Development 112, 231-240 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991


# Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions

ERIC J. LAMBIE and JUDITH KIMBLE

*Department of Biochemistry, College of Agriculture and Life Sciences, and Laboratory of Molecular Biology, Graduate School, University of Wisconsin-Madison, 53706, USA*

## Summary

**Two homologous genes, *lin-12* and *glp-1*, encode transmembrane proteins required for regulatory cell interactions during *C. elegans* development. Based on their single mutant phenotypes, each gene has been thought to govern a distinct set of cell fates. We show here that *lin-12* and *glp-1* are functionally redundant during embryogenesis: Unlike either single mutant, the *lin-12 glp-1* double mutant dies soon after hatching. Numerous cellular defects can be observed in these Lag (for lin-12 and glp-1) double mutants. Furthermore, we have identified two genes, *lag-1* and *lag-2*, that appear to be required for both *lin-12* and *glp-1*-mediated cell interactions. Strong loss-of-function *lag* mutants are phenotypically indistinguishable from the *lin-12 glp-1* double; weak *lag* mutants have phenotypes typical of *lin-12* and *glp-1* single mutants. We speculate that the *lin-12* and *glp-1* proteins are biochemically interchangeable and that their divergent roles in development may rely largely on differences in gene expression.**

Key words. *lin-12*, *glp-1*, *lag* genes, evolution of regulatory genes, functional redundancy.

## Introduction

Two *Caenorhabditis elegans* regulatory genes, *lin-12* and *glp-1*, are remarkably similar in structure and function. Genetic and phenotypic analyses have demonstrated that these genes control cell fate decisions that are normally regulated by intercellular communication. The *lin-12* gene is required in somatic tissues for lateral signalling between cells of equivalent developmental potential (Greenwald *et al.* 1983; Sternberg, 1988), while *glp-1* is required both in the germ line and in the early embryo for inductive signalling between cells with different developmental potential (Austin and Kimble, 1987; Priess *et al.* 1987). Furthermore, each gene acts cell autonomously (Austin and Kimble, 1987; Seydoux and Greenwald, 1989). Therefore, *lin-12* and *glp-1* both act in the receiving cell to influence regulatory cell interactions, but each gene controls a distinct type of fate decision. More recently, molecular studies have revealed that the predicted protein products of *lin-12* and *glp-1* are similar both in amino acid sequence (50–60% identical) and overall molecular organization (Yochem *et al.* 1988; Yochem and Greenwald, 1989; Austin and Kimble, 1989). Each gene encodes a putative transmembrane protein with multiple EGF-like repeats in the extracellular domain and six *cdc10/SWI6*-like repeats in the intracellular domain (Yochem and Greenwald, 1989; Austin and Kimble, 1989).

Given the similarities between *lin-12* and *glp-1*, we wondered whether the roles of these homologous genes might overlap during development. If either *lin-12* or *glp-1* were sufficient for a given process, no defect would be observed in either single mutant. Three previously reported results provided clues that *lin-12* and *glp-1* might indeed be redundant. First, an unusual allele of *glp-1*, *q35*, mimics one aspect of gain-of-function (gf) mutations of *lin-12*: both *lin-12(gf)/+* and *glp-1(q35)/+* hermaphrodites have multiple ectopic vulvae (the Muv phenotype) (Greenwald *et al.* 1983; Austin and Kimble, 1987). Second, some *glp-1* RNA is present in somatic tissues (Austin and Kimble, 1989), where only *lin-12* is known to function. And third, the expression of *lin-12* by certain cells of the somatic gonad is required to prevent anchor cell-dependent mitotic proliferation of the proximal germ line (Seydoux *et al.* 1990), Seydoux *et al.* have proposed that the anchor cell produces a signal that can interact with either *lin-12* or *glp-1*, and that this signal is ordinarily intercepted by *lin-12* expressed by cells of the somatic gonad before it can reach *glp-1* in the germ line.

One way to test the idea that *lin-12* and *glp-1* are redundant is to construct a double mutant that has a loss-of-function (*lf*) mutation in each gene. If the two genes function independently, the effects of *lin-12(lf)* and *glp-1(lf)* should be additive in the double mutant, but if they are redundant, a new phenotype might be observed. In this paper, we report that the *lin-12 glp-1* double mutant has a novel phenotype, which we call the Lag phenotype (for lin-12 and glp-1). In addition, we

report the isolation of mutations in two genes, *lag-1* and *lag-2*, which also cause a Lag phenotype. We propose that the functions of *lin-12* and *glp-1* are overlapping and that *lag-1* and *lag-2* are required for the activities of both *lin-12* and *glp-1*.

## Materials and methods

### *Strains*

The following mutations were used (Hodgkin *et al.* 1988):LGI, *dpy-5(e61)*; LGII, *rol-1(e91)*; LGIII, *dpy-19(e1259ts)*, *sma-2(e502)*, *unc-32(e189)*, *lin-12(n137)*, *unc-69(e587)*; LGIV, *dpy-13(e184)*, *unc-5(e53)*, *unc-8(e15)*, LGV, *unc-34(e315)*, *unc-60(e723)*, *unc-46(e177)*, *dpy-11(e224)*, *unc-42(e270)*, LGX *lon-2(e678)*. In addition, *rol-9(sc148) V* (R. Edgar, personal communication) was obtained from the *Caenorhabditis* Genetics Center. Single mutant phenotypes of *glp-1* alleles *q46*, *q224ts* and *q231ts* have been described (Austin and Kimble, 1987); postembryonic phenotypes of *lin-12(n941)* and *lin-12(q269)* have been described (Seydoux *et al.* 1990) We also used *sDf50*, a deletion that removes *lag-2* (Johnsen and Baillie, 1988; Johnsen, 1990).

### *Construction of* lin-12 glp-1 *double mutants*

The *lin-12(q269) glp-1(q231)* double mutant chromosome was constructed by picking Unc non-Sma recombinants from the strain *sma-2(e502) unc-32(e189) glp-1(q231) unc-69(e587)/lin-12(q269)* grown at 15°. None of the recombinants segregated progeny with the combination of *lin-12* and *glp-1* phenotypes expected if the effects of *lin-12* and *glp-1* were additive. However, several recombinants segregated L1 lethals (Lags), suggestive of a synergistic effect of the *lin-12* and *glp-1* mutations. Complementation testing confirmed that these recombinants had the genotype *lin-12 glp-1 unc-69/sma-2 unc-32 glp-1 unc-69*. The *unc-69* marker was removed from the double mutant chromosome prior to its use in experiments.

The *lin-12(n941) glp-1(q46)* double mutant chromosome was obtained by crossing males of genotype *dpy-19(e1259) glp-1(q46)/lin-12(n941) unc-69(e587)* with hermaphrodites of genotype *dpy-19(e1259) unc-69(e587)* and picking non-Dpy, non-Unc recombinant progeny The desired recombinant had the genotype *lin-12 glp-1/dpy-19 unc-69*. Complementation testing was performed with isolates that segregated Lag progeny to confirm the presence of the double mutant chromosome.

### *Assessment of maternal requirement for* lin-12

The percentage of viability among *lin-12* homozygous progeny of a heterozygous mother was estimated as follows. To calculate the number of unhatched eggs resulting from background lethality, the total number of eggs from *lin-12* heterozygotes was multiplied by the fraction of unhatched eggs from homozygous wild-type mothers The number of unhatched eggs in excess of background was divided by the total number of homozygous mutant progeny (estimated as 1/4 of the total number of eggs laid) to obtain the fraction of unhatched homozygous mutant eggs. To estimate the percentage of viability among progeny of *lin-12* homozygous mothers, eggs were dissected from gravid worms with protruding vulvae, transferred to Petri plates, counted and scored at appropriate intervals.

### *Characterization of the Lag phenotype*

Heterozygous hermaphrodites were allowed to lay eggs at 20° for 1–2 days, then worms were rinsed off the plate and transferred to a microfuge tube to allow the larger animals to settle out. After a few minutes, worms remaining in suspension were transferred to a new tube and centrifuged briefly. Larvae were mounted for Nomarski microscopy (Sulston and Hodgkin, 1988) and data were recorded for those with Lag characteristics.

Indirect immunofluorescence was performed as described by Priess and Hirsh (1986). Images were taken using an MRC-500 confocal microscope.

### *Isolation of* lag-1 *and* lag-2 *alleles*

All alleles of *lag-1* and *lag-2* were obtained after mutagenesis with ethylmethane sulfonate (EMS) (Brenner, 1974). Wild-type L4 hermaphrodites were mutagenized with EMS and allowed to self. Gravid $F_1$ progeny were picked to individual plates at 25° and their offspring were screened by dissecting scope for the presence of L1 lethals. L1 lethals were inspected by Nomarski to find those with a Lag phenotype. New isolates were crossed with wild-type males and the resulting heterozygotes mated with appropriate tester or balancer stocks

### *Mapping and complementation testing of* lag-1 *and* lag-2 *alleles*

Mapping and complementation of *lag-1* and *lag-2* mutations were done using standard genetic techniques (Sulston and Hodgkin, 1988).

Three factor mapping with the strain, *lag-1(q385)/dpy-13(e184) unc-5(e53)*, indicated that *lag-1(q385)* is either to the right of, or immediately to the left of, *unc-5*. 22/22 Dpy non-Unc and 0/18 Unc non-Dpy recombinants segregated Lag progeny Further mapping with *dpy-13(e184) lag-1(q385)/unc-8(e15)* indicated that *lag-1(q385)* is either to the left of, or immediately the right of, *unc-8*. 25/25 Dpy non-Lag recombinants segregated Unc progeny. Subsequently isolated alleles of *lag-1* were identified based on their tight linkage to *unc-5(e53)* and their failure to complement *lag-1(q385)*.

*lag-2(q387)* was mapped to the left arm of chromosome *V* using *lag-2(q387)/unc-34(e315) rol-9(sc148)* 18/21 Rol non-Unc and 1/18 Unc non-Rol recombinant $F_1$ clones segregated Lag progeny. The refined position of *lag-2(s1486)* on the left arm of *V* was determined by deficiency mapping performed by R Johnsen (Johnsen, 1990). Subsequently isolated alleles were assigned to *lag-2* based on their location on the left arm of chromosome *V* and their failure to complement *q387*.

The phenotype of *lag-2(q387)/sDf50* was assessed by crossing males of genotype *lag-2(q387)/+* with hermaphrodites of genotype *sDf50 unc-46(e177)/dpy-11(e224) unc-42(e270)* *sDf50* homozygotes almost invariably arrest prior to hatching, so Lag offspring were known to be *lag-2(q387)/sDf50* These were scored by Nomarski optics for the various Lag traits. In this and other experiments, it was found that males and hermaphrodites exhibit essentially identical Lag phenotypes (males were identified by the presence of the blast cell B in the tail region)

## Results

### *The* lin-12 glp-1 *double mutant has a novel phenotype*

The single mutant phenotypes of *lin-12(lf)* and *glp-1(lf)* are distinct (Table 1). *lin-12(lf)* mutants usually survive to adulthood; however, they exhibit various morphological abnormalities (the Lin-12 phenotype) due to numerous cell fate transformations in somatic tissues (Greenwald *et al.* 1983; Seydoux *et al.* 1990). In general,

**Table 1.** *Penetrance of L1 lethal, Glp-1 and Lin-12 phenotypes**

| Genotype | % L1 lethal | % Glp-1† | % Lin-12‡ |
|---|---|---|---|
| *lin-12(q269)* | 2 | 0 | 98 |
| *lin-12(n941)* | 9 | 0 | 91 |
| *glp-1(q231)§* | 0 | 100 | 0 |
| *glp-1(q46)* | 0 | 100 | 0 |
| *lin-12(q269) glp-1(q231)* | 100 | 0 | 0 |
| *lin-12(n941) glp-1(q46)* | 100 | 0 | 0 |

* Data are presented as percentages of homozygous animals with a given phenotype, homozygotes were derived from heterozygous mothers by selfing at 20° At least 100 homozygotes were scored in each case

† Glp-1 animals were identified by dissecting scope based on their severely reduced germ line.

‡ Lin-12 animals were identified by dissecting scope based on their expression of the protruding vulva phenotype

§ Grown at 25°C

the cells affected by mutations in *lin-12* are members of equivalence groups, i.e. cells of equivalent developmental potential that adopt distinct fates as the result of lateral signalling (Greenwald *et al.* 1983; Sternberg, 1988). The simplest equivalence group consists of two cells that cooperate so that one assumes a preferred (primary) fate and the other assumes a secondary fate. The primary fate is defined as that adopted by the surviving member of an equivalence group when its counterpart has been removed by laser microsurgery (Sulston and White, 1980; Kimble, 1981; Sulston *et al.* 1983). In *lin-12(lf)* mutants, both equivalent cells typically follow the primary fate, whereas in *lin-12(gf)* mutants both follow the secondary fate (Greenwald *et al.* 1983). Therefore, *lin-12* activity appears to be required for adoption of the secondary fate. There is some indication that *lin-12* may be involved in cell fate decisions required for viability since a low frequency of lethality among *lin-12(lf)* homozygotes has been observed (I. Greenwald and P. Sternberg, personal communication); however, the basis for this lethality has not been investigated.

*glp-1(lf)* mutants derived from a *glp-1(lf)/+* mother always survive to adulthood; however, these animals are sterile due to a defect in germline proliferation (the Glp-1 phenotype) (Austin and Kimble, 1987; Priess *et al.* 1987). Germline proliferation normally depends on a single somatic cell, the distal tip cell (DTC) (Kimble and White, 1981). If the DTC is removed by laser microsurgery, germline nuclei that are normally mitotic enter meiosis This is the same phenotype that is seen in *glp-1(lf)* mutants (Priess *et al.* 1987; Austin and Kimble, 1987). Genetic mosaic analysis has shown that mitotic proliferation of the germ line requires expression of *glp-1* in the germ line, but not in the DTC (Austin and Kimble, 1987). Some of the *glp-1* expressed in the germ line is contributed to developing oocytes, and subsequently performs essential functions during embryogenesis. Analyses of temperature-sensitive *glp-1* mutants have revealed that both the *glp-1/glp-1* and *glp-1/+* progeny of a *glp-1/glp-1* mother die as embryos that are defective in the induction of the anterior portion of the pharynx (Priess *et al.* 1987; Austin and Kimble, 1987). Thus, *glp-1* is required for two inductive interactions, both of which depend on germline expression.

We constructed the *lin-12(lf) glp-1(lf)* double mutant in order to determine the developmental consequences of removing the activities of both genes. We find that, unlike either single mutant, the *lin-12 glp-1* double mutant invariably arrests in the first larval stage (L1) (Table 1). The same effect is observed using either putative null alleles or weaker alleles for each gene. The *lin-12(q269)* allele causes a slightly less severe phenotype than the putative null allele *lin-12(n941)* (Seydoux *et al.* 1990), suggesting that *lin-12(q269)* retains some *lin-12* activity. Further, the *glp-1(q231)* allele is temperature sensitive, whereas the putative null allele *glp-1(q46)* is non-conditional (Austin and Kimble, 1987). The phenotypes of *lin-12(q269) glp-1(q231)* and *lin-12(n941) glp-1(q46)* are virtually identical (Tables 1, 2). Henceforth, each allele combination will be referred to simply as *lin-12 glp-1*.

The *lin-12 glp-1* double mutant exhibits three major anatomical defects (Fig. 1). (1) There is no detectable excretory cell or excretory duct and a small protrusion is present at the normal location of the excretory pore (Fig. 1A, B). (2) The nose of the worm is often twisted sideways or backwards (Fig. 1C). (3) The rectum is undetectable and a protrusion is present at the normal location of the anal opening (Fig. 1D,E). The frequency at which each of these defects is observed is presented in Table 2. We refer to this combination of anatomical defects as the Lag phenotype (for lin-12 and glp-1). Henceforth, the term Lag will be used to describe animals that exhibit one or more of these anatomical characteristics. Lag animals also have a behavioral phenotype: They are inactive and do not eat (although they can live for several days after hatching). This behavior may result from the lack of the excretory cell, which is known to be essential for viability (Nelson and Riddle, 1984), or it may be due to some unknown anatomical or physiological defect.

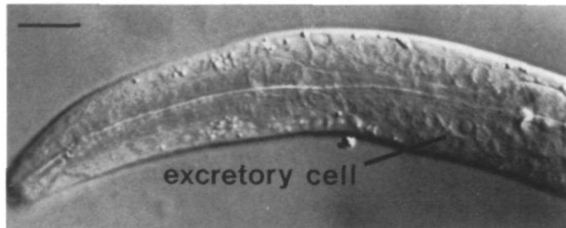
We examined the epithelia and muscles of *lin-12 glp-1* double mutants in more detail. Using an antibody that recognizes epithelial belt desmosomes, we observed a duplicated structure near the excretory pore (Fig. 2). The presence of a second pore cell could explain the protrusion occurring at this location in Lag animals (Fig. 1B). With the same antibody, we also saw that certain cells were missing in the posterior region. The cells of the intestinal/rectal valve (virR and virL) and the anterior rectum (rect D, rect VL and rect VR) appeared to be present as usual, but one or both of the next two pairs of rectal cells (K,K′ and F,U) appeared to be missing (data not shown). Using an antibody that recognizes muscle myosin, we found that the anal depressor muscle and at least one intestinal muscle were absent in the double mutant (Fig. 3A,B). Intriguingly, in some double mutant animals, a muscle resembling the anal depressor was present at the normal location of the intestinal muscle (Fig. 3C).

**Table 2.** *Frequencies of different Lag traits**,†

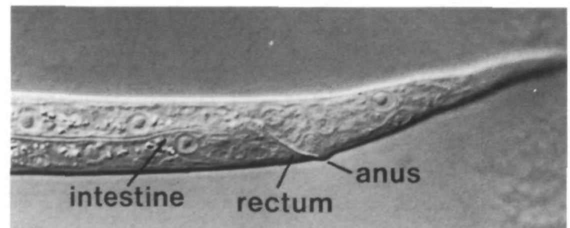
| Genotype | Excretory cell and rectum/anus both absent (%) | Either excretory cell or rectum/anus absent (%) | Twisted nose (%) |
|---|---|---|---|
| *lin-12(n941)* | 36 | 64 | 0 |
| *lin-12(q269) glp-1(q231)* | 100 | 0 | 56 |
| *lin-12(n941) glp-1(q46)* | 100 | 0 | 90 |
| *lag-1(q385)* | 98 | 2 | 12 |
| *lag-1(q418)* | 98 | 2 | 0 |
| *lag-1(q410)* | 72 | 28 | 0 |
| *lag-1(q386)* | 64 | 36 | 4 |
| *lag-1(q416)* | 78 | 22 | 0 |
| *lag-1(q426)* | 92 | 8 | 0 |
| *lag-2(q387)* | 100 | 0 | 76 |
| *lag-2(q431)* | 98 | 2 | 60 |
| *lag-2(q389)* | 100 | 0 | 6 |
| *lag-2(q411)* | 98 | 2 | 4 |
| *lag-2(s1486)* | 98 | 2 | 2 |
| *lag-2(q393)* | 92 | 8 | 4 |
| *lag-2(q420)* | 88 | 12 | 2 |
| *lag-2(q387)/sDf50*‡ | 100 | 0 | 63 |

*Data are presented as percentages of L1 lethal animals with various Lag traits See Materials and methods for more detail
† At least 50 L1 lethals were scored for each genotype
‡ *sDf50* is a deficiency for the *lag-2* locus (Johnsen and Baillie, 1988)

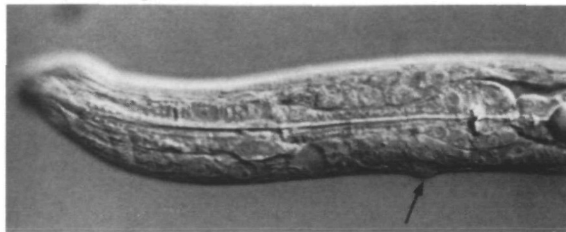
A. wild type



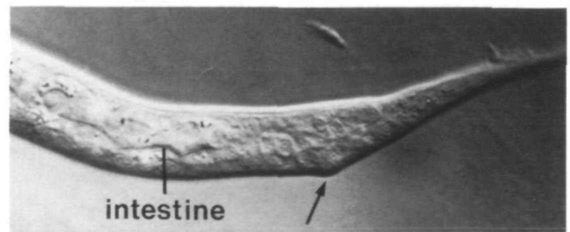

D. wild type




B. *lin-12 glp-1*



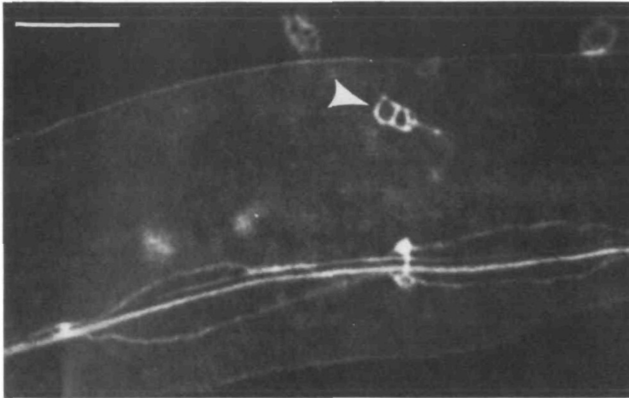
E. *lin-12 glp-1*




C. *lin-12 glp-1*

**Fig. 1.** The *lin-12 glp-1* double mutant phenotype. (A) Wild-type, head; large nucleus of excretory cell is indicated; excretory duct and excretory pore not visible in this focal plane. (B) *lin-12(q269) glp-1(q231)*, head, excretory cell nucleus and excretory duct are undetectable in any plane of focus; arrow indicates subesophageal bump (C) *lin-12(q269) glp-1(q231)*, head, nose is twisted backwards. (D) Wild type, tail; intestine, rectum and anus are indicated. (E) *lin-12(q269) glp-1(q231)* tail; intestine is indicated; rectum and anus are absent; arrow indicates bump at normal location of anus Nomarski microscopy performed as described by Sulston and Hodgkin (1988). Anterior is to the left All worms are L1 larvae Scale bar=10 $\mu$m.

A. wild type



B. *lin-12 glp-1*

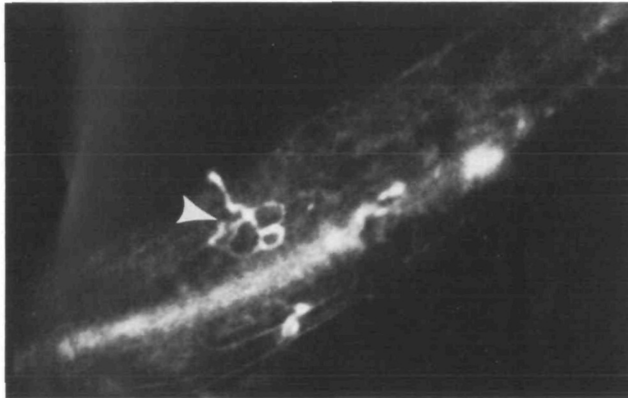
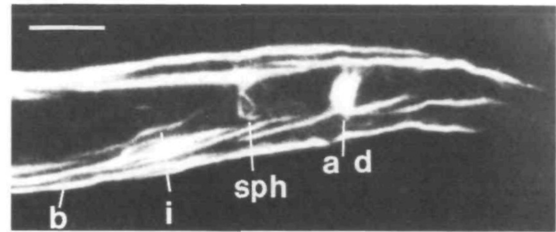
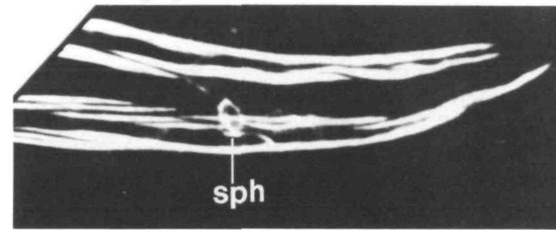


**Fig. 2.** A duplicated structure in the *lin-12 glp-1* double mutant. (A) Wild type; arrowhead indicates two rings of belt desmosomes associated with excretory the pore. (B) *lin-12(q269) glp-1(q231)*, duplication of the rings associated with the excretory pore is indicated. Antibody used was mouse monoclonal MH27, directed against belt desmosomes (Waterston, 1988). Anterior is to the left. All worms are L1 larvae. Scale bar=10 μm.

A. wild type




B. *lin-12 glp-1*




C. *lin-12 glp-1*

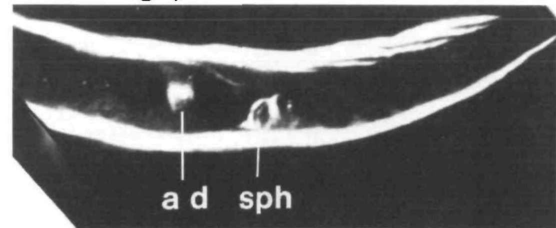



**Fig. 3.** Missing cells in the *lin-12 glp-1* double mutant. (A) Wild type; a body muscle (b), an intestinal muscle (i), intestinal–rectal sphincter muscle (sph), and anal depressor muscle (a d) are indicated (B) *lin-12 (q269) glp-1(q231)*; body muscles and sphincter muscle are present as in A, but intestinal muscles and anal depressor muscle are absent. (C) *lin-12(q269) glp-1(q231)*, body muscles and sphincter muscle are present as in A; ectopic anal depressor is indicated Antibody used was mouse monoclonal 5–6, directed against myosin heavy chain A (Miller *et al* 1983). Anterior is to the left. All worms are L1 larvae. Scale bar=10 μm.

### lin-12 *and* glp-1 *single mutants can also be Lag*

We also examined *lin-12* and *glp-1* single mutants to ask if they ever exhibit a Lag phenotype. For *lin-12*, we found that a fraction (<10 %) of both *lin-12(q269)* and *lin-12(n941)* single mutants are Lag (Tables 1, 2). However, the twisted nose typical of the double mutant is not observed in *lin-12* single mutants (Table 2). The low frequency of Lag animals among *lin-12* single mutant progeny raised the possibility that some animals might be rescued by maternal *lin-12(+)* product. We therefore compared the percentage of *lin-12* homozygotes that are Lag when derived from *lin-12/+ versus lin-12/lin-12* mothers, and found that the maternal genotype of *lin-12* has little effect on the viability of homozygous *lin-12* offspring (Table 3).

For *glp-1*, we found that Lag worms could be observed among single mutant homozygotes, but only under special circumstances. For example, when progeny from a homozygous *glp-1(q224ts)* hermaphrodite are shifted to restrictives temperatures during early embryogenesis, a small fraction (<1 %) develop into larvae with twisted noses or lacking both the excretory cell and the rectum (data not shown). In contrast, Lag animals are never observed among the progeny of wild-type mothers (Table 3). Thus, the absence of either *glp-1* or *lin-12* can result in a Lag phenotype, but in the absence of both genes, it occurs at high frequency.

### *Identification of* lag-1 *and* lag-2

The distinctive phenotype of the *lin-12 glp-1* double mutant allowed us to screen for genes required for the activities of both *lin-12* and *glp-1*. A loss-of-function mutation in such a gene might result in a Lag phenotype. We screened 13 000 EMS mutagenized

**Table 3.** *The effect of maternal* lin-12 *genotype on viability of mutant offspring**

| Genotype of progeny | Maternal genotype | Unhatched eggs (%) | Lag (%) |
|---|---|---|---|
| +/+ | +/+ | 2 | 0 |
| *lin-12(n941)/lin-12(n941)* | *+/lin-12(n941)* | 3 | 9 |
| *lin-12(n941)/lin-12(n941)* | *lin-12(n941)/lin-12(n941)* | 10 | 5 |

* See Materials and methods for methods used in counting and estimating

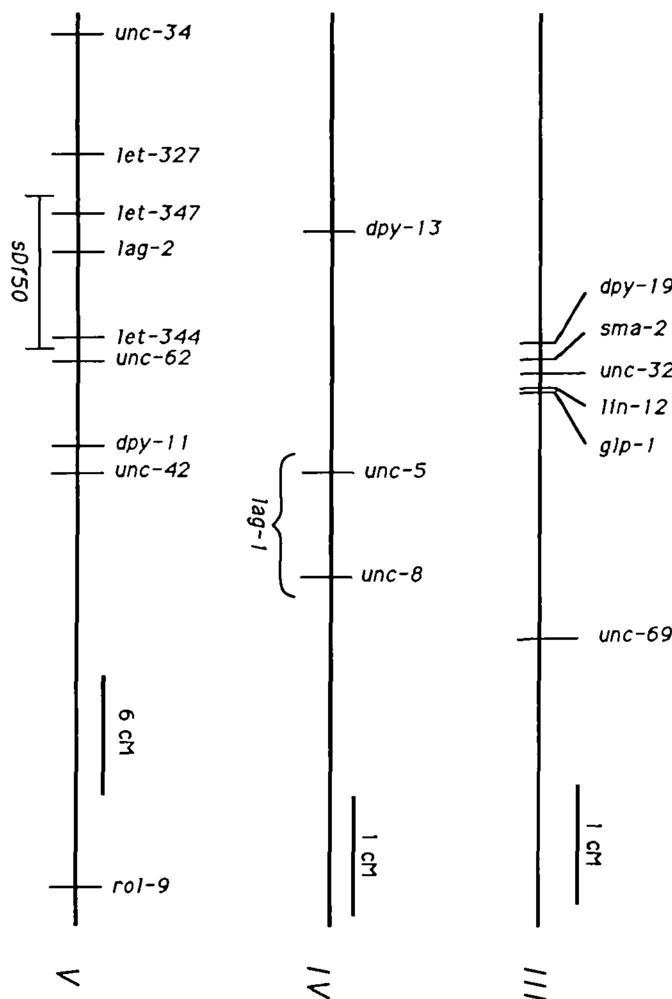



**Fig. 4.** Genetic maps of linkage groups *III*, *IV* and *V*. Only the central regions of chromosomes *III* and *IV* are shown. The location of *sDf50* is as described by Johnsen (1990).

haploid genomes for zygotically acting recessive mutations with a Lag phenotype (see Materials and methods). From this screen, we isolated 11 Lag mutations, all of which are fully recessive.

Mapping and complementation tests (see Materials and methods) revealed that the Lag mutations define two separate loci, each of which is distinct from *lin-12* and *glp-1* (Fig. 4). Five Lag mutations map to chromosome *IV* and all fail to complement *lag-1(q385)*. We call this newly identified gene *lag-1*. The six other Lag mutations map to chromosome *V* (Fig. 4) and fail to complement *lag-2(387)*, but do complement *lag-1(q385)*. Mutations of the locus on *V* had previously been isolated: One recessive allele, *s1486*, was isolated as a lethal (Johnsen, 1990) and two dominant alleles were isolated as *lin-12(gf)* suppressors (F. Tax, J. Thomas and R. Horvitz, personal communication). We designate this locus *lag-2* because of its distinctive Lag phenotype and the argument (see below) that the Lag phenotype is the loss-of-function phenotype of this gene.

*Phenotypic characterization of* lag-1 *and* lag-2 *mutants*

We compared the *lag-1* and *lag-2* mutant phenotypes to the Lag phenotype of the *lin-12 glp-1* double mutant. For *lag-1*, the five original alleles plus *lag-1(q426)*, which was not isolated based on a Lag phenotype (see below), were inspected. For *lag-2*, the six original alleles, plus *lag-2(s1486)*, were examined. Based on the relative severity of their mutant phenotypes, the *lag-1* and *lag-2* alleles can be ranked according to strength (Table 2). For *lag-1*, *q385* is the strongest allele: 100% of *q385* homozygotes arrest as L1 larvae (data not shown). Nearly all *q385* homozygotes examined lacked the excretory cell and were defective in the rectal/anal region; however, only about 10% of them had a twisted nose. *lag-1(q416)* is classified as the weakest allele because, unlike other alleles, some *q416* homozygotes are viable and fertile at 20°. *lag-1(q426)* is an unusual allele that exhibits a low frequency of larval lethality.

For *lag-2*, *q387* is the strongest mutation: 100% of *q387* homozygotes arrest as L1 larvae (data not shown). All *lag-2(q387)* homozygotes lacked both the excretory cell and the rectum/anus and most had a twisted nose. This phenotype was not enhanced when *lag-2(q387)* was placed in *trans* to a deficiency for the *lag-2* locus (Table 2). *lag-2(q420)* is classified as the weakest allele because some *q420* homozygotes are viable and fertile at 20°.

We propose that the Lag phenotype of both *lag-1* and *lag-2* results from a decrease in function at each locus. This hypothesis is based on the recessiveness of the *lag-1* and *lag-2* alleles, the relatively high frequency at which they were isolated (approximately $5\times10^{-4}$) and the allelic series available for each gene. Loss-of-function mutations in other genes occur at a similar frequency (Brenner, 1974; Greenwald and Horvitz, 1980). In addition, it seems likely that *lag-2(q387)* is a null allele: It is the most severe and its phenotype does not change when placed over a deficiency.

The epithelia and muscles of *lag-1(q385)* and *lag-2(q420)* were examined by immunofluorescence as described above for the *lin-12 glp-1* double mutant. In

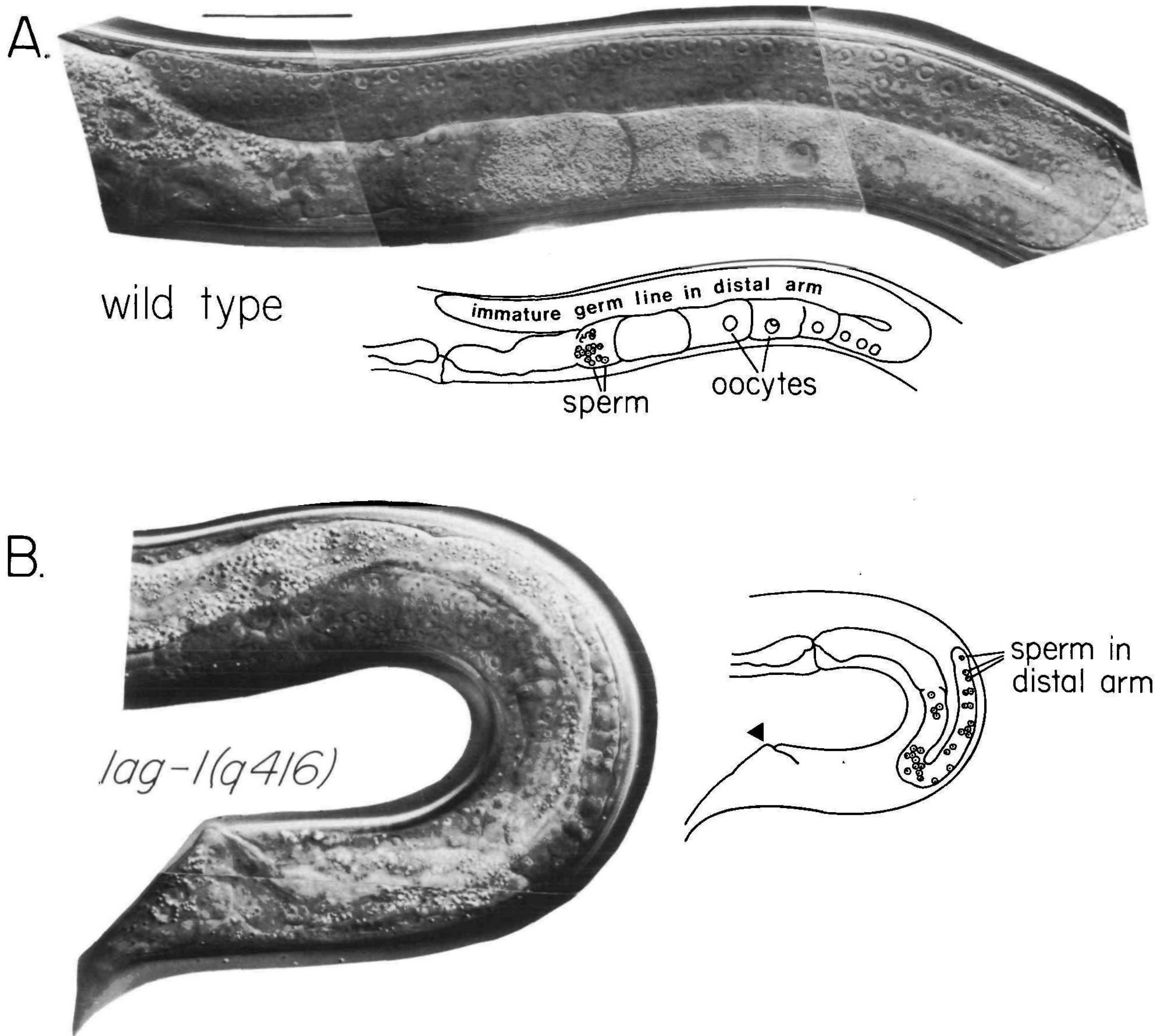


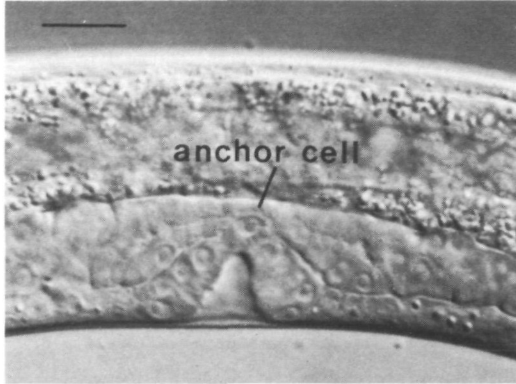

**Fig. 5.** A *lag-1* mutant with a Glp-1 germline phenotype. (A) Wild type, adult hermaphrodite gonad; locations of immature germ line, oocytes and sperm are indicated in line drawing. (B) *lag-1(q416)*; mitotic proliferation of the germ line is minimal; all germline descendants have left mitosis, completed meiosis and differentiated as sperm; arrowhead indicates expansion at base of tail observed in some *lag-1* and *lag-2* mutants. Anterior is to the left. Scale bar=50 $\mu$m.

each case, the phenotype of *lag* animals resembled that seen in the *lin-12 glp-1* double mutant. In the case of *lag-2(q420)*, occasional animals were found in which there appeared to be two anal depressor muscles, one ectopic (at the position of the intestinal muscle) and one at the normal location in the tail (data not shown).

### lag-1 *and* lag-2 *single mutants exhibit both* lin-12 *and* glp-1 *single mutant phenotypes*

Since some alleles of *lag-1* and *lag-2* permit homozygotes to survive to adulthood, we could observe the postembryonic phenotypes of these mutants. For weak *lag-1* and *lag-2* mutants, adults were often found in which the germ line had failed to proliferate. In these animals, there was no mitotic germ line; instead germ cells that would normally be in mitosis entered meiosis and differentiated into sperm (Fig. 5). This phenotype is identical with that of *glp-1* single mutants. The *lag-1* allele, *q426*, is particularly striking. Few *lag-1(q426)* animals are Lag (<10 %), and the rest have a *glp-1*-like germ line. In the case of *lag-2* (but not *lag-1*), adults often possessed a protruding vulva similar to that of *lin-12(lf)* mutants. This vulval defect is associated with the production of two anchor cells (Greenwald *et al.* 1983). When examined during the L3 or L4 stages, some *lag-2(q389)* and *lag-2(q420)* animals were found to have two anchor cells (Fig. 6). Finally, mature *lag-1(q416)* homozygotes often display an unusual expansion of the anal region (Fig. 5). This phenotype may be caused by the absence of the anal depressor muscle, since anti-myosin staining of such animals revealed that this muscle was missing. The absence of the intestinal muscle may also be involved in the production of this

A. wild type




B. lag-2(q389)

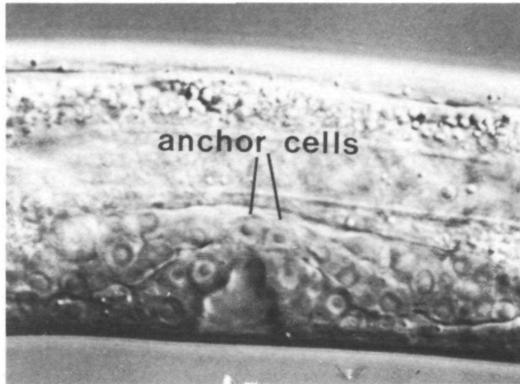



**Fig. 6.** A *lag-2* mutant with a Lin-12 phenotype. (A) Wild type; a single anchor cell is adjacent to the developing vulva (B) *lag-2(q389)* Two anchor cells are adjacent to the developing vulva. Both worms are L4 larvae Scale bar=10 μm

phenotype. We also examined *glp-1* and *lin-12* single mutants for this phenotype of tail expansion. It was found occasionally in *lin-12* single mutants, and less frequently among the progeny of *glp-1(q224ts)* homozygotes shifted to restrictive temperature during embryogenesis (data not shown).

## Discussion

### lin-12 *and* glp-1 *probably function by the same basic mechanism*

In this paper, we present two lines of evidence that *lin-12* and *glp-1* function by similar mechanisms. First, *lin-12* and *glp-1* are functionally redundant during embryonic development. Whereas both *lin-12* and *glp-1* single mutants usually reach adulthood (Greenwald *et al.* 1983; Seydoux *et al.* 1990; Austin and Kimble, 1987; Priess *et al.* 1987), the *lin-12 glp-1* double mutant invariably arrests in the first larval stage, apparently as the result of one or more transformations in cell fate. There are two models that might explain this result. One possibility is that *lin-12* and *glp-1* act in separate cells during embryogenesis and that these cells function redundantly in controlling specific cell fates. Alternatively, *lin-12* and *glp-1* could act in the same cells during embryogenesis and function redundantly within them to control cell fates. Given the known functions and molecular similarity of the two genes, the second model seems more likely. Thus, the simplest interpretation is that these two homologous genes encode interchangeable proteins that mediate a common function during embryogenesis.

Second, we have identified two genes required for both *lin-12* and *glp-1* functions. The *lin-12 glp-1* double mutant dies with a distinctive combination of defects, the Lag phenotype (for lin-12 and glp-1). Single mutants of *lag-1* and *lag-2* have this same Lag phenotype. Because mutations in both *lag* genes result in a decrease in gene function (see Results), these genes are likely to be required during embryogenesis for both *lin-12* and *glp-1* activities. In addition, because weak mutations of each *lag* gene exhibit phenotypes diagnostic of *lin-12* and/or *glp-1* single mutants, we suggest that the *lag* genes are also involved in postembryonic functions of *lin-12* and/or *glp-1*. Furthermore, a role for *lag-2* in postembryonic *lin-12* activity is supported by the identification of gain-of-function (*gf*) mutations of *lag-2* that suppress postembryonic defects in *lin-12(gf)* mutants (F. Tax, J. Thomas and R. Horvitz, personal communication).

The role of each *lag* gene in the functioning of *lin-12/glp-1* is not understood. One or both might be a positive regulator of *lin-12/glp-1*, act together with *lin-12/glp-1*, or function downstream of *lin-12/glp-1*. The suppression of *lin-12(gf)* by *lag-2(gf)* indicates an interaction between *lin-12* and *lag-2* (F. Tax, J. Thomas and R. Horvitz, personal communication), but the nature of that interaction is unknown. Preliminary epistasis experiments show that *lin-12(gf)*; *lag* double mutants are Lag (E. Lambie, unpublished observations.). Therefore, *lin-12(gf)* does not circumvent the *lag* gene requirement, but this result does not distinguish between the possible relationships among these genes. A further understanding of the relationship of the *lag* genes to *lin-12/glp-1* must await the results of genetic mosaic and molecular analyses of these genes.

### *Lag transformations of cell fate*

The cellular defects observed in Lag animals (either the *lin-12 glp-1* double or strong *lag* mutants) may result from transformations in cell fate during embryogenesis. Certain cells are missing (e.g. the excretory cell and the anal depressor muscle) and others appear to be duplicated (e.g. the excretory pore). Most missing cells would normally arise in the lineage of blastomere AB.pl (Sulston *et al.* 1983). In *lin-12* single mutants, one AB.pl descendant, called $G_2$, is often transformed into its AB.pr homolog, W (Greenwald *et al.* 1983). The effect of *lin-12* mutations on the G2/W equivalence group is notable because in this case *lin-12* is required for the primary fate (G2), unlike other cases, in which *lin-12* is required for specification of the secondary fate. A similar atypical transformation could explain the

coincident absence of the excretory duct cell, and apparent duplication of its homolog, the excretory pore cell (G1). Laser ablation studies have shown that the duct cell and the pore cell constitute an equivalence group, with the duct cell fate being primary (Sulston *et al.* 1983). Other cell fate transformations in Lag animals are less certain. The excretory cell (AB.plpappaap) may be transformed into a neuron, its AB.pr homolog (Sulston *et al* 1983), but it is difficult to detect one extra neuron. The anal depressor muscle does not appear to be transformed into the intestinal-rectal sphincter muscle, its AB.pr homolog (Sulston *et al.* 1983).

The absence of AB.pl-derived muscles in Lag mutants is striking in light of the maternal requirement for *glp-1* to induce the AB.a-derived muscles of the anterior pharynx (Priess *et al.* 1987). Although there are no known cell interactions that influence the development of the intestinal muscles (one of which is derived from MS) or the anal depressor muscle, an induction that is mediated by either *lin-12* or *glp-1* may be required for the production of AB-derived muscles in the posterior part of the animal.

### lin-12/glp-1 *and the* lag *genes may be the worm homologs of fly neurogenic genes*

The structural similarity of *lin-12* and *glp-1* indicates that these two genes are probably divergent products of an ancient duplication event (Yochem and Greenwald, 1989; Austin and Kimble, 1989). One enticing possibility is that the ancestral gene was functionally equivalent to the Notch gene of *Drosophila*. Like *lin-12* and *glp-1*, Notch is involved in numerous cell fate decisions that depend on intercellular signalling (Poulson, 1940; Shellenbarger and Mohler, 1975; Campos-Ortega, 1988; Doe and Goodman, 1985). Furthermore, Notch is similar in sequence and overall molecular organization to *lin-12* and *glp-1* (Wharton *et al.* 1985; Kidd *et al.* 1986). However, unlike *lin-12* and *glp-1*, Notch appears to be unique in the *Drosophila* genome. No other *Drosophila* gene has been identified with all the molecular features of the *lin-12*, Notch and *glp-1* family. If *lin-12* and *glp-1* together execute the worm equivalent of Notch function, which seems likely, the *lag* genes may be homologs of other neurogenic genes of *Drosophila* (Lehman *et al.* 1983). It is difficult to guess which neurogenic genes might be the most likely candidates because the roles played by *lag-1* and *lag-2* in *lin-12/glp-1* function are not understood (see above).

### *Evolution of developmentally separate functions for functionally redundant regulatory genes*

The *lin-12* and *glp-1* genes are poised at an interesting point in evolution from their ancestral gene. They have overlapping functions during embryogenesis, but have acquired distinct roles during postembryonic development. One way to achieve this situation would be to place proteins with identical biochemical activities under separate controls of gene expression. From analyses of single mutants (Greenwald *et al.* 1983; Seydoux and Greenwald, 1989; Austin and Kimble, 1987; Priess *et al.* 1987) and the tissue specificity of *lin-12* and *glp-1* RNAs (Austin and Kimble, 1987), it appears that *lin-12* is expressed zygotically and preferentially in somatic tissues, while *glp-1* is expressed preferentially in the germ line and has maternal effects. Therefore, the separate postembryonic roles of *lin-12* and *glp-1* would be achieved by somatic expression of *lin-12* to govern fate decisions in equivalence groups and germline expression of *glp-1* to control the decision between mitosis and meiosis and to provide maternal *glp-1* product to the embryo. An additional mechanism that could generate the postembryonic specificity of *lin-12* and *glp-1* function depends on tissue-specific regulatory proteins that interact differentially with *lin-12* and *glp-1*. Such genes may emerge from the current searches for tissue-specific suppressors or enhancers of *lin-12* or *glp-1* phenotypes. Further analyses of the similarities and differences in regulation and function of *lin-12* and *glp-1* should provide one of the clearest examples of the evolution of separate regulatory functions by homolagous and functionally similar genes.

We acknowledge the superb assistance of Phil Balandyk in generating all *lag* mutations described. Also, we thank Sean Carroll, Diane Church, Bill Dove and Ron Ellis for critical reading of this manuscript and Bob Johnsen, David Baillie, Susan Strome, Iva Greenwald and the *Caenorhabditis* Genetics Center for providing strains, Jim Thomas, Franz Tax, Bob Horvitz, John Sulston and John White for communicating unpublished results, and David Miller and Robert Waterston for gifts of antibodies. We are indebted to Steve Paddock and the UW-IMR for use of the confocal microscope. Leanne Olds is responsible for the excellent technical illustrations This research was supported by NIH grant GM31816 E.J.L. was supported by grant DRG-989 from the Damon Runyon-Walter Winchell Cancer Fund.

## References

Austin, J and Kimble, J (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C elegans* *Cell* **51**, 589–599

Austin, J and Kimble, J. (1989) Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of *C elegans*. *Cell* **58**, 565–571

Brenner, S (1974) The genetics of *Caenorhabditis elegans* *Genetics* **77**, 71–94

Campos-Ortega, J A (1988) Cellular interactions during early neurogenesis of *Drosophila melanogaster* *Trends Neurosci* **11**, 400–405

Doe, C Q and Goodman, C S (1985) Early events in insect neurogenesis II The role of cell interactions and cell lineage in the determination of neuronal precursor cells *Devl Biol* **111**, 206–219

Greenwald, I S and Horvitz, H R (1980) *unc-93(e1500)*. A behavioral mutant of *Caenorhabditis elegans* that defines a gene with a wild-type null phenotype *Genetics* **96**, 147–164

Greenwald, I S, Sternberg, P W and Horvitz, H R (1983) The *lin-12* locus specifies cell fates in *Caenorhabditis elegans*. *Cell* **34**, 435–444

Hodgkin, J, Edgley, M., Riddle, D and Albertson, D G (1988). In The nematode *Caenorhabditis elegans*, W Wood, ed. (Cold Spring Harbor, New York Cold Spring Harbor Laboratory), pp 491–584

Johnsen, R C (1990) Genetic analysis of the left half of linkage group *V* in *Caenorhabditis elegans* PhD. thesis, Simon Fraser University

JOHNSEN, R C AND BAILLIE, D L (1988) Formaldehyde mutagenesis of the *eT1* balanced region in *C elegans* Dose-response curve and the analysis of mutational events *Mutation Res.* **201**, 137–147

KIDD, S, KELLEY, M R AND YOUNG, M W. (1986). Sequence of the Notch locus of *Drosophila melanogaster* Relationship of the encoded protein to mammalian clotting factor and growth factors *Molec cell Biol* **6**, 3094–3108

KIMBLE, J (1981). Alterations in cell lineage following laser ablation of cells in the somatic gonad of *Caenorhabditis elegans. Devl Biol* **87**, 286–300

KIMBLE, J E AND WHITE, J G (1981) On the control of germ cell development in *Caenorhabditis elegans Devl Biol* **81**, 208–219

LEHMAN, R, JIMENEZ, F, DIETRICH, U AND CAMPOS-ORTEGA, J A (1983) On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster Wilhelm Roux's Arch devl Biol* **192**, 62–74

MILLER, D M, ORTIZ, I, BERLINER, G C AND EPSTEIN, H F (1983). Differential localization of two myosins within nematode thick filaments *Cell* **34**, 477–490

NELSON, F K. AND RIDDLE, D L (1984) Functional study of the *Caenorhabditis elegans* secretory-excretory system using laser microsurgery *J exp Zool* **231**, 45–56

POULSON, D F. (1940) The effects of certain X chromosome deficiencies on the embryonic development of *Drosophila melanogaster. J exp Zool* **83**, 271–325

PRIESS, J R, SCHNABEL, H AND SCHNABEL, R (1987). The *glp-1* locus and cellular interactions in early *C elegans* embryos. *Cell* **51**, 601–611.

PRIESS, J R AND THOMSON, J N. (1987) Cellular interactions in early *C elegans* embryos *Cell* **48**, 241–250.

PRIESS, J R AND HIRSH, D. I (1986) *Caenorhabditis elegans* morphogenesis The role of the cytoskeleton in elongation of the embryo. *Devl Biol* **117**, 156–173

SEYDOUX, G AND GREENWALD, I (1989) Cell autonomy of *lin-12* function in a cell fate decision *Cell* **57**, 1237–1245

SEYDOUX, G, SCHEDL, T AND GREENWALD, I. (1990) Cell–cell interactions prevent a potential inductive interaction between soma and germline in *C elegans Cell* **61**, 939–951

SHELLENBARGER, D L AND MOHLER, D. J (1975) Temperature sensitive mutations of the Notch locus of *Drosophila melanogaster Genetics* **81**, 143–162

STERNBERG, P. W (1988) Lateral inhibition during vulval induction in *Caenorhabditis elegans Nature* **335**, 551–554

SULSTON, J E AND HODGKIN, J (1988) In The nematode *Caenorhabditis elegans*, W Wood, ed. (Cold Spring Harbor, New York Cold Spring Harbor Laboratory), pp. 587–606.

SULSTON, J E., SCHIERENBERG, E, WHITE, J G. AND THOMSON, J N. (1983) The embryonic cell lineage of the nematode *Caenorhabditis elegans Devl Biol* **100**, 64–119

SULSTON, J E AND WHITE, J G (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans Devl Biol* **78**, 577–597

WATERSTON, R H (1988) In The nematode *Caenorhabditis elegans*, W Wood, ed (Cold Spring Harbor, New York Cold Spring Harbor Laboratory), pp 281–335

WHARTON, K A, JOHANSEN, K. M, XU, T AND ARTAVANIS-TSAKONAS, S (1985) Nucleotide sequence from the neurogenic locus Notch implies a gene product that shares homology with proteins containing EGF-like repeats *Cell* **43**, 567–581

YOCHEM, J AND GREENWALD, I (1989) *glp-1* and *lin-12*, genes implicated in distinct cell–cell interactions in *C elegans*, encode similar transmembrane proteins *Cell* **58**, 553–563

YOCHEM, J, WESTON, K AND GREENWALD, I S (1988) *C elegans lin-12* encodes a transmembrane protein similar to *Drosophila* Notch and yeast cell cycle gene products *Nature* **335**, 547–550

(*Accepted 1 February 1991*)