Development 116, 755-766 (1992)
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Characterization of a germ-line proliferation mutation in *C. elegans*

MAUREEN J. BEANAN* and SUSAN STROME†

Department of Biology, and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405, USA

*Current address: Department of Biochemistry, Morehouse School of Medicine, Atlanta, GA 30310, USA
†To whom reprint requests should be sent

Summary

The *C. elegans* germ line is generated by extensive proliferation of the two germ-line progenitor cells present in newly hatched larvae. We describe genetic and phenotypic characterization of *glp-4*, a locus whose product is required for normal proliferation of the germ line. *glp-4(bn2ts)* mutant worms raised at the restrictive temperature contain approximately 12 germ nuclei, in contrast to the 700-1000 present in wild-type adults. The few germ cells present in sterile *glp-4* adults appear to be arrested at prophase of the mitotic cell cycle. This cell-cycle disruption prevents the germ cells from entering meiosis and differentiating into gametes. Shifting sterile *glp-4* worms to the permissive temperature enables their germ cells to undergo extensive proliferation and form gametes, demonstrating that the *bn2*-induced cell-cycle arrest is reversible and that proliferation and differentiation of germ cells can be uncoupled from development of the somatic gonad. The *glp-4(bn2ts)* mutation can be used to generate large populations of worms that are severely depleted in germ cells, facilitating determination of whether any gene of interest is expressed in the germ line or soma or both.

Key words: *Caenorhabditis elegans*, germ-line proliferation, *glp-1*,*glp-4*.

Introduction

A crucial problem faced by all multicellular organisms is the correct spatial and temporal development of functional organs and tissues. Cells must proliferate, differentiate into their specialized cell type, and undergo correct morphogenesis into a functional tissue. Each of these developmental steps must be highly regulated, as errors in any step can lead to the formation of a dysfunctional tissue.

In this paper, we focus on the control of proliferation. Significant progress has been made in recent years towards understanding some of the proteins involved in cell cycle control (reviewed by Nurse, 1990; Nasmyth, 1990). Biochemical and genetic analyses in *Xenopus*, clams and yeast have identified a mitosis promoting factor (MPF) that consists of a ~34×10^3 M_r protein kinase ($p34^{cdc2}$) and a 45-60×10^3 M_r 'cyclin' protein whose level increases and decreases in a cell-cycle-dependent manner (Nurse and Thuriaux, 1980; Dunphy et al., 1988; Gautier et al., 1988; Draetta et al., 1989; Gautier et al., 1990). Entry into mitosis is controlled by the activity of the cdc2 kinase, which requires dephosphorylation by the cdc25 protein and association of cdc2 with cyclin (Gould and Nurse, 1989; Strausfeld et al., 1991; Solomon et al., 1990). In addition to its role in inducing mitosis, cdc2 along with a G_1-specific cyclin appears to be involved in another crucial cell cycle control point between G_1 and DNA synthesis (Wittenberg et al., 1990).

At present it is not known whether homologs of these cell cycle factors are involved in the control of cell proliferation in the nematode *Caenorhabditis elegans*. One tissue in which some of the factors controlling proliferation have been identified is the germ line. Both laser microsurgery and genetic experiments provide evidence for control of germ-line proliferation via a signal from the distal tip cell of the somatic gonad to the proliferating germ cells within the gonad (Kimble and White, 1981; Austin and Kimble, 1987). Although the signalling molecule has not yet been identified, the product of the *glp-1* (for germ-line proliferation defective) gene appears to function in the germ line to receive and/or interpret the distal tip cell signal. Consistent with this, the *glp-1* locus encodes a putative transmembrane protein and shares three repeated sequence motifs with the *lin-12* gene from *C. elegans* and the *Notch* homologs from *Drosophila*, *Xenopus* and human (Yochem and Greenwald, 1989; Wharton et al., 1985; Coffman et al., 1990; Ellisen et al., 1991); both *lin-12* and the *Drosophila Notch* gene products participate in cell-cell interactions (Seydoux and Greenwald, 1989; Hoppe and Greenspan, 1986; Technau and Campos-Ortega, 1987). Mutations in *glp-1* cause severely reduced germ-cell proliferation; in worms carrying strong alleles, only 4-8 germ cells are

formed, all of which prematurely enter meiosis and differentiate into sperm (Austin and Kimble, 1987).

In addition to *glp-1*, several other gene products that participate in controlling germ-line proliferation have recently been identified. Four maternal-effect sterile loci, *mes-2*, *mes-3*, *mes-4* and *mes-6*, encode maternally supplied products that are required for normal postembryonic germ-line development; the progeny of mutant mothers develop into agametic adults with 10- to 1000-fold reductions in number of germ cells (Capowski et al., 1991). In contrast to *glp-1* and *mes* mutations, loss-of-function mutations in *gld-1* (for germ-line development defective) lead to overproliferation of the germ line, indicating that one of the functions of the locus is to negatively regulate germ-cell proliferation, perhaps by controlling progression through meiosis (T. Schedl, personal communication).

We report here the identification and characterization of *glp-4*, another locus that appears to be involved in control of proliferation of the germ line. Like *glp-1* and the *mes* mutations, a mutation in *glp-4* leads to a greatly reduced germ line. In *glp-4* mutants, the small number of germ nuclei do not enter meiosis or differentiate into gametes and instead appear to be arrested at prophase of the mitotic cell cycle.

Materials and methods

Nematode strains and maintenance

Worms were cultivated on agar plates seeded with *Escherichia coli* strain OP50 (Brenner, 1974). The permissive temperature for *C. elegans* is 16°C. The restrictive temperature used in this study was 25°C. Wild-type N2 var. Bristol and the following mutant strains were obtained from the *Caenorhabditis* Genetics Center: *dpy-5(e61)*I, *unc-75(e950)*I, *lev-10(x17)*I, *unc-54(e675)*I, *egl-23(n601)*IV, *him-3(e1147)*IV, *him-5(e1490)*V. Two triply marked stocks, MT465 *dpy-5(e61)*I; *bli-2(e768)*II; *unc-32(e189)*III and MT464 *unc-5(e53)*IV; *dpy-11(e224)*V; *lon-2(e678)*X, were obtained from Robert Horvitz. *unc-32(e189) glp-1(q224ts)*III, *unc-32(e189) glp-1(q175)/eT1*, and *unc-32(e189) glp-1(q172)/eT1* were provided by Judith Kimble. *eT1(III;V)* is a translocation balancer for the region of LGIII containing *unc-32* and *glp-1* (Rosenbluth and Baillie, 1981). Genetic manipulations were performed as detailed by Brenner (1974).

Isolation of the glp-4(bn2) allele

Homozygous *egl-23(n601) him-3(e1147)* L4 hermaphrodites were mutagenized by a 4-hour treatment with 20 mM (final concentration) ethyl methane sulphonate (Brenner, 1974) and transferred to *E. coli* spread plates at 16°C. F_1 progeny of mutagenized parents were picked onto individual plates at 25°C. From those plates on which one-fourth of the F_2 adult progeny were sterile, fertile siblings were picked to propagate the sterile mutation in the heterozygous state. Subsequent analysis revealed that the *bn2* mutation is a temperature-sensitive allele and can be maintained in the homozygous state at the permissive temperature, 16°C.

Genetic mapping

The *bn2* allele was found to be on LGI by performing standard linkage analysis with two separate triply marked stocks, MT465 *dpy-5(e61)*I; *bli-2(e768)*II; *unc-32(e189)*III and MT464 *unc-5(e53)*IV; *dpy-11(e224)*V; *lon-2(e678)*X. A series of two-factor mapping experiments using *lev-10(x17)* and *unc-54(e675)* positioned *bn2* on the right arm of LGI (Table 2). The suppressor alleles *bn2bn39* and *bn2bn40* were positioned by performing three-factor crosses with *unc-75(e950)* and *lev-10(x17)* (Table 2). Homozygous *lev-10(x17)* animals are resistant to exposure to 1 mM levamisole. The levamisole-resistance phenotype (Lev^r) was assessed as described by Lewis et al. (1980). Briefly, adults were transferred to agar plates containing 1 mM levamisole (Sigma) for 12 to 16 hours. Non-Lev^r animals died; surviving adults were scored as homozygous *lev-10(x17)*.

Screens for other alleles of glp-4

Noncomplementation screen

Homozygous *him-5(e1490)* males were mutagenized by a 4-hour treatment with 23.5 mM, 38 mM, or 57 mM (final concentrations) ethyl methane sulphonate (Brenner, 1974) or irradiation with cesium (1500, 6000, or 8000 rad) and mated to homozygous *glp-4(bn2) unc-54(e675)* hermaphrodites at 16°C. Non-Unc outcross F_1 progeny were raised at 16°C and picked onto individual plates at the L4 stage. An egg lay/transfer step was used to enable propagation of any new noncomplementing allele. Each heterozygous F_1 hermaphrodite was first allowed to lay progeny on one plate, plate A, at 16°C. The F_1 hermaphrodite was then transferred to a second plate, plate B, at 16°C, and the F_2 progeny on plate A were shifted to 25°C. A noncomplementing allele would be expected to result in an F_2 plate with sterile Unc homozygous *glp-4(bn2) unc-54(e675)* worms plus either sterile or dead worms of the genotypes *glp-4(bn2) unc-54(e675)/glp-4(new allele)* and *glp-4(new allele)/glp-4(new allele)*. We expected to be able to retrieve the new allele of *glp-4* from the corresponding 16°C plate. 6250 F_1 hermaphrodites were scored; no noncomplementing alleles were identified.

Marked chromosome screens

Homozygous *unc-75(e950)*, *unc-54(e675)*, or *dpy-5(e61) lev-10(x17)* hermaphrodites were mutagenized with 28 mM, 38 mM, or 59 mM (final concentrations) ethyl methane sulphonate or irradiation with cesium (8000 rad). The mutagenized hermaphrodites were mated to homozygous *him-5(e1490)* males. Non-Unc or non-Dpy F_1 outcross L4 progeny were picked onto individual plates and shifted to 25°C. Sterile mutations on LGI were identified by screening for sterile Uncs or Dpys in the F_2 progeny. Heterozygous animals from plates displaying a linked sterility phenotype were used to perform complementation tests with *glp-4(bn2)*. The complementation tests were scored for both sterility and lethality. 5300 F_1 hermaphrodites were screened; no noncomplementing alleles were identified.

Isolation of suppressors of the glp-4(bn2) allele

Homozygous *glp-4(bn2)* L4 hermaphrodites raised at 16°C were mutagenized with 20 mM or 38 mM (final concentrations) ethyl methane sulphonate. The F_1 progeny were raised en masse on large plates at 16°C. All plates were shifted to 25°C when the F_1 hermaphrodites began producing progeny. The F_2 progeny from 3.9 million F_1s were screened for the presence of fertile worms. Two suppressor alleles, *bn39* and *bn40*, were isolated from separate screens.

Construction of glp-4; glp-1 double mutants

To construct *glp-4(bn2); unc-32(e189) glp-1(q224ts)/+ +*, *glp-4(bn2)* males raised at 16°C were mated to *unc-32(e189) glp-1(q224)* hermaphrodites raised at 16°C. Non-Unc F_1 outcross progeny were picked onto individual plates and allowed to produce self progeny at 16°C. Individual F_2 hermaphrodites were allowed to lay some eggs at 16°C and then shifted to 25°C. Those that produced all sterile progeny at 25°C and Unc progeny at both temperatures were judged to be homozygous for *glp-4* and heterozy-

gous for *unc-32 glp-1*. The double mutant was maintained heterozygous for *glp-1(q224)* because of the high maternal-effect embryonic lethality associated with *q224* homozygotes even at the permissive temperature.

To construct *glp-4(bn2); unc-32(e189) glp-1(q172 or q175)/eT1, glp-4(bn2)* males raised at 16°C were mated to *unc-32 glp-1/eT1* hermaphrodites. The F_1 male progeny were again mated to *unc-32 glp-1/eT1* hermaphrodites, and the F_2 hermaphrodite progeny were picked onto individual plates. Among the F_3 progeny, worms of the desired genotype (*glp-4*; *unc-32 glp-1/eT1*) were detected by the production of all sterile progeny at 25°C, and sterile Unc-32 animals and fertile Unc-36 animals (the phenotype displayed by *eT1/eT1*) at 16°C.

Laser killing of distal tip cells

The nuclei of distal tip cells were destroyed by the laser microbeam method of Sulston and White (1980), using the same model of laser (VSL-337 from Laser Science, Inc.) and procedures as described by Avery and Horvitz (1987). The laser was attached to a Zeiss Axioplan using a laser-to-microscope adapter from Zeiss. *glp-4(bn2)* larvae were raised at 25°C to L3, and one distal tip cell per worm was ablated. Operated worms were scored for an altered appearance or absence of the distal tip cell 4-12 hours after ablation. Some of the operated worms were kept at 25°C to assess the ability of mutant germ nuclei to differentiate into sperm. Other operated worms were shifted down to 16°C to serve as controls for the effectiveness of killing; the precocious appearance of sperm in the operated arms indicated that the distal tip cells were effectively killed and that the surrounding germ cells were not damaged.

Characterization of brood sizes and percent embryonic lethality

L4 hermaphrodites of the desired genotype were individually cloned onto agar plates at 25°C. The hermaphrodites were transferred to fresh plates at 8- to 16-hour intervals. An embryo was scored as being dead if it had not hatched after 24 hours at 25°C. Hatched progeny were allowed to reach adulthood and were scored as being fertile or sterile. The brood size of each animal is the sum of non-hatched and hatched progeny. Embryonic lethality is the number of non-hatched embryos divided by the sum of non-hatched and hatched progeny. Percent sterility is the number of sterile progeny divided by the total number of hatched progeny.

Temperature-shift experiments

Animals were developmentally staged by observing the L1 lethargus and molt and by Nomarski examination of the morphology of hypodermal and vulval cells in early L3s through adults (Sulston and Horvitz, 1977). For the shift-up experiments shown in Table 3, embryos were prepared by NaOCl/KOH digestion of gravid *glp-4(bn2)* hermaphrodites raised at 16°C (Johnson and Hirsh, 1979). The embryos were allowed to hatch at 16°C on plates lacking *E. coli*. The hatched L1 larvae were transferred to, and allowed to develop on, plates spread with *E. coli* until they were of the correct age to shift up to 25°C. Individual hermaphrodites were staged, shifted to 25°C, and grown to adulthood. They were scored as being fertile or sterile by screening for the presence of F_1 progeny on the plates. Young adult hermaphrodites were also shifted up and their gonad morphology and function were monitored. For the shift-down experiments shown in Table 3, embryos were prepared as above and allowed to hatch on unspread plates at 25°C. The hatched larvae were transferred to spread plates and allowed to develop until they were of the correct age to shift down to 16°C. They were scored as adults for being fertile or sterile and their hatched progeny were counted. Young adult hermaphrodites were also shifted down to 16°C and monitored for fertility until their time of death.

For the shift-up experiments shown in Table 4, individual *glp-4; unc-32 glp-1(q172 or q175)/eT1* and *glp-4; unc-32 glp-1(q224ts)/+* + hermaphrodites were transferred to drops of M9 buffer (Brenner, 1974) on gelatin-coated slides and incubated in a humid chamber. Newly hatched L1s were collected every 3-5 hours and transferred to spread plates at 16°C. Larvae at progressively later stages were shifted to 25°C and grown to young adulthood. The Unc worms (homozygous for *glp-1*) were stained with anti-sperm and anti-P-granule antibody (see below) and scored for the presence of sperm and undifferentiated germ cells.

DAPI staining of nuclei in intact worms

Nuclear patterns and morphology were visualized in intact larvae and adults by transferring the worms to a drop of 0.01 mg/ml (final concentration) DAPI (diamidinophenolindole) in 0.125% tricaine, 0.04% tetramisole on a slide. A thin layer of high vacuum grease (Dow Corning) was spread on two parallel edges of a coverslip, and the coverslip was placed over the drop with the high vacuum grease side down. The slide was placed on a flat piece of dry ice and put through five to ten cycles of freezing and thawing. The stained worms were viewed immediately.

Immunofluorescence staining of sperm and germ cells

To facilitate identifying and counting sperm and the undifferentiated germ cells characteristic of *glp-4* animals, worms were cut in half, fixed and stained with antibody, as described in Strome and Wood (1982). Briefly, worms were cut in a drop of M9 buffer on a gelatin/polylysine-coated slide, frozen in liquid nitrogen, fixed in cold methanol followed by cold acetone and air dried. They were stained with a combination of two monoclonal antibodies: SP56 stains sperm (Roberts et al., 1986; Strome, 1986b), and OIC1D4 stains P granules (Strome, 1986b). The secondary antibody was fluorescein-conjugated goat anti-mouse IgG, which binds to both primary antibodies. DAPI was added to one of the final washes, to stain the nuclei of the cut worms. Sperm and undifferentiated germ cells were easily distinguished by nuclear morphology (see Fig. 3) and by the pattern of antibody staining; the sperm antigen is on the cell surface, whereas P granules are punctate and perinuclear.

Microscopy and photography

Microscopy was performed on a Zeiss microscope (ICM405 inverted, Photomicroscope III, or Axioscope) equipped with Nomarski differential interference contrast and epifluorescence optics. For visualization of DAPI-stained DNA, specimens were examined with 365 nm epi-illumination. For visualization of fluorescein immunofluorescence, specimens were examined with 440-490 nm epi-illumination. Photographs were taken using Kodak Tri-X film at ASA 1600. The film was developed using Diafine two-bath developer (Acufine Inc., Chicago, Il.).

Results

Review of gonadogenesis and germ-line development in C. elegans

The cell lineage and development of the *C. elegans* gonad have been studied in detail (Hirsh et al., 1976; Kimble and Hirsh, 1979; Kimble and White, 1981). Gonadogenesis in *C. elegans* hermaphrodites and males is shown schematically in Fig. 1. A 4-cell gonad primordium is present in newly hatched larvae of both sexes. Of these four cells, the

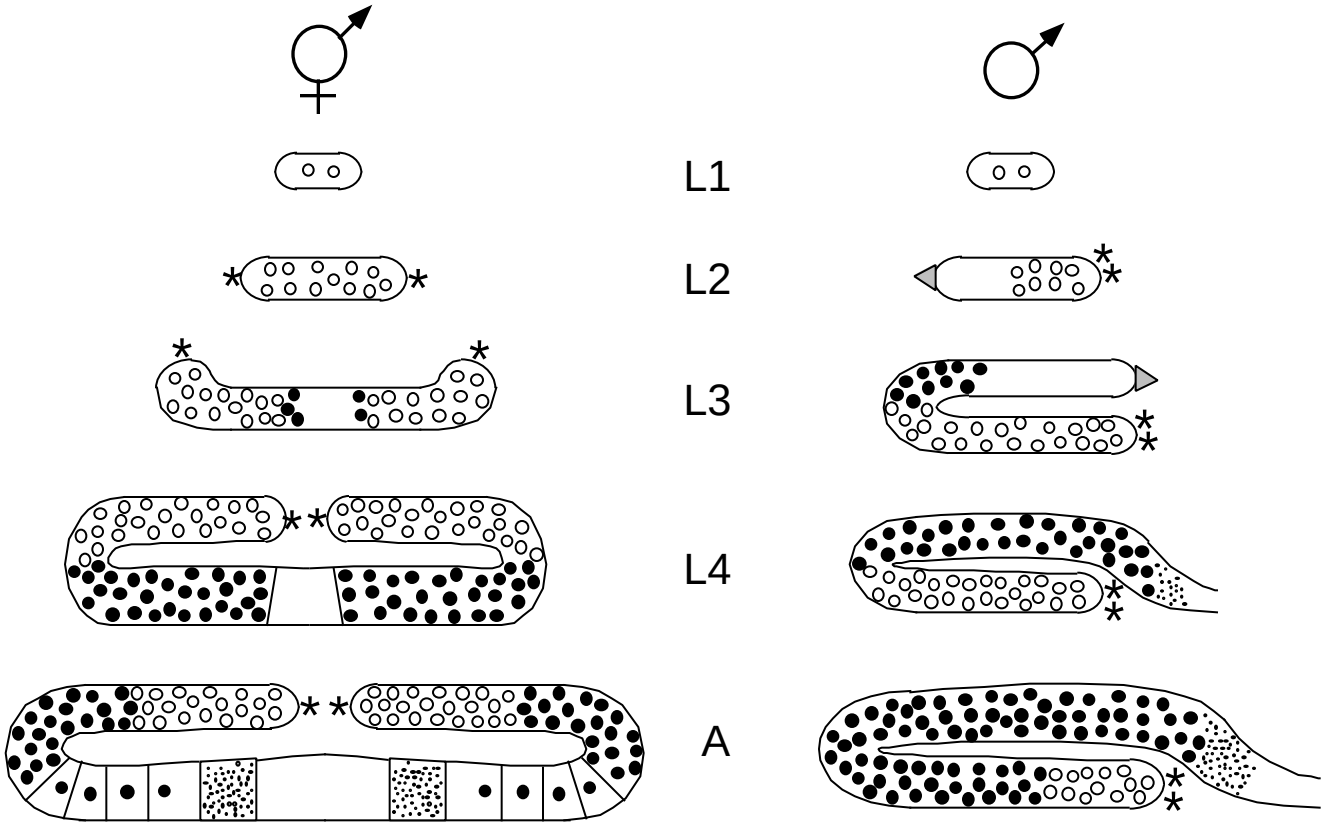


Fig. 1. Gonadogenesis and germ-line development in wild-type hermaphrodites and males. These diagrams represent developing gonads during the postembryonic stages. Anterior is to the left; ventral is down. L1, L2, L3, and L4 are the four larval stages. A is adult. Distal tip cells are marked by asterisks, mitotic germ nuclei by open circles, meiotic germ nuclei by closed circles, and sperm by dots; the large blocks in adult hermaphrodites are oocytes. Adapted from Austin and Kimble (1987).

two somatic precursor cells, Z1 and Z4, proliferate and differentiate into the somatic structures of the gonad, which include the distal tip cells, sheath cells, spermathecal cells and uterine cells. The two germ-line precursors, Z2 and Z3, proliferate, enter meiosis and differentiate into gametes. In hermaphrodites, the distal tip cells, located at the distal end of each gonad arm, direct the elongating arms to extend and reflex 180 degrees. As a result, the adult hermaphrodite gonad consists of two U-shaped arms attached to a single uterus. *C. elegans* males have a single gonad structure whose elongation is led by a different somatic cell, the linker cell. The mitotic and meiotic germ nuclei in both hermaphrodites and males are located at the periphery of the gonadal cylinder and are incompletely cellularized (Hirsh et al., 1976; Strome, 1986a). Although we and others (Austin and Kimble, 1987; Maine and Kimble, 1989) use the term ‘germ cells’, the germ nuclei in fact proliferate and develop within a syncytium.

Within each reflexed gonad arm, an ordered progression of mitosis, meiosis and gamete formation occurs (Kimble and White, 1981). The nuclei in the distal-most region of each gonad arm close to each distal tip cell remain mitotically active. In late L3 hermaphrodites, the germ cells most distant from the distal tip cell enter meiosis and during L4 differentiate into sperm, which become localized to the spermatheca. After the L4 to adult molt, the gonad converts from spermatogenesis to oogenesis; hermaphrodites continue to produce oocytes throughout adulthood. Young adult hermaphrodites contain a total of approximately 700 germ-line nuclei (Table 1). This number increases to a total of approximately 1200 germ-line nuclei as the adults age.

Identification and characterization of glp-4(bn2)

The *glp-4(bn2)* mutation was isolated in a screen for sterile hermaphrodites. It maps to the right arm of chromosome

Table 1. *Germ-line proliferation in hermaphrodites raised at 25°C**

	Total number of germ nuclei in:			
Stage	N2	*n*	*glp-4(bn2)*	*n*
L1	2		2	
mid-L3	48±17	23	8±2	14
early-mid L4	266±27	25	10±2	13
young adult	676±157†	5	12±1	7
2-day adult	1185±127‡	5	28±12	5
5-day adult	N.D.		76±25	5
9-day adult	N.D.		277±61	5

*These numbers were obtained by DAPI staining staged hermaphrodites and counting the germ nuclei present in the gonad at each time point. Values are the mean±standard deviation.

†This number includes mitotic, meiotic and sperm nuclei.

‡This number includes mitotic, meiotic, oocyte and sperm nuclei.

n=number of hermaphrodite gonads counted per experiment.

N.D.=not determined.

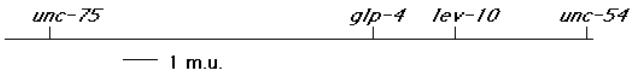


Fig. 2. Map position of *glp-4*. This figure shows the rightmost 17 map units of chromosome I. *glp-4* maps 2.3 map units to the left of *lev-10*. See Table 2 for further details.

I (Fig. 2, Table 2) and is a recessive, temperature-sensitive mutation that results in sterility only when the animals are grown at the restrictive temperature, 25°C. Young adult *glp-4* mutant hermaphrodites raised at 25°C have non-reflexed gonad arms that contain an average of only 12 germ nuclei (Fig. 3B, Table 1), compared to the approximately 700 germ nuclei in young adult wild-type hermaphrodites (Fig. 3A, Table 1). The few germ nuclei present in young *glp-4* adults continue to proliferate as the adults age at 25°C (Table 1), but a functional germ line never develops and the hermaphrodites remain sterile. The germ nuclei in *glp-4* mutant animals (Fig. 3D) appear morphologically similar to a subset of the nuclei found in the mitotic region of wild-type gonad arms (Fig. 3C); in DAPI-stained gonads, the nuclei contain discrete, condensed bodies that are probably individual chromosomes. The somatic structures of *glp-4* gonads appear to have wild-type morphology when visualized by Nomarski optics. In general, the mutant animals appear morphologically normal and healthy. The only obvious phenotype is the absence of gametes and embryos.

As shown in Table 1, the reduction in the number of germ-line nuclei in mutant worms raised at 25°C appears to be due to a very slow rate of proliferation of the germ-line progenitor cells, Z2 and Z3, during larval development. We have seen no evidence for proliferation, followed by rapid disintegration of the germ cells, although that remains a formal possibility.

The germ-line proliferation defect described for hermaphrodites is also observed in males. Similar to *glp-4(bn2)* hermaphrodites, young adult *glp-4(bn2)* males raised at 25°C have only 16±2 germ nuclei. In contrast to hermaphrodites, however, these males contain a normally reflexed gonad. Sterility appears to be the only defect of *glp-4(bn2)* males raised at 25°C.

At the permissive temperature, 16°C, homozygous *glp-4(bn2)* hermaphrodites display a weak germ-line proliferation phenotype. These animals produce embryos 12 hours later than wild-type hermaphrodites and have a reduced brood size (Table 3). Otherwise, *glp-4(bn2)* hermaphrodites raised at 16°C appear normal and exhibit wild-type behavior.

At present *bn2* is the only allele of the *glp-4* locus; our attempts to isolate other mutant *glp-4* alleles have been unsuccessful. We have used both a noncomplementation screen for sterile and lethal mutations (6250 F_1s screened) and several marked chromosome I screens (5300 F_1s screened). In the latter screens, we isolated sterile muta-

Table 2. *Mapping of* glp-4(bn2) *and* bn2 *revertants*

A. *Two-factor mapping of* glp-4(bn2)

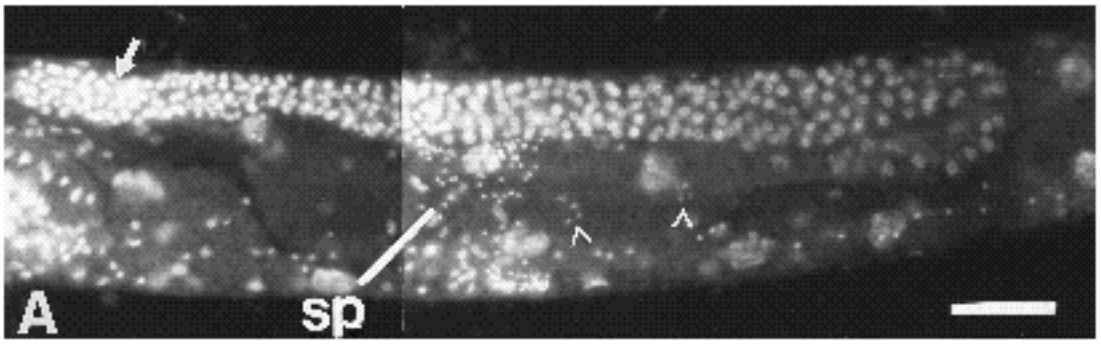
Allele	Heterozygous parent	Segregants	Map distance
bn2	*glp-4(bn2) unc-54/++*	773 wild type 132 Glp Unc 22 Glp 22 Unc	4.8±1.5 m.u. from *unc-54*
bn2	*glp-4(bn2) lev-10/++*	497 Glp Lev^r 23 Lev^r	2.3±0.9 m.u. from *lev-10*

B. *Three-factor mapping of* glp-4(bn2)

Allele	Parental genotype	Recombinant phenotype	Recombinant genotype	Number
bn2	$\frac{glp\text{-}4(bn2)++}{lev\text{-}10\ unc\text{-}54}$	Lev^r non-Unc	$\frac{lev\text{-}10+}{lev\text{-}10\ unc\text{-}54}$	28
			$\frac{glp\text{-}4(bn2)\ lev\text{-}10+}{lev\text{-}10\ unc\text{-}54}$	0

C. *Three-factor mapping of* (bn2bn39) *and* (bn2bn40)

Allele	Parental genotype	Recombinant phenotype	Recombinant genotype	Number
bn2bn39	$\frac{unc\text{-}75\ (bn2bn39)}{glp\text{-}4(bn2)\ lev\text{-}10}$	Lev^r	$\frac{unc\text{-}75\ (bn2bn39)\ lev\text{-}10}{glp\text{-}4(bn2)\ lev\text{-}10}$	5
			$\frac{unc\text{-}75\ glp\text{-}4(bn2)\ lev\text{-}10}{glp\text{-}4(bn2)\ lev\text{-}10}$	44
bn2bn40	$\frac{unc\text{-}75\ (bn2bn40)}{glp\text{-}4(bn2)\ lev\text{-}10}$	Lev^r	$\frac{unc\text{-}75\ (bn2bn40)\ lev\text{-}10}{glp\text{-}4(bn2)\ lev\text{-}10}$	8
			$\frac{unc\text{-}75\ glp\text{-}4(bn2)\ lev\text{-}10}{glp\text{-}4(bn2)\ lev\text{-}10}$	43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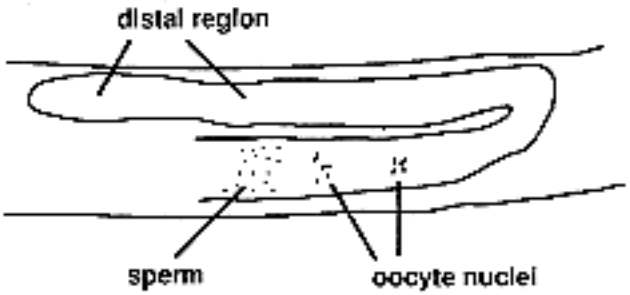
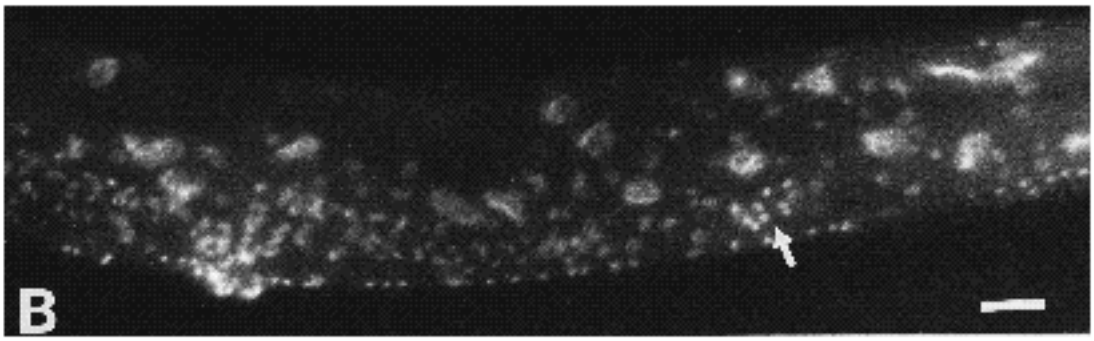

distal region
sperm
oocyte nuclei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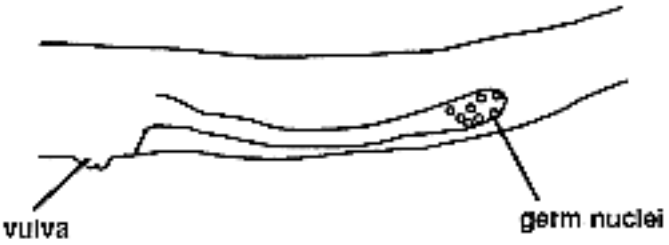
vulva
germ nuclei

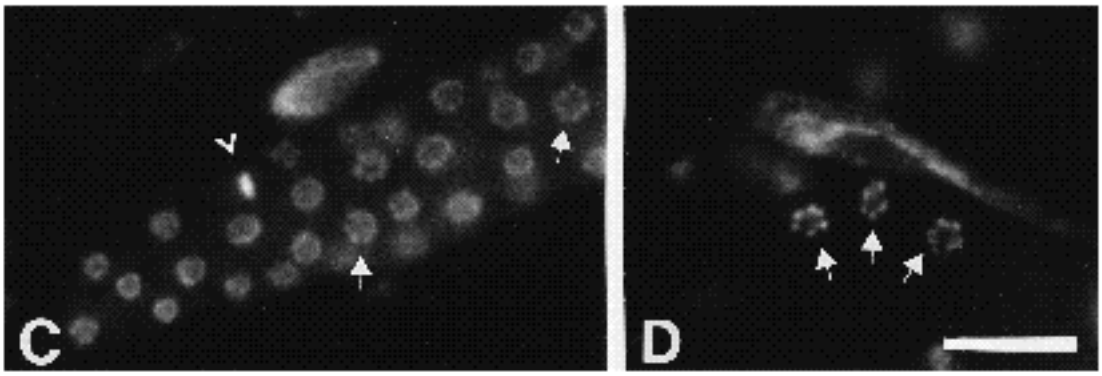
C
D

Fig. 3. Germ nuclei stained with DAPI in wild-type and *glp-4(bn2)* young adult hermaphrodites raised at 25°C. Intact animals were stained with DAPI. The vulva in each animal is down and to the left. (A) Wild-type hermaphrodite. This reflexed gonad arm has mitotic nuclei in the distal region (arrow), meiotic nuclei, oocytes (arrowheads), and sperm (sp). (B) *glp-4(bn2)* hermaphrodite. This non-reflexed gonad arm contains only 9 germ nuclei (arrow). (C) Wild-type hermaphrodite, high-magnification view of the distal region. Two germ nuclei like those present in *glp-4* animals are marked with arrows. The arrowhead marks a germ cell in metaphase. (D) *glp-4(bn2)* hermaphrodite. The three germ nuclei in this focal plane are marked with arrows. Bars = 50 μm for A and B and 10 μm for C and D.

tions linked to chromosome I and performed complementation tests. None of these screens produced mutations that failed to complement *glp-4(bn2)* (see Materials and methods, and Discussion).

We also performed complementation tests to determine whether deficiencies that remove *unc-54*, *lev-10*, and other genes on the right arm of LGI (Anderson and Brenner, 1984) also delete *glp-4*. Using each of the four largest deficiencies (*eDf3*, *eDf6*, *eDf7*, and *eDf12*), *glp-4/Df* hermaphrodites were fertile at 25°C and 16°C, and their brood sizes were similar to the brood sizes of heterozygous *glp-4(bn2)/+* hermaphrodites raised at 25°C and 16°C (data not shown). These results indicate that either *glp-4(bn2)* is a recessive hypermorph, or more likely, the deficiencies do not remove the *glp-4* locus.

Table 3. *Temperature shift analysis of* glp-4(bn2) *hermaphrodites*

A. *Unshifted control animals raised at 16°C*

Genotype	Number of fertile hermaphrodites	Average brood size*	*n*
glp-4(bn2)	22/22	159±56	22
N2	10/10	316±47	10

B. *Shift of* glp-4(bn2) *from 16°C to 25°C*

Stage of shift up to 25°C	Number of fertile hermaphrodites	Total number of germ nuclei in adults	*n*
L1	0/15	17±3	5
L2	0/19	32±9	3
L3	0/17	77±14	5
L4	0/17	N.D.	
N2 raised at 25°C†		676±157	5

C. *Shift of* glp-4(bn2) *from 25°C to 16°C*

Stage of shift down to 16°C	Number of fertile hermaphrodites	Average brood size*	*n*
L1	18/18	122±30	5
L2	20/20	132±46	5
L3	20/20	21±7	5
L4	3/20	4±3	3
Adult	5/21	9±5	5

*Numbers represent hatched progeny only. Brood sizes are expressed as the mean±standard deviation.

†Taken from Table 1.

n=number of hermaphrodites per average brood size measurement or determination of number of germ nuclei.

N.D.=not determined.

The germ nuclei in glp-4(bn2) hermaphrodites are blocked from undergoing gametogenesis

The few germ nuclei present in *glp-4* animals raised at 25°C do not form gametes. This could be due to their proximity to the distal tip cell, which functions to maintain a proliferating population of germ cells (Kimble and White, 1981), or to an inability of the germ nuclei to enter meiosis and form gametes. We took two approaches, laser ablation and double mutant analysis, to discriminate between these possibilities. Laser ablation of the distal tip cells in wild-type hermaphrodites causes all of the germ cells within the operated gonad arm to enter meiosis and differentiate into gametes (Kimble and White, 1981). When the ablation is done during L1, L2 or early L3, all germ cells differentiate into sperm (Kimble and White, 1981). As shown in Table 4, after ablation of one of the two distal tip cells in L3 stage *glp-4* hermaphrodites raised at 25°C, the germ nuclei in the operated gonad arms did not differentiate into sperm. Furthermore, the similarity of the DAPI-stained germ nuclei in the operated and unoperated arms suggests that the germ nuclei in *glp-4* animals are blocked from entering meiosis. As a control, operated *glp-4* animals were shifted from 25°C to the permissive temperature, 16°C. All of the germ nuclei in the operated gonad arms of the shifted

Table 4. *Assessing the ability of* glp-4(bn2) *germ nuclei to differentiate into gametes*

A. *Laser ablation of distal tip cells in* glp-4(bn2) *hermaphrodites**

Temperature after ablation	Time of examination	Number of hermaphrodites with sperm in ablated gonad arm
25°C	24-26 hours post-ablation	0/1†
	48-60 hours post-ablation	0/5†
16°C	96 hours post-ablation	4/4‡

B. *Analysis of* glp4; glp-1 *double mutants*

Genotype	Stage of shift up to 25°C	Number of gonad arms containing sperm§	Number of germ nuclei in spermless arms§
glp-4(bn2)	early L1	0/13	4-10
glp-4(bn2); *glp-1(q224ts)*	early L1	0/48	0-3
	mid L1	0/20	0-6
	late L1	1/18	0-3
	L1/L2	15/27	0-4
	early L2	26/26	N.A.
glp-4(bn2); *glp-1(q172)*	early L1	0/16	0-2
	mid L1	0/9	0-2
	late L1	4/6	0-2
	L1/L2	10/13	0
	early L2	7/7	N.A.

**glp-1(bn2)* hermaphrodites were raised at 25°C to the L3 stage. The distal tip cell of one arm of the gonad was ablated.

†The ablated arms contained germ nuclei that by DAPI staining resembled those in the unablated arms.

‡The ablated arms contained only sperm. The unablated arms contained sperm and undifferentiated germ cells.

§Adult worms were cut in half, fixed, and stained with a combination of two monoclonal antibodies: SP56 stains sperm and OIC1D4 stains the P granules that surround undifferentiated germ-line nuclei in wild-type and *glp-4* gonads. The numbers shown are the range of undifferentiated germ nuclei counts.

N.A. = not applicable. These gonad arms contain all sperm.

animals differentiated into sperm, and in two of the four animals sperm were seen earlier in the operated than in the unoperated arm. These results demonstrate that the distal tip cells were effectively killed by the laser treatment, and that nearby germ cells were not impaired in their ability to enter meiosis and form gametes.

The ability of *glp-4* germ nuclei to differentiate into gametes was also tested by placing them in a *glp-1* mutant background. As previously described, *glp-1* mutant animals have reduced germ lines in which all of the germ cells differentiate into gametes (Austin and Kimble, 1987). We constructed double mutant strains containing *glp-4(bn2)* in combination with a non-conditional *glp-1* allele (*q172* or *q175*) or a temperature-sensitive *glp-1* allele (*q224ts*). As shown in Table 4, sperm were not present in the gonads of adult *glp-4; glp-1* hermaphrodites that had been shifted to 25°C during the first half of L1. The gonads of these sperm-less animals either lacked germ cells altogether or contained a very small number of undifferentiated germ nuclei typical of *glp-4* mutants. Double mutant hermaphrodites shifted to 25°C during the second half of L1 contained sperm in some gonad arms and no or very few undifferentiated germ cells in other gonad arms. Hermaphrodites shifted to 25°C during or after early L2 resembled *glp-1* adults; all gonad arms contained only sperm. Similar results were obtained with all three *glp-1* alleles tested.

The above results suggest that the germ nuclei in *glp-4* animals raised at 25°C are unable to enter meiosis and form gametes, even after being released from the influence of the distal tip cell. The *glp-4; glp-1* shift-up experiments define a window of development, during late L1 and early L2, when the double mutants switch from being blocked to being able to enter meiosis. This probably reflects competition between two processes: loss of functional *glp-4* product causes germ cells to arrest at a premeiotic stage, and loss or absence of functional *glp-1* product causes germ cells to enter meiosis prematurely, at late L2 (Austin and Kimble, 1987). In upshifted L1s, the *glp-4* block goes into effect before germ cells are signalled to enter meiosis. In upshifted L2s, germ cells become committed to meiosis before the *glp-4* block is effected.

The temperature-sensitive period of glp-4(bn2)

Temperature-shift experiments were done to determine when during the *C. elegans* life cycle the *glp-4* product is required for germ-line development. This analysis involved performing a series of shift-up and shift-down experiments at each of the four larval stages and subsequently observing whether the shifted hermaphrodites were fertile or sterile. Larval animals were staged using Nomarski optics to visualize ventral hypodermal cells, as described by Sulston and Horvitz (1977).

The results of the shifts from permissive (16°C) to restrictive (25°C) temperature demonstrate that interference with production of functional *glp-4* product at any time during larval development interferes with proliferation of the germ line and leads to sterility (Table 3). As expected, animals shifted later in larval development contained more germ nuclei than animals shifted early, but even late-shifted larvae developed into sterile adults.

The results of the shifts from 25°C to 16°C demonstrate that the negative effect of being at the restrictive temperature is reversible. Young adults raised at 25°C and containing only 6-10 germ nuclei at the time of the downshift contained 100-200 germ nuclei three days later and 400-800 germ nuclei seven days later (data not shown). Furthermore, downshifted animals can become fertile (Table 3). Animals shifted down to 16°C during L1 or L2 developed morphologically wild-type gonads and had fairly normal brood sizes when compared to unshifted *glp-4* hermaphrodites. Animals shifted down during L3 developed into fertile adults, but with reduced brood sizes. Only a small percentage of animals shifted down during L4 or early adulthood became fertile adults, and all of these had greatly reduced brood sizes. The reduction in brood size was probably due to the reduction in number of germ nuclei relative to wild type (see above) and an aberrant spatial organization of the germ-line tissue; in DAPI-stained specimens, sperm and oocytes were observed to be intermingled with each other and with undifferentiated germ cells (not shown). Two conclusions can be drawn from the young adult shift-down experiments: (1) At least a few of the germ nuclei present in *glp-4* animals raised at 25°C remain competent to undergo significant proliferation, enter meiosis and form gametes when shifted down to the permissive temperature. (2) Development of the germ line in *C. elegans* hermaphrodites can be uncoupled from development of the somatic gonad. In wild-type animals, these processes are temporally coupled. However, in *glp-4* animals raised to adulthood at 25°C and then shifted down to 16°C, somatic gonad development precedes germ-line proliferation and differentiation. In such downshifted animals, the germ tissue is disorganized and the number of germ cells reduced compared to wild-type gonad arms, but as shown in Table 3, the germ line can still be functional.

In addition to the effect on germ-line proliferation observed when *glp-4* larvae were shifted to the restrictive temperature, we observed an effect on production of viable progeny when *glp-4* adults were shifted to the restrictive temperature. When fertile *glp-4* hermaphrodites (raised at 16°C) were shifted to the restrictive temperature, the germ line became disorganized and approximately 48 hours later began to produce and accumulate reduced-size oocytes (Fig. 4B). The embryos that were produced by these upshifted adults exhibited variably abnormal early division patterns and usually arrested between the 30- and 100-cell stages of embryogenesis (data not shown). Some embryos developed into morphologically abnormal L1 larvae, which also died. This embryonic/larval lethality is most likely a secondary effect of the *glp-4* germ line and/or somatic gonad of the mother becoming functionally altered after being shifted to the restrictive temperature. The alternative possibility, that the observed embryonic/larval lethality is due to a requirement for the *glp-4* product during embryogenesis, is argued against by the fact that the defect was not observed until 48 hours after the shift. Approximately 35 normal embryos per hermaphrodite were produced during this 48-hour period. In addition, *glp-4* embryos produced at 16°C undergo normal embryogenesis after being shifted to 25°C. Furthermore, when these animals are shifted back down to 16°C after hatching, they develop into fertile adults. Thus, in adult hermaphrodites, wild-type *glp-4* product appears to

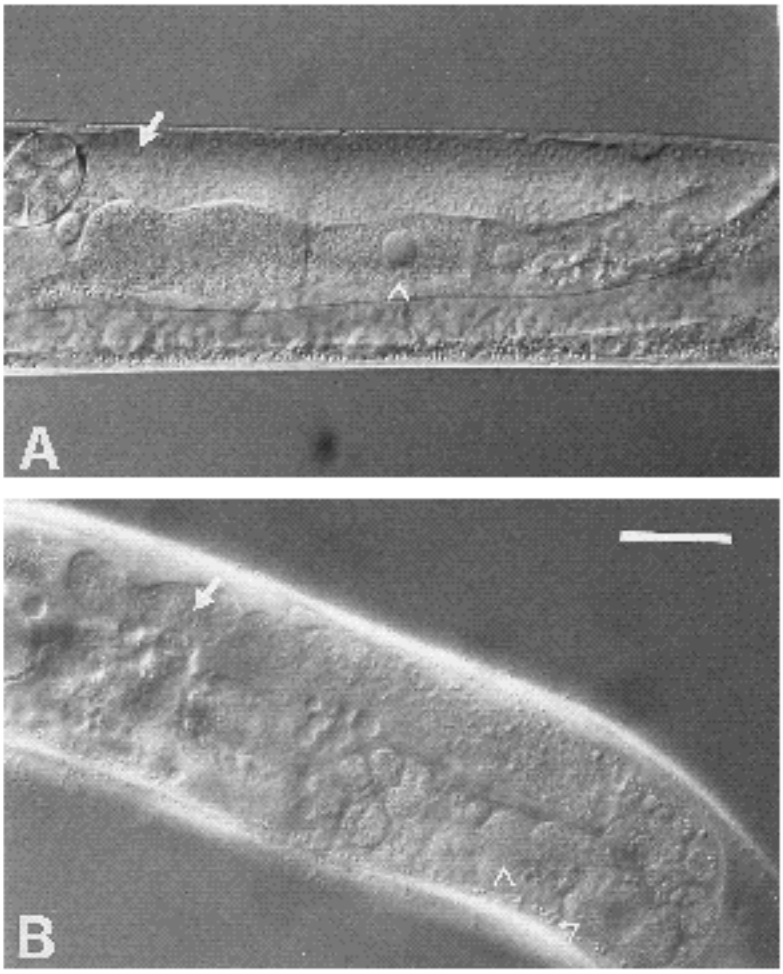

Fig. 4. Abnormal oocytes produced by *glp-4(bn2)* hermaphrodites shifted to 25°C. Animals were raised at 16°C until they began producing embryos and then were shifted to 25°C for 44 hours. The vulva in each animal is down and to the left. The arrows point to the distal region of each gonad arm. (A) *unc-75(e950)* hermaphrodite. The germ line is morphologically wild-type. Oocytes (arrowhead) and embryos are still being produced. (B) *unc-75(e950) glp-4(bn2)* hermaphrodite. The oocyte region of the arm is filled with small, morphologically abnormal "oocytes" (arrowhead). Bar = 50 μm.

be continuously required for maintenance of a functional germ line.

In addition to shift-up and shift-down experiments, *glp-4* larvae were transiently shifted to the restrictive temperature for 11- to 15-hour intervals at various stages during larval development. No significant effects were observed; the hermaphrodites were fertile and began producing embryos at about the same time as control hermaphrodites (data not shown). These results, taken together with the other temperature-shift analyses, demonstrate that the *glp-4* product functions throughout larval development and is not absolutely required during any individual larval stage. As described above, the *glp-4* product is also required in the adult hermaphrodite to maintain a functional germ line.

Intragenic revertants of glp-4(bn2)

We screened through approximately 8×10^6 EMS-mutagenized haploid genomes and isolated two suppressors of the *glp-4(bn2)* sterile phenotype. Both map to the same region of LGI as *glp-4(bn2)* and appear to be intragenic revertants of *glp-4* (Table 2). Alternatively, these suppressors may be extragenic and tightly linked to *glp-4*. Both suppressors, referred to as *glp-4(bn2bn39)* and *glp-4(bn2bn40)*, are presumed to carry the original *bn2* allele as well as a second mutation, because neither restores germ-line proliferation or fertility to wild-type levels (Table 5).

To assess the suppression strength of each allele, we measured the average brood sizes, percent embryonic lethality, percent sterile and fertile progeny and average number of germ nuclei per gonad (Table 5). Homozygous *glp-4(bn2bn39)* animals are only weakly fertile at 25°C. Fertile *glp-4(bn2bn39)* hermaphrodites have a greatly reduced average brood size of about 17 progeny. Of these progeny, 15% arrest during embryogenesis and 43% become sterile adults. Sterile *glp-4(bn2bn39)* hermaphrodites exhibit defective germ-line proliferation. Fertile *glp-4(bn2bn39)* hermaphrodites contain morphologically normal gonads with mitotic nuclei, meiotic nuclei, oocytes and sperm, but the total number of germ nuclei is reduced. Thus, these gonads are functional, but not completely wild type. The embryonic lethality is most likely due to fertilization of defective oocytes, as is seen in *glp-4(bn2)* animals shifted to 25°C.

Homozygous *glp-4(bn2bn40)* hermaphrodites also have a reduced average brood size and display somewhat elevated embryonic lethality relative to control hermaphrodites. However, in contrast to homozygous *glp-4(bn2bn39)* hermaphrodites, only 3% of the progeny develop into sterile adults. Similar to fertile *glp-4(bn2bn39)* hermaphrodites, the gonad morphology of fertile *glp-4(bn2bn40)* hermaphrodites is normal, but again the total number of germ nuclei is reduced.

Table 5. *Analysis of germ-line proliferation and fertility of* glp-4(bn2bn39) *and* glp-4(bn2bn40) *hermaphrodites at 25°C†*

Genotype	Average brood size‡	% Embryonic lethality‡	% Sterile progeny§	Average number of germ nuclei per gonad in: Sterile adults	Fertile adults
+/+*	141±45	5	0	−	531±66
	n=6				*n*=10
bn2/bn2	0	N.A.	N.A.	12±1	−
				n=7	
bn2bn39/bn2bn39	17±6	15	43	81±41	252±56
	n=6			*n*=5	*n*=7
bn2bn40/bn2bn40	38±20	9	3	N.D.	257±76
	n=5				*n*=11
bn2bn39/bn2	0	N.A.	N.A.	42±17	−
				n=5	
bn2bn40/bn2	10±6	0	71	37±15	343±93
	n=8			*n*=5	*n*=4
bn2bn39/bn2bn40	72±10	26	0	−	N.D.
	n=5				

*Counts for homozygous +, *bn2bn39* and *bn2bn40* animals were done with adults produced and raised at 25°C. Counts for *bn2/bn2* and heterozygous combinations were done with F_1 progeny produced at 16°C and raised at 25°C.

†These counts were done on animals in an *unc-75* background, so +/+=*unc-75/unc-75*.

‡Brood measurements are the total number of non-hatched and hatched embryos per hermaphrodite, expressed as the mean±standard deviation. % embryonic lethality was calculated by dividing the number of non-hatched embryos by the total number of non-hatched and hatched embryos.

§Percent sterile progeny was calculated by dividing the number of sterile progeny by the total number of hatched embryos.

n=number of hermaphrodites per experiment.

N.A.=not applicable. These animals are sterile at 25°C.

N.D.=not determined.

Thus, these two apparent intragenic revertant mutations when homozygous are able to suppress partially the germ-line proliferation and sterile phenotypes of *glp-4(bn2)* at 25°C. The *bn40* mutation is a slightly stronger suppressor than the *bn39* mutation and can partially suppress *glp-4(bn2)* sterility even when heterozygous (Table 5). As described in the Discussion, isolation and analysis of these revertants argue against one possible explanation of the nature of the *glp-4(bn2)* mutation.

Discussion

glp-4(bn2) is a recessive, temperature-sensitive mutation that interferes with proliferation of the germ line during the larval and adult stages of *C. elegans* development. Young homozygous mutant adults raised at the restrictive temperature contain only about 12 germ nuclei, which resemble a subset of the nuclei in the mitotic region of wild-type gonads. This suggests that the germ nuclei in *glp-4* animals are arrested at a stage of the normal mitotic cell cycle. The condensed appearance of the DNA is consistent with arrest in prophase. The arrest is not absolute since the number of germ nuclei increases as *glp-4* adults age (Table 1).

The few germ nuclei present in *glp-4* hermaphrodites raised from hatching at the restrictive temperature do not undergo meiosis and gametogenesis, even after laser ablation of the distal tip cells or when placed in a *glp-1* mutant background, both of which interfere with the distal tip cell-germ cell signalling pathway. These results argue against the hypothesis that the few germ nuclei present in *glp-4* animals are capable of entering meiosis but are prevented from doing so by the distal tip cells. Instead, the *glp-4(bn2)*-induced disruption of the cell cycle apparently prevents the germ nuclei from switching from mitosis to meiosis.

The distal tip cell-*glp-1* signalling pathway is thought to function either to inhibit meiosis or to promote mitosis (Kimble and White, 1981). The finding that *glp-4; glp-1* double mutant larvae and adults contain even fewer germ nuclei than *glp-4* worms (Table 4) provides support for the latter role; functional *glp-1* product appears to be required for what limited germ-line proliferation is seen in *glp-4* mutant animals. Another possibility is that functional *glp-1* product is required for germ-cell viability in a *glp-4* mutant background.

The temporal requirement for *glp-4*$^+$ function was addressed by temperature-shift analysis. While the shift-up results indicate that *glp-4* functions during all four larval stages, the shift-down results indicate that *glp-4* function is not required during L1 and L2, but is required during later larval stages. In addition to its role in germ-line proliferation, *glp-4*$^+$ product is also required for maintenance of a functional germ line in adults. The young adult shift-down experiment further demonstrates that development of the germ line in *C. elegans* hermaphrodites can be uncoupled from development of the somatic gonad.

What is the role of the *glp-4* gene product? (1) The *glp-4* gene may encode a cell cycle factor that is expressed or functions exclusively in the germ line. (2) It is also possible that *bn2* represents a tissue-specific allele of a locus that

functions in proliferation of the germ line and other tissues. This could occur if *bn2* is a hypomorphic allele and if the germ line is more sensitive to the level of functional *glp-4* product than other tissues. (3) *glp-4*$^+$ product may be required for the normal distal tip cell function that controls germ-line proliferation in hermaphrodites and males (Kimble and White, 1981). (4) *glp-4*$^+$ product may function elsewhere in the somatic gonad to create the proper environment or signals for proliferation of the germ cells within the gonad.

Knowledge of the null phenotype of *glp-4* would help us discriminate between these and other possibilities. However, apparently there are no deficiencies that remove the *glp-4* locus. Thus, we have been unable to place *bn2* over a deficiency of the locus to test the likelihood that *glp-4(bn2)* animals display the null phenotype.

As stated previously, our attempts to isolate other mutant alleles of the *glp-4* locus have been unsuccessful. We have used both an F_1 noncomplementation screen for sterile and lethal mutations (6250 F_1 screened) and a series of F_2 screens for sterile mutations on chromosome I (5300 F_1 screened). None of these screens resulted in the isolation of mutations that failed to complement *glp-4(bn2)*. There are several possible explanations for the failure to isolate noncomplementing alleles using either of our screens. (1) *glp-4* may be a very small gene and therefore a small target for inactivation by mutagenesis. (2) The F_1 noncomplementation screen involved placing a mutagenized chromosome over a marked *glp-4(bn2)* chromosome and relied on the *bn2/new allele* combination being fertile at 16°C (see Materials and methods for details). If this heterozygous combination leads to sterility or lethality at 16°C, we would not be able to recover the new allele. (3) *bn2* may be an unusual allele of a locus whose null phenotype is wild type, due to redundancy of function provided by another locus (Greenwald and Horvitz, 1980). For example, *bn2* could be an antimorphic allele that when homozygous acts as a poison and interferes with the normal functioning of the redundant gene. Our intragenic reversion analysis argues against this possibility, since we recovered intragenic revertants of the *bn2* mutant phenotype at a frequency of 2×10^{-7}. This frequency is approximately 2000-fold lower than the frequency of generating loss-of-function mutations in *C. elegans*. Thus, it appears that the *bn2* mutant phenotype cannot be reverted simply by loss of gene activity and that the *glp-4* product is likely to serve an essential role. Molecular analysis of the *glp-4* locus may reveal why it has been difficult to obtain other mutant alleles.

While this locus is not well understood genetically, the *glp-4(bn2)* mutation already has provided useful information about the dynamics and plasticity of germ-line development. Furthermore, it has proven very useful for addressing whether the transcripts for any gene of interest are expressed in the germ line or soma or both. For example, northern analysis using mRNA isolated from N2 hermaphrodites and young *glp-4(bn2)* hermaphrodites, which essentially lack a germ line, has revealed that the *glp-1* locus is expressed predominantly in the germ line during late larval development and in adults (Austin and Kimble, 1989). Similar analyses have demonstrated the production of germ-line-specific transcripts from *skn-1*, a newly identified maternal-effect gene required for specification of intestinal and pharyngeal cell fates (Bowerman et al., 1992), and *fem-3* and *tra-2*, two sex-determination genes (Rosenquist and Kimble, 1988; Okkema and Kimble, 1991).

We are especially grateful to Tim Schedl and Ross Francis (University of Washington) for providing the laser and assisting with the laser ablation experiments. We thank Judith Kimble for providing the *glp-1* alleles, and Ian Korf and Cindi Madej for assistance with the figures and tables. We appreciate the critical comments on the manuscript provided by Tom Blumenthal, Beth Capowski, Peg MacMorris, Tim Schedl, and two anonymous reviewers. This research was supported by NIH grant GM34059 to S.S. Some nematode strains used in this work were provided by the *Caenorhabditis* Genetics Center, which is funded by the NIH National Center for Research Resources (NCRR).

References

Anderson, P. and Brenner, S. (1984). A selection for myosin heavy-chain mutants in the nematode *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **81**, 4470-4474.

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.

Avery, L. and Horvitz, H. R. (1987). A cell that dies during wild-type *C. elegans* development can function as a neuron in a *ced-3* mutant. *Cell* **51**, 1071-1078.

Bowerman, B., Eaton, B. A. and Priess, J. R. (1992). *skn-1*, a maternally expressed gene required to specify the fate of ventral blastomeres in the early *C. elegans* embryo. *Cell* **68**, 1061-1075.

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

Capowski, E. E., Martin, P., Garvin, C. and Strome, S. (1991). Identification of grandchildless loci whose products are required for normal germ-line development in the nematode *Caenorhabditiselegans. Genetics* **129**, 1061-1072.

Coffman, C., Harris, W. and Kintner, C. (1990). *Xotch*, the *Xenopus* homolog of *DrosophilaNotch*. *Science* **249**, 1438-1441.

Draetta, G., Luca, F., Westendorf, J., Brizuela, L., Ruderman, J. and Beach, D. (1989). cdc2 protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. *Cell* **56**, 829-838.

Dunphy, W. G., Brizuela, L., Beach, D. and Newport, J. (1988). The *Xenopus* cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* **54**, 423-431.

Ellisen, L. W., Bird, J., West, D. C., Soreng, A. L., Reynolds, T. C., Smith, S. D. and Sklar, J. (1991). *TAN-1*, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649-661.

Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J. (1988). Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene *cdc2*$^+$. *Cell* **54**, 433-439.

Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T. and Maller, J. L. (1990). Cyclin is a component of maturation-promoting factor from *Xenopus*. *Cell* **60**, 487-494.

Gould, K. L. and Nurse, P. (1989). Tyrosine phosphorylation of the fission yeast cdc2$^+$ protein kinase regulates entry into mitosis. *Nature* **342**, 39-45.

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.

Hirsh, D., Oppenheim, D. and Klass, M. (1976). Development of the reproductive system of *Caenorhabditiselegans*. *Dev. Biol.* **49**, 200-219.

Hoppe, P. E. and Greenspan, R. J. (1986). Local function of the *Notch* gene for embryonic ectodermal pathway choice in *Drosophila*. *Cell* **46**, 773-783.

Johnson, K. and Hirsh, D. (1979). Patterns of proteins synthesized during development of *Caenorhabditiselegans*. *Dev. Biol.* **70**, 241-248.

Kimble, J. and Hirsh, D. (1979). Post-embryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditiselegans*. *Dev. Biol.* **70**, 396-417.

Kimble, J. E. and White, J. G. (1981). On the control of germ cell development in *Caenorhabditiselegans*. *Dev. Biol.* **81**, 208-219.

Lewis, J. A., Wu, C.-H., Berg, H. and Levine, J. H. (1980). The genetics of levamisole resistance in the nematode *Caenorhabditis elegans*. *Genetics* **95**, 905-928.

Maine, E. M. and Kimble, J. (1989). Identification of genes that interact with *glp-1*, a gene required for inductive cell interactions in *Caenorhabditiselegans*. *Development* **106**, 133-143.

Nasmyth, K. A. (1990). *FAR*-reaching discoveries about the regulation of START. *Cell* **63**, 1117-1120.

Nurse, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* **344**, 503-508.

Nurse, P. and Thuriaux, P. (1980). Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomycespombe*. *Genetics* **96**, 627-637.

Okkema, P. G. and Kimble, J. (1991). Molecular analysis of *tra-2*, a sex determining gene in *C. elegans*. *EMBO J.* **10**, 171-176.

Roberts, T. M., Pavalko, F. M. and Ward, S. (1986). Membrane and cytoplasmic proteins are transported in the same organelle complex during nematode spermatogenesis. *J. Cell Biol.* **102**, 1787-1796.

Rosenbluth, R. E. and Baillie, D. L. (1981). The genetic analysis of a reciprocal translocation, *eT1(III;V)*, in *Caenorhabditiselegans*. *Genetics* **99**, 415-428.

Rosenquist, T. A. and Kimble, J. (1988). Molecular cloning and transcript analysis of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. *Genes and Dev.* **2**, 606-616.

Seydoux, G. and Greenwald, I. (1989). Cell autonomy of *lin-12* function in a cell fate decision in *C. elegans*. *Cell* **57**, 1237-1245.

Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M. and Kirschner, M. W. (1990). Cyclin activation of $p34^{cdc2}$. *Cell* **63**, 1013-1024.

Strausfeld, U., Labbe, J. C., Fesquet, D., Cavadore, J. C., Picard, A., Sadhu, K., Russell, P. and Doree, M. (1991). Dephosphorylation and activation of a $p34^{cdc2}$/cyclin B complex in vitro by human CDC25 protein. *Nature* **351**, 242-245.

Strome, S. (1986a). Fluorescence visualization of the distribution of microfilaments in gonads and early embryos of the nematode *Caenorhabditiselegans*. *J. Cell Biol.* **103**, 2241-2252.

Strome, S. (1986b). Establishment of asymmetry in early *Caenorhabditis elegans* embryos: visualization with antibodies to germ cell components. In *Gametogenesis and the Early Embryo* (ed. J.G. Gall), pp. 77-95. Alan R. Liss: New York.

Strome, S. and Wood, W. B. (1982). Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of *Caenorhabditiselegans*. *Proc. Natl. Acad. Sci. USA* **79**, 1558-1562.

Sulston, J. E. and Horvitz, H. R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditiselegans*. *Dev. Biol.* **56**, 110-156.

Sulston, J. E. and White, J. G. (1980). Regulation and cell autonomy during postembryonic development of *Caenorhabditis elegans*. *Dev. Biol.* **78**, 577-597.

Technau, G. M. and Campos-Ortega, J. A. (1987). Cell autonomy of expression of neurogenic genes of *Drosophilamelanogaster*. *Proc. Natl. Acad. Sci. USA* **84**, 4500-4504.

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.

Wittenberg, C., Sugimoto, K. and Reed, S. I. (1990). G1-specific cyclins of *S. cerevisiae*: cell cycle periodicity, regulation by mating pheromone, and association with the $p34^{CDC28}$ protein kinase. *Cell* **62**, 225-237.

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.

(Accepted 30 July 1992)