# Isolation and Characterization of Lethal Mutation near the *unc-29* (LG I) Region of *Caenorhabditis elegans*

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The *unc-29* region on the chromosome I of *Caenorhabditis elegans* has been mutagenized in order to obtain lethal mutations. In this screen, the uncoordinated phenotype of *unc-29* (*e193*) mutant was used to identify any lethal mutations closely linked to the *unc-29* gene, which encodes a subunit of nicotinic acetylcholine receptors. We have isolated six independent mutations (*jh1* to *jh6*) out of approximately 5,200 ethyl methanesulfonate (EMS) treated haploids. Four of the six mutations demonstrated embryonic lethal phenotypes, while the other two showed embryonic and larval lethal phenotypes. Terminal phenotypes observed in two mutations (*jh1* and *jh2*) indicated developmental defects specific to posterior part of embryos which appeared similar to the phenotypes observed in *nob* (no back end) mutants. Another mutation (*jh4*) resulted in an interesting phenotype of body-wall muscle degeneration at larval stage. These mutations were mapped by using three-factor crosses and deficiency mutants in this region. Here we report genetic analysis and characterization of these lethal mutations.

The free-living nematode *C. elegans* has become an ideal genetic model system. Several characteristics of *C. elegans* make a genetic approach very powerful and easily accessible. First, *C. elegans* has a relatively short life cycle. From a fertilized embryo to reproductive adult stage, it takes 3 days at 22℃ (Wood, 1988). Second, *C. elegans* has a hermaphroditic reproduction system. That is, a single animal can produce both sperm and oocyte so that a particular strain can be maintained without male-female matings at each generation (Schedl, 1997). Third, it is possible to store strains for a longer period of time by freezing them in liquid nitrogen. This convenient storage of strains becomes very useful when conducting an extensive mutant screen.

Recently, rapid progress in sequencing the entire genome of *C. elegans* has been made (Coulson A and Waterston R, personal communication). Approximately 70 Mb of DNA sequences have been accumulated and by sequence analysis more than 12,000 genes are being identified. Among these genes, approximately half of them are related to the genes whose products had been studied in other organisms (Coulson A and Waterston R, personal communication). Therefore, molecular cloning of any homologous gene of interest in *C. elegans* is greatly facilitated. Once the gene of interest is cloned using homologous sequences, the next step forward would be a functional study of that gene. One of the best ways to study gene functions at an

organism level is to study mutants.

To obtain mutations in particular genes, two different approaches are available. One is the so-called "reverse genetic" approach. In this approach, mutagenesis is carried out first, then deletion mutations are identified by polymerase chain reaction (PCR) using the sequence information of the gene. The advantages of this approach are the powerful techniques of molecular biology to screen specific mutations in a targeted gene. On the other hand, it has a limitation of getting mostly deletion mutations because the PCR method can only detect such mutations. The other available approach is a classical mutant screen. After the mutagenesis is carried out, particular phenotypes are followed to isolate mutants. The advantages of this approach are that various kinds of mutations of the genes involved in particular cellular or developmental processes could be obtained since specific phenotypes were selectively screened. On the other hand, extensive characterization and mapping of mutations requires tremendous effort.

We have chosen the second approach to obtain mutations in the genes important for early developmental processes. Embryonic and larval lethal phenotypes were selected to isolate mutations occurring in essential genes for early development. However, instead of screening lethal mutations from the entire genome, we decided to focus on a specific region of the chromosome I near the *unc-29* region. Focused on a narrow region reduces much of the extensive mapping efforts. The *unc-29* region was chosen for the following reasons. First, the *unc-29* mutant shows an uncoordinated movement phenotype, Unc, which can be easily scored. This provides a tool to screen for lethal

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mutations tightly linked to the *unc-29* gene. Not only can this phenotype be observed under dissecting microscope but it can also be tested by levamisole treatment, which is known to be a strong agonist of the nicotinic acetylcholine receptor in *C. elegans*. A previous study has revealed that the *unc-29* gene encodes a subunit of nicotinic acetylcholine receptor and is expressed at neuromuscular junctions (Fleming et al., 1997).

Second, the *unc-29* region has been characterized as a gene-cluster region of chromosome I (Lewis et al., 1979), where many genes are densely located. This will increase the chance of isolating mutations in essential genes. Furthermore, this region of chromosome I has been completely sequenced (Coulson A and Waterston R, personal communication) and the sequence information is currently available. Once interesting lethal mutations are isolated from a genetic screen, molecular cloning and characterization of those genes carrying lethal mutations will be feasible.

Therefore, we have screened lethal mutations closely linked to the *unc-29* on chromosome I and isolated six independent mutations. Here we report the genetic analysis of these mutants.

## Materials and Methods

### C. elegans strains and culture

Animals were grown on the standard nematode growth medium plates (Sulston et al., 1988) at 20℃. The wild type (N2) strain and following mutant strains were obtained from *Caenorhabditis elegans* Genetic Center at the university of Minnesota, U.S.A.

CB193  *unc-29(e193)* (Brenner, 1974)
CB2167  *dpy-5(e61) unc-13(e1091)* (III, personal communication)
MT2180  *nDf23/unc-13(e1091) lin-11(n566)* (Ferguson et al., 1985)
MT2138  *nDf29/unc-13(e1091)I in-11(n566)* (Ferguson EL and Horvitz HR, personal communication)

Levamisole resistance tests for *unc-29* mutant animals were performed by incubating animals with 1 mM levamisole in M9 buffer (22 mM $KH_2PO_4$, 42 mM $NaHPO_4$, 86 mM NaCl, 1 mM $MgSO_4$) as described previously (Fleming et al., 1993; Lewis et al., 1980).

### Mutagenesis

Homozygous *unc-29(e193)* animals were mutagenized in 47.1 mM or 94.2 mM EMS (ethyl methanesulfonate) for 4 hours at room temperature. After EMS treatment, the animals were washed twice in M9 buffer (22 mM $KH_2PO_4$, 42 mM $NaHPO_4$, 86 mM NaCl, 1 mM $MgSO_4$), modified from Brenner (1974). Then L4-stage larvae ($P_0$) were picked onto plates in groups of five and mated with non-mutagenized males of *dpy-5(e61) unc-13(e1091)/unc-29(e193)*. These males were made by a

cross between *dpy-5(e61) unc-13(e1091)* homozygous hermaphrodites and *unc-29(e193)* homozygous males. From $F_1$, wild type animals carrying a mutagen-exposed *unc-29(e193)* chromosome and a non-mutagenized *dpy-5(e61) unc-13(e1091)* chromosome were picked onto individual plates and allowed to self-fertilize. In $F_2$, failure to segregate progeny showing Unc (uncoordinated movement) phenotype indicating a new lethal mutation is closely linked to the *unc-29* locus. Mutant candidates which failed to segregate any progeny with Unc phenotype were picked as a clone and maintained as heterozygotes [*unc-29(e193) let/dpy-5(e61) unc-13(e1091)*]. These heterozygous animals were used for the complementation test with deficiencies and for the three factor crosses.

### Characterization of mutant embryos and larvae

To observe terminal phenotypes of homozygous lethal mutant animals, dead embryos or larvae were obtained from self-fertilized progeny of heterozygotes [*unc-29 (e193) let/dpy-5(e61) unc-13(e1091)*]. Twelve to fifteen adult hermaphrodites heterozygous for deficiency or lethal mutation were allowed to lay eggs for 10 hours. After removal of these adults, the number of total embryos were counted under a dissecting microscope and these embryos were allowed to develop for 16 hours. Then the number of unhatched embryos was counted. After 24 hours, the number of arrested larvae was counted. All procedures were performed at 20℃. The unhatched embryos and arrested larvae were observed under the Nomarski microscope (Nomarski, 1955) and stained with antibody.

### Immunofluorescent staining

Immunofluorescent staining was performed as described previously (Ahnn et al., 1994; Lee et al., 1997). In brief, animals were attached on polylysine-coated multi-well slides. Slides were frozen on a dry ice-chilled aluminum block and permeabilized by freeze-cracking. Samples were dehydrated and rehydrated through a series of incubations in methanol solutions [100%, 75%, 50% and 25% (v/v)] and washed in Tween-TBS buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.8, 0.1% Tween 20). Primary antibody was incubated on the slides for 5 to 6 hours at room temperature in humidifying chamber. Slides were washed three times in Tween-TBS buffer, and then fluorescein-conjugated secondary antibody was incubated for 5 to 6 hours. After washing three times in Tween-TBS buffer, samples were mounted in mounting medium (80% glycerol, 1% N-propyl gallate) and observed with a fluorescent microscope Olympus BX50.

### Three-factor crosses

To map the lethal mutations, we determined the position of mutations relative to the *dpy-5* and the *unc-13* loci.

**Table 1.** Mapping by three-factor crosses

| Position of lethal mutation | Phenotype of recombinant (F₁) | Progenies expected from recombinant (F₂) |
|---|---|---|
| *let*    *unc-29* / *dpy-5*   *unc-13* | Dpy-5 | Dpy-5, Dpy-5Unc-13, Dpy-5 Unc-29 |
| | Unc-13 | Unc-13, Dpy-5Unc-13, Let |
| *let*    *unc-29* / *dpy-5*   *unc-13* | Dpry-5 | Dpy-5, Dpy-5Unc-13, Let Dpy-5, Dpy-5Unc-13, Dpy-5 Unc-29 |
| | Unc-13 | Unc-13, Dpy-5Unc-13 |
| | | Unc-13, Dpy-5Unc-13, Let |
| *unc-29* *let* / *dpy-5*   *unc-13* | Dpy-5 | Dpy-5, Dpy-5Unc-13, Let |
| | Unc-13 | Unc-13, Dpy-5Unc-13 |

Recombinants (Dpy-5 and Unc-13) were picked and put on separate plates and the phenotypes of their progeny were observed. For example, in the case that a lethal mutation was located at the left side of *dpy-5* locus, recombination with the Dpy-5 phenotype [genotype should be *dpy-5(e61) unc-29(e193)/dpy-5(e61) unc-13(e1093)*] segregates only Dpy-5, Dpy-5 Unc-13, and Dpy-5 Unc-29. Recombination with the Unc-13 phenotype [genotype should be *let · unc-29(e193)/dpy-5(e61) unc-13(e1093)*] segregates only Unc-13, Dpy-5 Unc-13, and lethal embryos or larvae. The scheme for these three-factor crosses is shown in Table 1.

## Complementation test with deficiency

To map lethal mutations, we tested whether the mutation could be complemented by deficiencies (*nDf23* and *nDf29*) deleting the *unc-29* region. Males carrying a lethal mutation were obtained by crossing *dpy-5(e61) unc-13(e1091)/++* males into *unc-29(e193) let/dpy-5(e61) unc-13(e1091)* hermaphrodites. Eight wild type males were picked from the progeny and mated with four *nDf23/unc-13(e1091) lin-11(n566)* or *nDf29/unc-13(e1091) lin-11(n566)* hermaphrodites. As shown in Fig. 1, one out of six offspring from the cross will be heterozygotes of *unc-29(e193) let* over deficiency chromosome. If the lethal mutation is located within the deleted region, these heterozygotes should be lethal. If the lethal mutation is located outside of the deleted region, these heterozygotes should be the Unc-29 phenotype. Therefore, the phenotypes of male progeny from the cross were carefully observed. More than three crosses were tested for each mutation.

## Results

### Isolation of lethal mutations near the unc-29 region of the chromosome I

In order to study genes involved in early development of *C. elegans*, a genetic screen for embryonic and larval lethal mutation was designed. In wild type animals, embryogenesis occurs very rapidly as reviewed in Fig. 2.
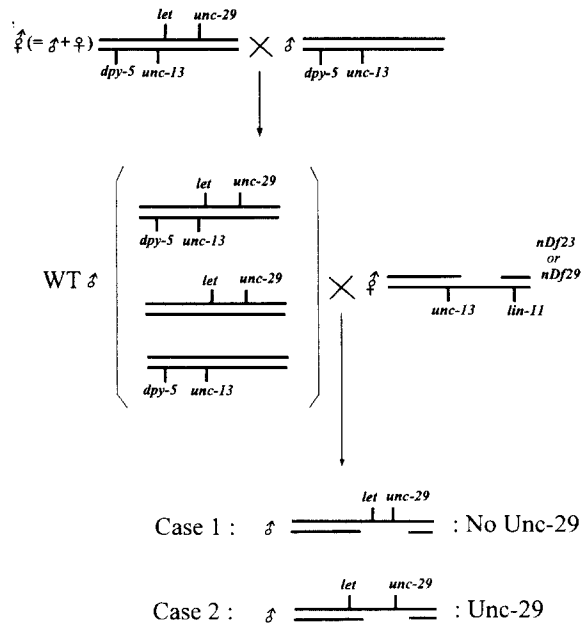


Case 1 : ♂ =ᴵᵉᵗ ᵘⁿᶜ⁻²⁹= : No Unc-29

Case 2 : ♂ =ᴵᵉᵗ ᵘⁿᶜ⁻²⁹= : Unc-29

**Fig. 1.** A schematic diagram of complementation test with deficiency. For simplicity, only a pair of chromosome I is drawn for male(♂) and hermaphrodite (♀). Each horizontal bar represents a haploid chromosome with genetic markers shown by vertical lines (*let*, *unc-29*, *dpy-5*, and *unc-13*). The gap within this horizontal bar shows the deleted region by the deficiencies (*nDf23* and *nDf29*). First, in order to obtain males which have a chromosome containing a lethal mutation, we crossed *dpy-5(e61)unc-13(e1093)/++* males into *unc-29(e193) let/dpy-5(e61) unc-13(e1093)* hermaphrodites. Wild type male progeny were picked and crossed into *nDf23* or *nDf29 /unc-13(e1091) lin-11(n566)* hermaphrodites. Mathematically, one third of crossed male gametes should contain the chromosome with the lethal mutation. The progeny from the second cross were observed to score any male progeny with Unc-29 phenotype.

After hatching, a larva (L1 stage) goes through three additional stages (L2, L3 and L4) to become a fully developed adult during the next 50 hours (Wood, 1980). Because we were interested in early events of development, phenotypes of embryonic lethal and early larval (L1 or L2) lethal were selected in this screen. Another aspect of this screen is that uncoordinated movement phenotype of the *unc-29(e193)* mutant was used to narrow down the genetic region where we intended to obtain mutations. The overall scheme of the screen is shown in Fig. 3. Details of manipulations are described in Materials and Methods.
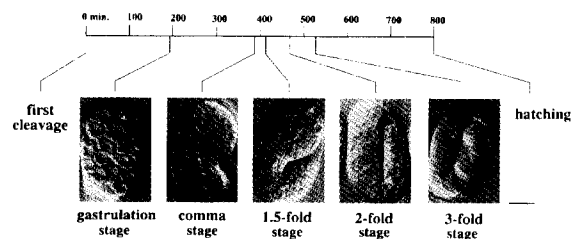


**Fig. 2.** Embryogenesis of wild type *C. elegans* at 22℃. The horizontal line at the top indicates the progression of embryogenesis from the first cleavage until hatching in minutes (Sulston et al., 1983). Representative stages of development are shown with Nomarski images of wild type embryo. Scale bar=50 μm.
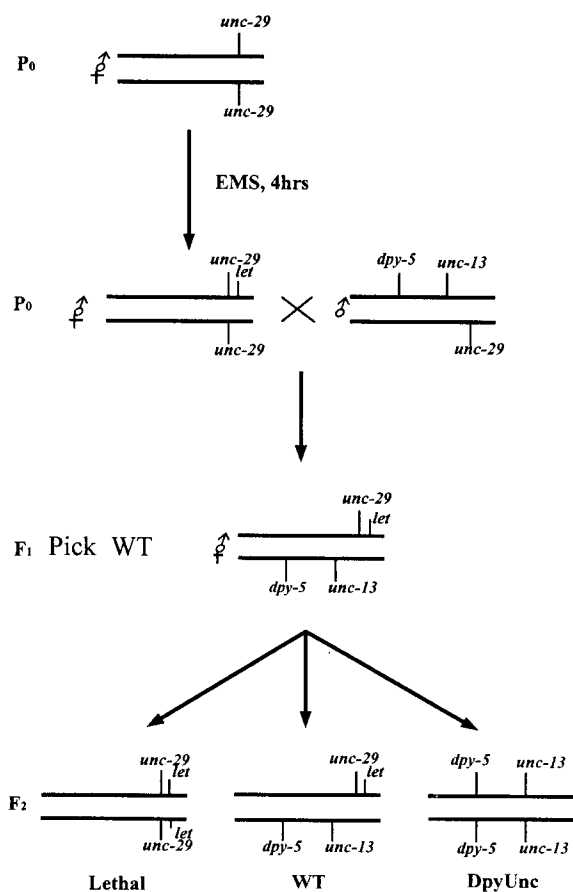
Fig. 3. A schematic diagram of mutagenesis at the region near the *unc-29* on LG I. For simplicity, only a pair of chromosome I is drawn for male ($\male$) and hermaphrodite ($\female$). Each horizontal bar represents haploid chromosome with genetic markers shown by vertical lines (*let*, *unc-29*, *dpy-5*, and *unc-13*), where (*let*) represents lethal mutation produced by this study.

Approximately 5,200 ethyl methanesulfonate (EMS) mutagenized haploids were screened. As a result, 6 lethal mutations were isolated near the *unc-29* region on chromosome I. These six lethal mutations were

designated as *jh1* to *jh6* and grouped into three classes based on their terminal phenotypes (Table 2). Our screen was for lethal mutations linked to *unc-29(e193)*, so new lethal mutations have been maintained as heterozygotes of [*unc-29(e193) let/unc-13(e1091) lin-11(n566)*], where *let* is a designation for lethal mutation. Eighteen to 27 percent of progeny from these heterozygotes were lethal (Table 2). For each homozygous lethal mutant, phenotypes were analysed in two ways. First, the arrested stages and the development of major tissues (gut granules, body-wall muscles, and pharynx) were observed under the Nomarski microscope to assess the degree of development. Second, immuno-staining with antibody against *myo-3* gene product, a myosin heavy chain isoform of body-wall muscles, was performed to assay formation of body-wall muscles. All the animals homozygous for mutations produced gut granules, body-wall muscles, and pharynx and these results are summarized in Table 2. Among these mutants, interesting phenotypes were characterized in detail:

*jh1* and *jh2*: Homozygous animals of *jh1* and *jh2* both arrested at embryonic stage and were very similar in their terminal phenotypes when these embryos were observed under the Nomarski microscope (Fig. 4). Interestingly, the anterior parts of embryos where the pharyngeal structure forms seemed to develop fully, but the posterior parts developed abnormally. That is, morphology of the pharyngeal structures seemed normal and pharyngeal muscles showed active pumping. However, the posterior half of the embryo failed to elongate and remained as clumped cells (Fig. 4B and 4C). In the case of the *jh1* homozygotes, during elongation of the embryo hypodermal cells failed to constrict at the posterior part of the embryo, which would result in similar phenotypes (Fig. 4A and 4B). For the homozygous embryos of *jh2*, many extra pro-grammed cell deaths were observed (Fig. 4D). To assess the formation of body-wall muscles in these embryos,

**Table 2.** Characterization of mutants

| Group[a] | Mutation | Concentration of EMS (mM) | Arrested stage | Percent of arrested animals[c] (n) | Terminal phenotypes[d] | | | Antibody staining with 5-6[e] |
|---|---|---|---|---|---|---|---|---|
| | | | | | Gut granules | Body-wall muscles | Pharynx | |
| 1 | *jh1* | 47.1 | embryonic[b] | 26.8(3,230) | + | + | + | + |
| | *jh2* | 94.2 | embryonic[b] | 25.3(3,396) | + | + | + | + |
| 2 | *jh3* | 94.2 | 3-fold | 12.6(911) | + | + | + | + |
| | | | early larval | 7.1(911) | + | + | + | + |
| | *jh4* | 94.2 | 2-fold to 3-fold | 5.2(2,178) | + | + | + | + |
| | | | early larval | 14.1(2,178) | + | + | + | + |
| 3 | *jh5* | 94.2 | before-comma to 3-fold | 17.9(1,062) | + | + | + | + |
| | *jh6* | 94.2 | 3-fold | 23.9(1,351) | + | + | + | + |

[a]Mutations were grouped into three classes based on their arrested phenotypes.
[b]Pharyngeal parts of these mutations developed well, but posterior parts were abnormal (see Results and Fig. 4).
[c]Percent of arrested animals out of progeny obtained from the heterozygotes [ *unc-29(e193)let/unc-13(e1091)lin-11(n566)*, *let* is a designation for lethal mutation]. (n) indicates the total number of embryos counted.
[d]"+" indicates that development of each tissue was normally initiated, or positive staining.
[e]5-6: a monoclonal antibody against the *myo-3* gene product, one of the two isoforms of myosin heavy chain of body-wall muscles.
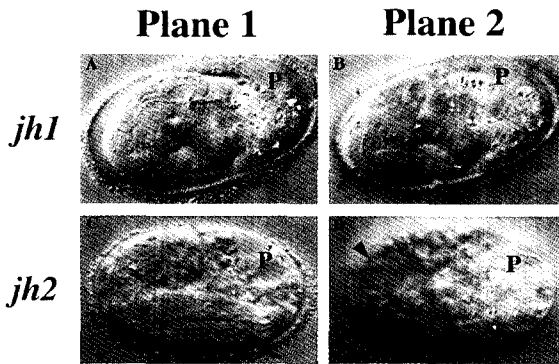
## Plane 1    Plane 2



**Fig. 4.** Nomarski images of embryos homozygous for *jh1* and *jh2* mutations. Images of the same arrested embryos which are homozygous for *jh1* [(A) and (B)] and *jh2* [(C) and (D)] at two different focal planes. Morphology of pharynx and pharyngeal muscle structure seemed to be normal in *jh1* and *jh2* homozygotes (arrows). Posterior parts (upper right corners marked by "P") of the *jh1* and the *jh2* homozygous embryos showed no indication of morphogenesis and seemed to have ruptured. In the case of the *jh2* homozygote, many programed cell deaths were observed (arrow heads). Scale bar=10 μm.

immunofluorescent staining with antibody against the *myo-3* gene product was performed. As shown in Fig. 5, the four quadrants of body-wall muscles stained strongly at the anterior part of embryos. However, staining was not observed at the posterior parts of embryos, which suggested that body-wall muscles failed to develope at posterior part of the embryo (Fig. 5A and 5C). In summary, homozygotes of *jh1* and *jh2* were embryonic lethal and defective in the formation of posterior structures including body-wall muscles.

*jh4*: Homozygous animals of *jh4* were arrested at late embryonic stages (2-fold and 3-fold stages) and at early larval stage. Embryos arrested at 2-fold and 3-fold stages did not show any morphological defects when observed under Nomarski microscope. The immunofluorescent staining pattern also looked quite
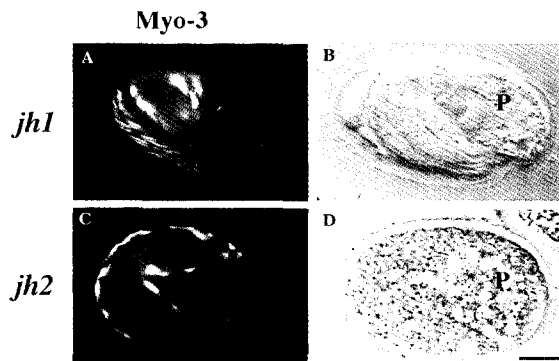
## Myo-3



**Fig. 5.** Immunofluorescent staining with antibody against myosin heavy chain isoform on homozygous embryos for *jh1* and *jh2*. Arrested embryos were stained with antibody against the *myo-3* gene products [(A) and (C)]. (B) and (D) show the same embryos with Nomarski view of (A) and (C) respectively. Two muscle quadrants are in focus (A, C) and body-wall muscles are formed normally only in pharyngeal part of the embryos [compare (A) and (B), and (C) and (D)]. Posterior parts of embryo which failed to stain with antibody were marked as "P" in (B) and (D). Scale bar=10 μm.
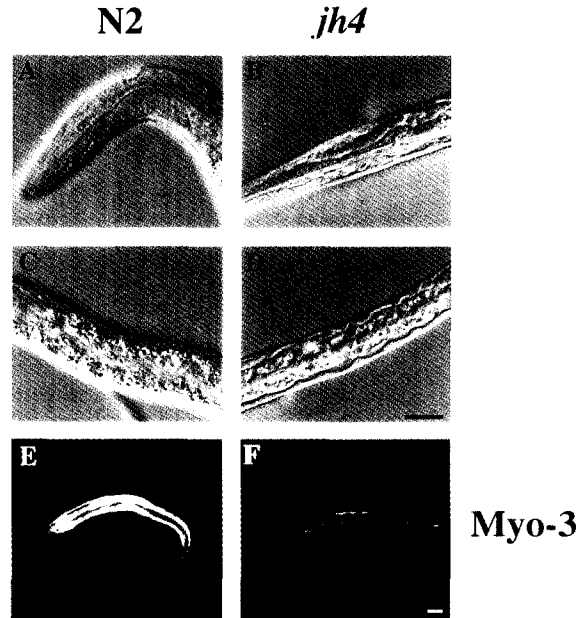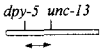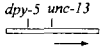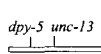
## N2    jh4



**Myo-3**
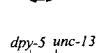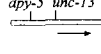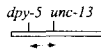
**Fig. 6.** Nomarski and immunofluorescent staining images of wild type and *jh4* homozygote larvae. (A) and (C) are Nomarski images of a wild type (N2) larva at similar stage to that of the *jh4* homozygotes. (B) and (D) are Nomarski im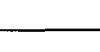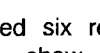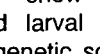ages of the *jh4* homozygotes. The body of the *jh4* homozygote is much thiner than wild type and the body wall muscle cells seemed degenerated, resulting large gaps between gut and hypodermis. (E) and (F) show N2 and *jh4* homozygote larvae stained with antibody against *myo-3* gene products. The staining of the *jh4* homozygote is much weaker compared to wild type (N2) and staining pattern was disorganized. Scale bar=10 μm.

normal when stained with antibody against the *myo-3* gene product (data not shown). However, the arrested larvae showed severe abnormalities (Fig. 6). Homozygous *jh4* larvae seemed much thinner than wild type larvae (Fig. 6B and D) and moved very slowly. When stained with antibody against the *myo-3* gene product, homozygotes *jh4* larvae showed much reduced staining compared to wild type (Fig. 6E and F). This appeared due to very thin body-wall muscles, which resulted from a failure of body-wall muscle formation during larval stage or degeneration of body-wall muscles. Upon observing terminal phenotypes of homozygotes *jh4* larvae closely, large gaps between gut and hypodermis were found in many of these animals (Fig. 6D). Since body-wall muscle formation seemed normal during embryogenesis of these embryos, it seemed most likely that body-wall muscles have degenerated during larval stage. Similar degeneration phenotypes had been observed in neuronal cells of *deg-1* (Chalfie et al., 1990), *mec-4* (Driscoll et al., 1991), and *deg-3* mutants (Treinin et al., 1995). Pharynx of homozygote *jh4* larva seemed to pump normally and the enteric muscles contracted well. Therefore, defects seen in homozygous *jh4* animals appeared to be specific to body-wall muscle.

### Genetic mapping of mutations

All the lethal mutations were mapped by two methods,

**Table 3.** Results of three-factor crosses

| Mutation | Phenotype of recombinant (F₁) | Progenies from recombinant (F₂) | Number of cases scored | Position of lethal mutation |
|---|---|---|---|---|
| jh1 | Dpy-5 | ND[a] | | *dpy-5 unc-13* |
| | Unc-13 | Unc-13, Dpy-5Unc-13<br>Unc-13, Dpy-5Unc-13, Let | 13<br>2 | |
| jh2 | Dpy-5 | Dpy-5, Dpy-5Unc-13, Let | 4 | *dpy-5 unc-13* |
| | Unc-13 | Unc-13, Dpy-5Unc-13 | 7 | |
| jh3 | Dpy-5 | Dpy-5, Dpy-5Unc-13, Let<br>Dpy-5, Dpy-5Unc-13, Dpy-5Unc-29 | 4<br>1 | *dpy-5 unc-13* |
| | Unc-13 | Unc-13, Dpy-5Unc-13<br>Unc-13, Dpy-5Unc-13, Let | 4<br>4 | |
| jh4 | Dpy-5 | Dpy-5, Dpy-5Unc-13, Let | 13 | *dpy-5 unc-13* |
| | Unc-13 | Unc-13, Dpy-5Unc-13 | 13 | |
| jh5 | Dpy-5 | Dpy-5, Dpy-5Unc-13, Let | 9 | *dpy-5 unc-13* |
| | Unc-13 | Unc-13, Dpy-5Unc-13 | 10 | |
| jh6 | Dpy-5 | Dpy-5, Dpy-5Unc-13, Let<br>Dpy-5, Dpy-5Unc-13, Dpy-5Unc-29 | 2<br>8 | *dpy-5 unc-13* |
| | Unc-13 | Unc-13, Dpy-5Unc-13<br>Unc-13, Dpy-5Unc-13, Let | 1<br>10 | |

[a]ND : Not determined.

three-factor crosses and deficiency complementation tests. By three-factor crosses, positions of lethal mutations were determined relative to two genetic markers, *dpy-5* and *unc-13*. The basis of mapping by three-factor crosses is to look at the frequencies of recombinations between the two genetic markers as illustrated on Table 1 (see Materials and Methods). The results of three-factor crosses indicated that three mutations (*jh1*, *jh3* and *jh6*) were located between the two genetic markers (*dpy-5* and *unc-13*) and the other three mutations were located right side of the *unc-13* gene (Table 3). For the deficiency complementation tests, two small deficiencies (*nDf23* and *nDf29*) deleting the region of the *unc-29* gene were used. Because mutations were recessive, lethal, and linked to the *unc-29* gene, *trans*-heterozygotes with the deficiency should show Unc-29 phenotype or lethal phenotype depending on the position of mutations (see Fig. 1, and Materials and Methods). The results of deficiency complementation test are summarized in Table 4. The positions of each mutation determined by both methods of mapping were consistent except for *jh6* (see Table 3 and 4). In the deficiency complementation test for *jh6*, no progeny showing Unc-29 phenotype were obtained from the mating between males carrying the *jh6* and hermaphrodites carrying deficiencies (*nDf23* and *nDf29*). This result suggested that the *jh6* mutation was located at the deleted region by the two deficiencies. However, by three-factor crosses the *jh6* mutation was mapped to the region between the *dpy-5* and the *unc-13* (Table 3) which could not be deleted by the deficiencies. This discrepancy between the two mapping results for the *jh6* mutation will be discussed in detail in the discussion. In summary, all six mutations were mapped by the two genetic methods and their approximate loci are shown in Fig. 7.

## Discussion

In this study, we have isolated six recessive lethal mutations that appeared to show developmental defects during embryonic and larval stages. These mutations were isolated by a genetic screen designed to select recessive lethal mutations closely linked to the *unc-29* gene on the chromosome I. Uncoordinated-movement phenotype (Unc) of the *unc-29(e193)* mutant was used as a marker to screen new recessive lethal mutations which were co-segregated with Unc phenotype. Therefore, in the F2 generation a clone of animals that do not segregate any progeny showing Unc phenotype due to a linked lethal mutation was selected out of approximately 5,200 mutagenized haploid clones (Fig. 3). As a result, six (*jh1* to *jh6*) recessive lethal mutations were isolated and characterized (Table 2). As summarized in Table 2, three mutations (*jh1*, *jh2*, and *jh6*) segregated arrested embryos at the rate of approximately 25% of the total progeny suggesting that these are recessive lethal mutations. On the other hand, the other three mutations (*jh3*, *jh4*, and *jh5*) showed less than 25% in segregation of arrested progeny (19.7%, 19.3%, and 17.9% respectively). There are two possible explanations for these results. First, because we screened for arrested phenotypes at early stages of development, we could have failed to score some of the animals arrested at later stages (L2 or L3 stages). The second possibility is that these mutations may be showing less than a hundred percent penetrance. That is, the proportion of animals with these mutations manifesting their phenotypes may be less than a hundred percent. The nature of each mutation should be further investigated to test these possibilities.

Two genetic mapping methods, three-factor crosses and complementation tests with deficiencies, were
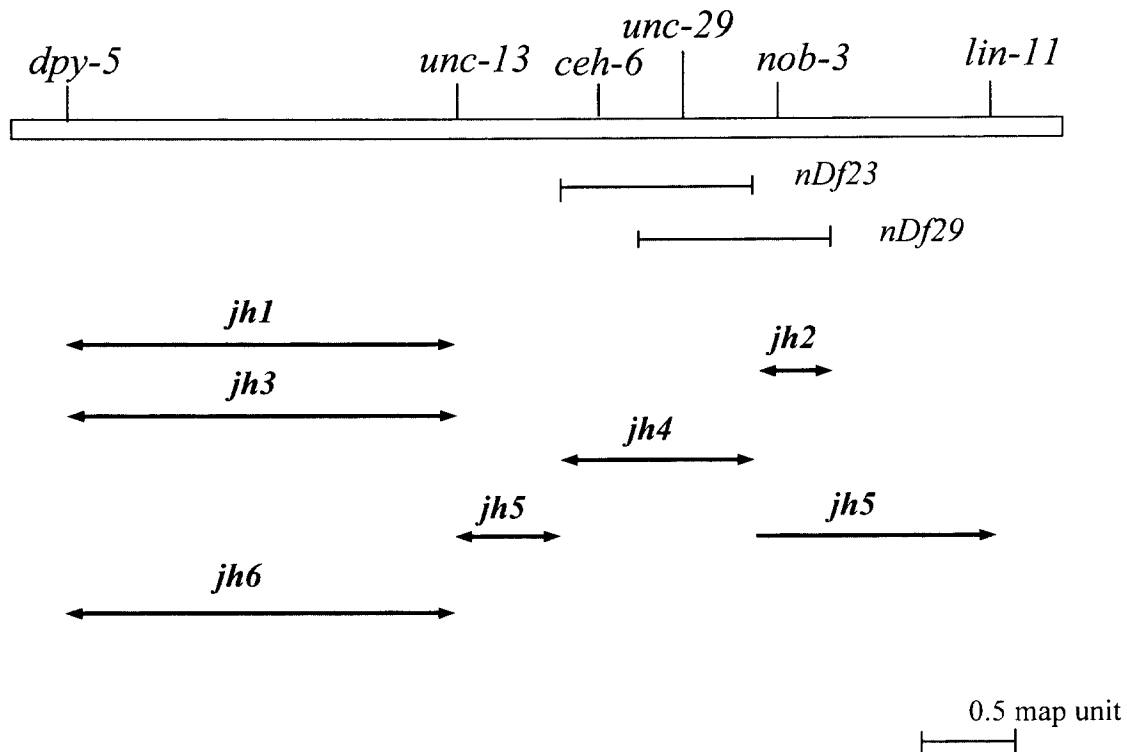
**Fig. 7.** A Genetic map of lethal mutations near the *unc-29* region. The horizontal open bar indicates the right side of chromosome I with selected markers. Below the chromosome, regions deleted by the two deficiencies (*nDf23* and *nDf29*) are shown. Putative positions of new mutants (*jh1, jh2, jh3, jh4, jh5,* and *jh6*) are shown by bold arrows. These positions of new mutants were determined by three-factor crosses and complementation test with deficiency.

used to locate these lethal mutations (Table 3 and 4). The relative genetic positions of each mutation determined by both methods were consistent except for the *jh6* mutation. In the case of *jh6*, the three-factor crosses located the *jh6* mutation between the *dpy-5* and the *unc-13* genes (Table 3), whereas the complementation tests with deficiencies mapped *jh6* to the right side of the *unc-13* gene (Table 4). Our interpretation of these conflicting results is that the three-factor crosses should be the primary method to locate a relative genetic position and complementation tests with deficiencies should be a secondary method to confirm the mapping data from three-factor crosses. In this point of view, mapping data of the *jh6* mutation by complementation tests with deficiencies had a potential weakness. That is, unlike other complementation test results of our other mutations (*jh1, jh2, jh3,* and *jh5*), the *jh6* mapping data were based on the fact that no Unc progeny were observed (Table 4). This could be interpreted as the *jh6* mutation being located within the deleted region by deficiency. Another possibility is that through recombination between *jh6* and *unc-29*, which should occur only at a very low frequency, the *unc-29* marker could have been lost during crosses of complementation tests. Therefore, we think that the *jh6* mutation should be placed to the region between the *dpy-5* and the *unc-13* as suggested by its three-factor

crosses. Based on the results of these genetic analyses, a relative genetic map of six mutations (*jh1* to *jh6*) was determined as shown in Fig. 7.

It is interesting to notice that two independently isolated mutants (*jh1* and *jh2*) showed similar phenotypes that were defective in the formation of posterior structure during embryogenesis (Fig. 4 and 5). These two mutations could be in the same gene, but these two mutations (*jh1* and *jh2*) mapped to two different locations. The *jh2* mutation was mapped to a narrow region (less than 0.3 map unit) near the *nob-3* gene by two different mapping methods (see Table 3, 4 and Fig. 7). On the other hand, mapping data of the *jh1* mutation suggested that it should be placed at the left side of the *unc-13* gene (Fig. 7). Therefore, these two mutations cannot be in the same gene. For the other mutations, the possibility that these mutations could be different alleles of the same locus remains. In order to determine whether any of these mutations are different alleles of the same loci, complementation tests against each of the mutations should be carried out. For this test, trans-heterozygous animals for each mutation should be made and their phenotypes must be examined. These genetic manipulations will be carried out in the near future.

The phenotypes observed in the *jh2* mutant animals were similar to previously described *nob* (no back end)

**Table 4.** Results of complementation test with *nDf23* and *nDf29*

| Mutation | Phenotype of unc-29let/nDf23[a] | Phenotype of unc-29let/nDf29[a] | Position of lethal mutation |
|---|---|---|---|
| *jh1* | Unc-29 | ND[b] | *dpy-5 unc-13 unc-29* |
| *jh2* | Unc-29 | No Unc-29 | *dpy-5 unc-13 unc-29* |
| *jh3* | Unc-29 | ND | *dpy-5 unc-13 unc-29* |
| *jh4* | No Unc-29 | ND | *dpy-5 unc-13 unc-29* |
| *jh5* | Unc-29 | ND | *dpy-5 unc-13 unc-29* |
| *jh6[c]* | No Unc-29 | No Unc-29 | *dpy-5 unc-13 unc-29* |

[a]The phenotypes of *unc-29(e193)let/nDf23* and *unc-29(e193)let/nDf29* were examined by observing the male progenies from the cross between males carrying the lethal mutation and hermaphrodites carrying *nDf23*. If there was any male progeny with Unc phenotype, the lethal mutation should be outside of the deleted region by the deficiency. If no male progeny with Unc phenotype was observed, this suggested that the lethal mutation was located within the region deleted by the deficiency (see also Fig. 1).
[b]ND: Not determined.
[c]Discrepancy was found between the data obtained from complementation test and the data from three-factor crosses. See Results and Discussion.

mutants (Powell-Coffman JA and Wood WB, personal communication). The *nob* mutants were reported to show defects in formation of the posterior structures during embryogenesis. One of these mutants (*nob-3*) had been mapped near the *unc-29* on the chromosome I (Powell-Coffman JA and Wood WB, personal communication), where we have mapped the *jh2* mutant (see Fig. 7). The possibility that the *jh2* mutation may be an allele of the *nob-3* gene can be tested by two different ways; a genetic method is to perform a complementation test of the *jh2* mutation with the *ct315* allele of the *nob-3* mutant (Powell-Coffman JA and Wood WB, personal communication). If the *jh2* mutation can not complement the *ct315* allele of the *nob-3* mutant as a trans-heterozygote, the *jh2* mutation must be in the *nob-3* gene. Another way to prove that the *jh2* mutation is in the *nob-3* gene is a molecular rescue experiment. In this rescue experiment, it can be tested whether the lethal phenotype of *jh2* can be rescued by microinjection of DNA clones containing the *nob-3* gene (Fire, 1986; Mello et al., 1995). Recently, the *nob-3* gene has been cloned by a genomic DNA fragment (less than 4 kb) from overlapping two cosmids (Powell-Coffman JA and Wood WB, personal communication). These genetic and molecular biological experiments will answer the question of whether the *jh2* mutation occurred in the *nob-3* gene.

Another mutant showing an interesting phenotype was *jh4* (Fig. 6). The *jh4* homozygous animals arrested at different developmental stages; that is, some of them arrested during embryogenesis and others arrested at an early larval stage. Terminal phenotypes of arrested embryos did not show any obvious defects when observed by Nomarski microscopy and stained positively with muscle specific antibodies (data not shown). However, the *jh4* mutants arrested at an early larval stage, showing body-wall muscle defects (Fig. 6). In addition to the defects of much thinner body-wall muscles compared to wild type, large gaps perhaps created by vacuolar swelling and lysis of body-wall muscle cells were observed. These types of degenerative cell death were morphologically distinct from the programmed cell deaths frequently observed in embryos during embryogenesis (Hengartner, 1997). Similar degenerative cell deaths were observed in some of neuronal cells of *deg-1*, *mec-4* and *deg-3* mutants (Chalfie et al., 1990; Driscoll et al., 1991; Treinin et al., 1995). Interestingly, all of these mutations were dominant alleles and gain of function mutations. Furthermore, cloning of these genes revealed that *deg-1* and *mec-4* genes encode membrane proteins (Chalfie et al., 1990; Driscoll et al., 1991) and *deg-3* to encode an alpha subunit of nicotinic acetylcholine receptor (Treinin et al., 1995). Dominant mutations in these genes were suggested to cause a steric hinderance of membrane proteins and channel hyperactivity, which might underlie the degeneration phenotypes in neuronal cells.

In contrast, the *jh4* mutation that we have isolated is a recessive lethal mutation, which is probably different from the neuro-degenerative mutations described above. Moreover, in the *jh4* mutants it appeared that most, if not all, degeneration had occurred in muscle cells rather than neuronal cells (Fig. 6). It is extremely important and interesting to understand underlying mechanisms of muscle cell degeneration, which may have significant implications for muscle dystrophic diseases. For example, defective dystrophin proteins which link skeletal muscle arrays to muscle cell membrane cause degeneration of muscle cells in muscle dystrophy patients (Hoffman et al., 1987; Bonilla et al., 1988; Ervasti et al. 1993). At this moment, without any molecular cloning data of the gene where the *jh4* mutation occurred, it would be speculative to predict what could have caused muscle degenerative phenotypes. Certainly, it will be very interesting to investigate the nature of the *jh4* mutation in the future, particularly with regard to its implication in muscle cell degeneration in *C. elegans*.

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