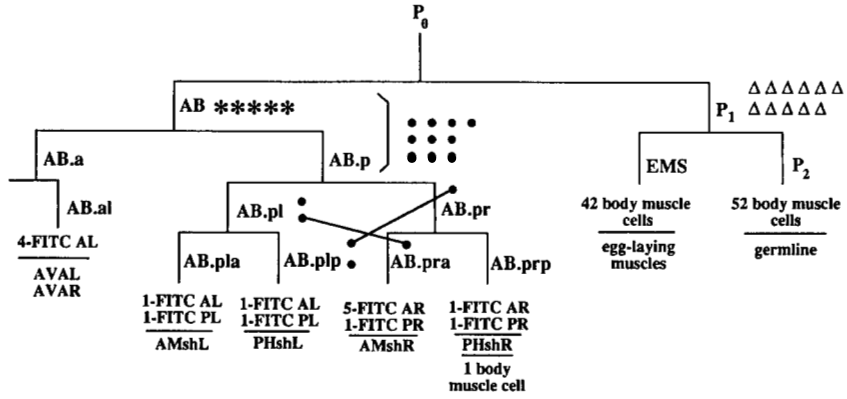

FIGURE 9.—Early embryonic lineages of cells relevant to the genetic markers used in mosaic analysis. Lineages are derived from SULSTON *et al.* (1983). Sixteen neurons in wild-type animals stain with FITC, six each of the left amphid (AL) and right amphid (AR) and two each of the left phasmid (PL) and right phasmid (PR). The single sheath cells of the left amphid (AMshL), right amphid (AMshR), left phasmid (PHshL) and right phasmid (PHshR) are responsible for the FITC staining conferred by *daf-6(+)*. All 11 $P_1(-)$ mosaics, denoted by open triangles in the lineage diagram, were non-Unc-7. Mosaic animals exhibiting the Unc-7 phenotype by virtue of *unc-7(+)* duplication loss were often found to be AB(−) or AB.p(−) mosaics. For example, among 14 *daf-6* mosaics, denoted by filled circles, 10 showed no FITC staining and were therefore either AB(−) or AB.p(−) mosaics. Each of the other four showed patterns of staining indicative of early losses in the AB.p lineage; two of these animals exhibited evidence of consecutive losses–AB.pl(−) and AB.pra(−) in one case and AB.pr(−) and AB.plp(−) in the other. Among 10 *osm-1* mosaics, five showed no FITC staining, indicative of loss at AB (denoted by asterisks); four of the others showed evidence of duplication loss among descendants of AB.p, but we did not identify the specific nonstaining cells and therefore have not marked specific points of duplication loss in the figure.

The focus of *unc-3* action, which has a different effect on animal movement, is largely among descendants of AB.p (HERMAN 1987). The Unc-3 phenotype is stronger than Unc-7 and hence epistatic to it. The *osm-1* mutation acts cell autonomously to prevent the staining by FITC of 16 neurons (HERMAN 1984; HEDGECOCK *et al.* 1985; PERKINS *et al.* 1986), which descend from AB.a and AB.p (Figure 9). $P_1(-)$ mosaics are non-Unc-3, FITC-staining (because of the presence of *unc-3(+)* and *osm-1(+)* among descendants of AB), and nonrubberband, and they segregate only Unc-3, rubberband, FITC-nonstaining self progeny. We screened for such animals and asked whether they were Unc-7 or not. Among about 30,000 duplication-bearing animals screened, 11 $P_1(-)$ mosaics satisfying all of the above criteria were found. All were non-Unc-7, *i.e.*, they showed wild-type coordination; and no Unc-7, nonrubberband animals were found. These results support the view that expression of *unc-7(+)* among descendants of P_1 is not needed to promote coordinated movement.

We used three other strains to screen for animals exhibiting an Unc-7 phenotype while carrying an *unc-7(+)* duplication in the P_1 lineage; such animals were analyzed with respect to another genetic marker carried by the duplication to ascertain where in the lineage the duplication was lost. The genotype of the first strain was *daf-6 unc-7(e5) sup-10(n983); mnDp14*. Self progeny lacking the duplication altogether displayed the rubberband phenotype (described above for *unc-93*) as a consequence of the gain-of-function *sup-10(n983)* mutation (GREENWALD and HORVITZ 1986), the focus of which is muscle (VILLENEUVE and MEYER 1990). Nonduplication-bearing animals also kink when moving in the forward direction as a consequence of the *unc-7* mutation, and they fail to stain with the dye FITC because of the *daf-6* mutation. The *daf-6(+)* function is required in the four sheath cells, AMshR, AMshL, PHshR and PHshL, to promote uptake of dye by neurons of four sensilla: right amphid, left amphid, right phasmid and left phasmid, respectively (ALBERT, BROWN and RIDDLE 1981; HERMAN 1987); the four sheath cells all descend from AB.p (Figure 9). Duplication-bearing animals are wild-type because the wild-type alleles are in each case dominant. The incidence of nonstaining sensilla in wild-type animals was less than about 2% per sensillum. We screened the self progeny of duplication-bearing animals for Unc-7 nonrubberband animals. Among about 13,500 duplication-bearing animals screened, 14 Unc-7 nonrubberband mosaics were found. Other Unc-7 candidates were isolated, but progeny testing showed that they resulted from duplication breakdown (HERMAN 1984; VILLENEUVE and MEYER 1990). All 14 Unc-7 nonrubberband mosaics segregated wild-type (including FITC-staining) self progeny (as well as *unc-7 osm-1 sup-10* self progeny), which showed that the mosaic parents had maintained *mnDp14* in their germ lines and hence the descendants of P_1. Ten of the 14 showed complete absence of FITC staining, suggesting that duplication loss had occurred at AB or AB.p (Figure 9). Of the remaining four animals, one showed FITC staining of neurons of right amphid and right phasmid only, which suggested duplication loss at AB.pl, the progenitor of both AMshL and PHshL (Figure 9). A second animal

showed staining of only the right phasmid neurons; the simplest explanation for this mosaic would be consecutive losses at AB.pl and AB.pra. Such consecutive losses have been previously described (E. HEDGECOCK, personal communication). The third mosaic animal showed neurons only in the left amphid staining; this pattern also could be explained by consecutive losses, at AB.pr and AB.plp. The fourth animal lacked FITC staining only in the neurons of the left phasmid, suggesting duplication loss at AB.plp or possibly later in the lineage.

We note that all 14 Unc-7 nonrubberband mosaics showed evidence of duplication loss by AB or AB.p or early descendants of AB.p. It therefore seems likely that the focus of action of *unc-7* is diffusely spread among several descendants of AB or AB.p. This conclusion was supported by mosaics generated from two other strains. One of these had the genotype *unc-7(bx5) osm-1 sup-10(n983) X; mnDp3(X;f)[unc-7(+) osm-1(+) sup-10(+)]*, and the other was identical except that the duplication *mnDp14* was used in place of *mnDp3*. The principal difference between these strains and the first one, apart from a different duplication in one case, was the substitution of *osm-1* for *daf-6*. As mentioned previously, the *osm-1* mutation acts cell autonomously to prevent the staining by FITC of 16 neurons which descend from AB.a and AB.p (Figure 9). Ten Unc-7 nonrubberband mosaics were found (one of which came from the *mnDp3* strain); all segregated wild-type (including FITC-staining) self progeny. Five of the 10 failed completely to stain with FITC; they thus appeared to be AB(−) mosaics (Figure 9). Among the other five animals, four showed some loss of staining. None of the four staining patterns was consistent with a simple duplication loss at AB.a(−) or AB.p(−); duplication losses appeared to have occurred later in the AB.p lineage, although some patterns were complex and may have involved more than one loss.

DISCUSSION

We have identified *unc-7* DNA by showing that restriction fragment dimorphisms are associated with each of nine independent *unc-7* alleles. Five of the alleles were spontaneous mutations identified in mutator strains, and four were induced by gamma irradiation. Additional evidence for our identification of *unc-7* was provided by examination of two spontaneous revertants derived from *unc-7(bx5::Tc1)*; in each case, reversion of the uncoordinated phenotype was correlated with excision of *bx5::Tc1*.

The region defined by these mutations encodes a 2.7-kb mRNA from which a predicted protein of 60 kd may be translated. The hydropathy profile of this protein suggests it is membrane associated, with as many as four transmembrane domains predicted. The primary sequence does not share homology with any other protein sequence currently entered in databanks.

Mutations in the large intron affect expression: A striking feature of the *unc-7* locus is the presence of an unusually large (approximately 18-kb) intron near the 5′ end of the message. The 5′ end of the wild-type *unc-7* message begins with the *trans*-spliced SL1 leader (KRAUSE and HIRSH 1987) joined to a 69-nucleotide noncoding exon, the 3′ end of which is spliced across the 18-kb intron to an exon that contains the beginning of the open reading frame. Surprisingly, all four of the gamma-ray-induced *unc-7* alleles are associated with DNA breakpoints restricted to the large intron; thus, these mutations appear to leave the protein coding sequence intact. Northern analysis revealed the presence of two transcripts for each of these mutants, one transcript smaller and one larger than the wild-type *unc-7* transcript. Two of the rearrangements appear to be translocations that completely separate the 5′ noncoding exon from the rest of the message; we suggest that for these mutations some transcription initiates within the large intron. A third rearrangement, *mn383*, is a large deletion mutation wholly within the large intron, and we confirmed by sequence analysis of RACE-PCR products that some transcriptional initiation in the *mn383*-bearing mutant does begin within what remains of the intron. The fourth gamma-ray-induced allele, *mn384*, confers a weak phenotype and has a 2-kb insertion in the large intron. RACE-PCR analysis also revealed the presence of transcripts initiating within the large intron in *mn384* mutants; some of the RACE-PCR products of these transcripts were shown to have been *trans*-spliced to SL1. We conclude that sequences within the 18-kb wild-type intron are necessary for proper transcriptional initiation.

Although we have clearly shown that transcriptional initiation is altered in two mutants containing rearrangements in the first intron, we do not know the relationship between the transcripts analyzed by RACE-PCR and the two novel transcripts defined by northern analysis of these mutants. It is not clear that altered transcriptional initiation alone accounts for the appearance of the novel transcripts. It is possible that two aberrant transcriptional initiation sites are involved in generating the two novel transcripts, but other possibilities accounting for their appearance may include alternative splicing, differences in poly(A) addition, or increased expression of an *unc-7* transcript not usually detected by northern analysis in wild-type. Although only a single *unc-7* transcript was apparently expressed in wild-type, additional transcripts of similar size or low abundance may be produced.

We do not know why the rearrangement mutants

exhibit the Unc-7 phenotype, since the protein coding sequence does not seem to be disrupted. One possibility is that translation of an ORF (open reading frame) originating at an upstream AUG may interfere with translational initiation from the wild-type site. Such an AUG is present in the intron 53 nucleotides upstream of the second exon, out of frame with respect to the predicted wild-type *unc-7* reading frame, and this AUG was included in many of the mutant transcripts analyzed (data not shown). This upstream AUG begins a short ORF that ends downstream of the wild-type translational initiation site. Alternatively, the rearrangement mutants may exhibit the Unc-7 phenotype because the level of translation of the mutant messages may be too low, or sequences within the large intron that are necessary for proper tissue-specific transcription may be disrupted.

Null phenotype: We cannot be certain that we have defined the phenotype of an *unc-7* null mutant. All of the Tc1 mutants produce a transcript of approximately wild-type size, and all of the gamma-ray-induced mutants produce transcripts that seem to be unaffected in the protein coding region of the gene. In each of these cases, it is possible that at least some wild-type *unc-7* protein is being produced. Northern analysis of *wd7* failed to detect any *unc-7* message, which suggests it may be a null mutation. However, the *wd7* rearrangement maps near the 3′end of the coding sequence, and it is possible that at least some functional *unc-7* protein is made before the message is rapidly degraded. The Unc-7 phenotype conferred by *wd7* is indistinguishable from that conferred by all other alleles we have studied except the weaker alleles *e65* and *mn384*. Some preliminary screens for lethal mutations associated with *unc-7* using gamma-irradiation have so far produced only large deficiencies (unpublished results).

Role of *unc-7* in coordinated locomotion: Our molecular and genetic characterization of the *unc-7* gene was undertaken to determine how the *unc-7* product is involved in coordinated locomotion. The results presented here suggest that the *unc-7* product is an integral membrane protein probably required in neurons but not required in muscle cells. Any further formulation of a model for *unc-7* function must also take into account three areas of study pertaining to the biological traits associated with *unc-7* mutants.

First, mutations in *unc-7* are able to suppress the hypersensitive response of *unc-79* and *unc-80* mutants to certain volatile anesthetics such as halothane (MORGAN, SEDENSKY and MENEELY 1990). We have noted that the *unc-7* alleles *mn382*, *wd2*, *wd6*, *wd7* and *bx5::Tc1* also suppress this hypersensitivity (data not shown). The traditional view of anesthetics has been that they exert their effects on ion channels, but indirectly, by binding to lipid membranes (discussed in MATTHEWS 1992). Recent evidence concerning the effects of anesthetic optical isomers on nerve ion channels, however, has been taken to support the view that the targets for anesthetic action are proteins associated with ion channels rather than the lipid bilayer (FRANKS and LIEB 1991; MATTHEWS 1992). MORGAN, SEDENSKY and MENEELY (1990) have suggested that mutations that affect the response of *C. elegans* to halothane–in the genes *unc-79*, *unc-80* and the suppressor genes such as *unc-7*–may be acting directly on proteins of ion channels. The proposed transmembrane location of the *unc-7* protein would be consistent with this hypothesis, whether the protein be a structural component or a regulatory subunit of an ion channel.

Second, evidence suggesting a role for the *unc-7* protein in the action of ion channels comes from studies of ivermectin resistance. Ivermectin is an anthelmintic that binds to membranes and is thought to modulate chloride channel activity. The compound was recently shown to affect chloride currents elicited in Xenopus oocytes after injection with *C. elegans* mRNA (ARENA *et al.* 1991). Resistance of *C. elegans* to ivermectin may in some instances involve mutations in the *unc-7* gene (C. JOHNSON and P. MORGAN, personal communication). The *unc-7* protein could thus be involved in the functioning of a chloride channel.

Third, reconstruction of the anterior ventral nerve cord of an *unc-7* mutant animal from electron micrographs of serial sections has revealed aberrant gap junction formation in some of the neurons involved in regulating locomotion (J. WHITE, E. SOUTHGATE and N. THOMSON, personal communication). A model for the roles of different classes of motor neurons in controlling the muscle contractions involved in locomotion has been proposed (CHALFIE *et al.* 1985; CHALFIE and WHITE 1988). The model is based on the known synaptic relationships among the various neurons and body muscles (WHITE *et al.* 1976, 1986), the effects on locomotion of laser ablation of different classes of neurons (CHALFIE *et al.* 1985), and extrapolation from electrophysiological and neurotransmitter localization studies of the similar but larger motor nervous system of the nematode *Ascaris lumbricoides* (JOHNSON and STRETTON 1985; STRETTON *et al.* 1985; WALROND and STRETTON 1985a,b; WALROND *et al.* 1985).

In the adult, body muscle cells on the dorsal side of the animal are innervated by the DA, AS, DB and DD classes of motor neurons. Their counterparts innervating body muscle on the ventral side include the motor neuron classes VA, VB and VD. The model proposes that the DD and VD classes are reciprocal inhibitors, receiving input from motor neurons on one side of the animal and inhibiting muscle contraction on the opposite side. The other motor neuron classes are believed to be stimulatory, and they receive input from several classes of interneurons. Two pairs

of large diameter interneurons, the AVA and AVB pairs, are presumed to play principal roles in regulating motor neuron activity. The DA, VA and AS motor neuron classes receive input from AVA and are necessary for backward movement. The DB and VB motor neurons receive input from AVB and together are necessary for directing forward locomotion.

In an *unc-7* animal examined by J. WHITE, E. SOUTHGATE and N. THOMSON (personal communication), the AVA interneurons formed gap junctions with VB and DB motor neurons in addition to their normal synaptic partners. This alteration in the wiring pattern might account for the uncoordination of *unc-7* animals: the AVA interneurons responsible for backward movement in wild-type animals may, in the mutants, be communicating with the motor neurons involved in forward locomotion.

How might a role in the function of ion channels be reconciled with the electron micrographic analysis showing that *unc-7* mutants display ectopic gap junctions between the AVA interneurons and the VB and DB motor neurons? It seems unlikely that *unc-7* encodes a component of the gap junctions. The *unc-7* protein sequence does not show any similarity to the conserved sequences which are characteristic of the known gap junction proteins (BEYER, PAUL and GOODENOUGH 1990; BENNET *et al.* 1991). Furthermore, loss-of-function mutations (which the *unc-7* alleles appear to represent) in a gap junction protein would be expected to decrease gap junction formation rather than lead to ectopic formation. It seems more likely that mutations in *unc-7* affect the membrane physiology of the involved cells, by affecting, for example, the function of an ion channel, with the result that the unusual gap junction connections are made.

How would a channel hypothesis account for the uncoordinated phenotype? As noted above, one possibility is that the ectopic gap junctions present in *unc-7* mutants result in neuronal miscommunications, which by themselves are sufficient to account for the uncoordination. Alternatively, the *unc-7* product may have an additional role in neuronal signal transmission, not revealed by electron microscopy of the mutants, that contributes to coordinated movement.

Focus of action: Another issue pertaining to *unc-7* function is whether the *unc-7* protein is required in all neurons or just a subset. We suggest from our mosaic analysis that the anatomical focus of *unc-7* action is diffusely distributed among descendants of AB.p; we cannot rule out a requirement in the AB.a lineage as well, but we did not identify any animals in which duplication loss at AB.a alone accounted for an Unc-7 phenotype. Both AVA interneurons, which may be involved in the ectopic gap junctions in *unc-7* animals (J. WHITE, E. SOUTHGATE and N. THOMSON, personal communication), descend from AB.al, so we think it unlikely that *unc-7(+)* is required solely in the AVA neurons. All but one of the 75 ventral and dorsal cord motor neurons descend from AB.p (the one exceptional neuron descends from AB.a); we therefore favor the idea that *unc-7(+)* is required in motor neurons (although a hypodermal requirement has not been excluded). In *C. elegans*, motor neurons arise both embryonically and post-embryonically (SULSTON 1976; SULSTON and HORVITZ 1977; SULSTON *et al.* 1983). Newly hatched animals, which exhibit a strong Unc-7 phenotype, have only 22 cord motor neurons–10 derived from AB.pl, 11 derived from AB.pr and one derived from AB.a (SULSTON *et al.* 1983). Seven of these are members of the DB class, which show the aberrant gap junctions to the AVAs; of these, three descend from AB.pl, three descend from AB.pr, and one descends from AB.a. The fact that *unc-7* mutants are severely uncoordinated as newly hatched larvae suggests that *unc-7(+)* is required in embryonically derived neurons. Two lines of evidence suggest that the *unc-7(+)* product also plays a role postembryonically. First, one of the VB neurons, all of which are postembryonically derived from AB.p, exhibited ectopic gap junctions to the AVA interneurons in an *unc-7* mutant animal examined by electron microscopy of serial sections (J. WHITE, E. SOUTHGATE and N. THOMSON, personal communication). Second, our northern analysis indicates that the level of *unc-7* transcript peaks postembryonically, during larval stages L1–L3; this suggests that the *unc-7* product is required during the periods when postembryonically derived motor neurons are generated and extending their processes. An interesting possibility is that the unusually large *unc-7* intron plays a role in regulating the temporal and cell-specific expression of *unc-7*. Information concerning the location and developmental timing of *unc-7* protein expression would obviously be very useful in further assessing the role of the *unc-7* gene in coordinated locomotion.

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Note added in proof: The predicted amino acid sequences of the Drosophila *passover* and *ogre* proteins appear to bear significant similarity to that of *unc-7* (S. KRISHNAN, E. FREI, G. SWAIN and R. WYMAN, personal communication).

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