Enhancers of *glp-1*, a Gene Required for Cell-Signaling in *Caenorhabditis elegans*, Define a Set of Genes Required for Germline Development

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ABSTRACT

The distal tip cell (DTC) regulates the proliferation or differentiation choice in the *Caenorhabditis elegans* germline by an inductive mechanism. Cell signaling requires a putative receptor in the germline, encoded by the *glp-1* gene, and a putative signal from the DTC, encoded by the *lag-2* gene. Both *glp-1* and *lag-2* belong to multigene gene families whose members are essential for cell signaling during development of various tissues in insects and vertebrates as well as *C. elegans*. Relatively little is known about how these pathways regulate cell fate choice. To identify additional genes involved in the *glp-1* signaling pathway, we carried out screens for genetic enhancers of *glp-1*. We recovered mutations in five new genes, named *ego* (*e*nhancer of *glp-1*), and two previously identified genes, *lag-1* and *glp-4*, that strongly enhance a weak *glp-1* loss-of-function phenotype in the germline. Ego mutations cause multiple phenotypes consistent with the idea that gene activity is required for more than one aspect of germline and, in some cases, somatic development. Based on genetic experiments, *glp-1* appears to act upstream of *ego-1* and *ego-3*. We discuss the possible functional relationships among these genes in light of their phenotypes and interactions with *glp-1*.

CELL–cell interactions control many cell fate choices during the development of multicellular organisms. A well-defined example in the nematode *Caenorhabditis elegans* is the regulation of proliferation *vs.* differentiation in the germline. Normally, *C. elegans* are able to make gametes throughout adulthood because the distal population of germ cells undergoes continuous mitosis. Germline proliferation depends on the presence of a pair of somatic cells called distal tip cells (DTC). If the DTCs are killed, germline mitosis stops and all germ cells enter meiotic prophase and undergo gametogenesis (KIMBLE and WHITE 1981). Hence, it is thought that the DTCs must signal proliferation and/or block differentiation in the germline. The *glp-1* (for germline proliferation defective) gene encodes a putative receptor protein that is essential for DTC control of germline proliferation. In the absence of *glp-1* gene function, germ cells that are normally mitotic instead enter meiosis (AUSTIN and KIMBLE 1987). GLP-1 is involved in cell signaling in other tissues during embryogenesis where it presumably acts as a receptor as well (PRIESS *et al.* 1987; LAMBIE and KIMBLE 1991; EVANS *et al.* 1994; HUTTER and SCHNABEL 1994; MANGO *et al.* 1994; MELLO *et al.* 1994; MOSKOWITZ *et al.* 1994; reviewed by PRIESS 1994).

glp-1 belongs to a gene family including *lin-12* from *C. elegans*, *Notch* from Drosophila and several vertebrate homologues that encode putative receptor proteins (YOCHEM and GREENWALD 1989; see ARTAVANIS-TSAKONAS *et al.* 1995; FORTINI and ARTAVANIS-TSAKONAS 1993; MAINE *et al.* 1995). In each case, the receptor is thought to mediate a cell-signaling event that determines cell fate. Thus, a common signal transduction pathway has been conserved to specify very different cell fates.

In certain tissues, GLP-1 and LIN-12 appear to interact with a common ligand, called LAG-2 (HENDERSON *et al.* 1994; TAX *et al.* 1994; WILKINSON *et al.* 1994). Both LAG-2 and another GLP-1 ligand, APX-1 (MELLO *et al.* 1994), are transmembrane proteins with structural similarity to Delta and Serrate, two putative ligands for Notch in Drosophila. In the gonad, LAG-2 is produced in the DTC as a signal, binds the GLP-1 extracellular domain, and becomes internalized by the germline tissue. The GLP-1 intracellular domain is predicted to specify cell fate—germline mitosis (KODOYIANNI *et al.* 1992; ROEHL and KIMBLE 1993; CRITTENDEN *et al.* 1994; HENDERSON *et al.* 1994).

To further understand the *glp-1* mediated cell-signaling process, we screened for enhancers of a weak *glp-1* mutation, *bn18ts*. We isolated enhancer mutations in five new genes, *ego-1, ego-2, ego-3, ego-4, ego-5* (for *e*nhancer of *glp-1*), and novel alleles of two previously known genes, *lag-1* (LAMBIE and KIMBLE 1991) and *glp-4* (BEANAN and STROME 1992). When referring to these genes collectively, we will call them *ego* genes. Mutations

We dedicate this manuscript to the memory of our colleague and friend, Dr. Kevin Van Doren.

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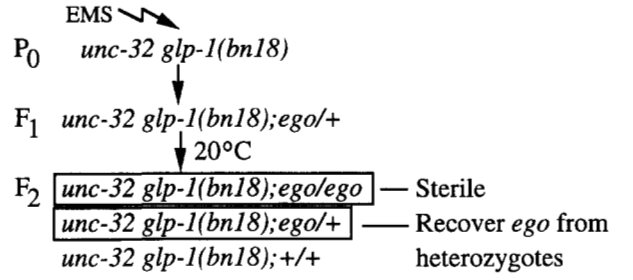

FIGURE 1.—Mutagenesis scheme used to isolate genetic enhancers of *glp-1(bn18ts). ego*, recessive enhancer of *glp-1* mutation.

in most *ego* genes have visible phenotypes besides *glp-1* enhancement, suggesting they function in multiple aspects of development.

MATERIALS AND METHODS

Strains: Worms were maintained on agar plates as described (BRENNER 1974). The wild-type strain *C. elegans* var. Bristol (N2) and most mutants are described in HODGKIN *et al.* (1988) unless indicated. Nomenclature follows the guidelines of HORVITZ *et al.* (1979). When referring to a region of a linkage group (LG), we use the following standard nomenclature: C, the cluster of closely spaced genes, and L or R, the arm of the chromosome to the left or right of the cluster, respectively.

Mutations used in this study were [*bli* (blister), *dpy* (dumpy), *fem* (feminized germline), *gld* (germline development defective), *glp* (germline proliferation defective), *him* (high incidence of males), *lag* (*lin-12* and *glp-1*), *let* (lethal), *lin* (lineage defective), *lon* (long), *ooc* (oocyte defective), *rol* (roller), *sdc* (sex determination and dosage compensation defective), *sma* (small), *sog* (suppressor of *glp-1*), *sqt* (squat), and *unc* (uncoordinated)]:

Linkage group (LG) I: *dpy-5(e61), dpy-14(e188ts), dpy-24(s71), gld-1(q268)* (FRANCIS *et al.* 1995), *glp-4(bn2ts)* (BEANAN and STROME 1992), *let-202(e1720), lin-10(e1439), sog-1(q298)* (MAINE and KIMBLE 1993), *unc-11(e47), unc-13(e51), unc-29(e129), unc-54(e1301ts, h1040), unc-75(e950), unc-101(m1), vab-10(e698), hDf9, nDf24, nDf25, ozDf5* (FRANCIS *et al.* 1995), *hT2, hIn(1)[unc-54(h1040)]* (ZETKA and ROSE 1992).

LGII: *bli-2(e768), dpy-10(e128), lin-31(e1414), rol-1(e91), rol-6(e187), sqt-2(sc108), unc-4(e120), unc-52(e444), mnC1.*

LGIII: *dpy-18(e364), dpy-19(e1259ts), glp-1(q224ts), glp-1(bn18ts)* (KODOYIANNI *et al.* 1992), *glp-1(e2142ts)* (PRIESS *et al.* 1987), *glp-1(oz112gf)* (provided by T. SCHEDL and L. WILSON BERRY), *lin-12(n302gf), ooc-4(e2078)* (provided by J. HODGKIN), *sma-2(e502), unc-32(e189), unc-36(e251), unc-69(e587), nDf40, eT1, hT2.*

LGIV: *bli-6(sc16), dpy-13(e164sd), dpy-20(e1282ts), fem-1(hc17ts), lag-1(q385, q476)* (LAMBIE and KIMBLE 1991; provided by J. KIMBLE), *sog-3(q294)* (MAINE and KIMBLE 1993), *unc-5(e53), unc-24(e138), unc-44(e362), nT1[unc-?(n754) let-?], nDf41.*

LGV: *dpy-11(e224), him-5(e1467ts), lon-3(e2175), rol-4(sc8), sdc-3(y52y180), sma-1(e30), unc-42(e270), unc-61(e228), unc-76(e911), nT1[unc-?(n754) let-?], eT1, eDf1, nDf42, sDf35, yDf8.*

LGX: *dpy-6(e14), lon-2(e678), unc-3(e151).*

Isolation and initial characterization of recessive enhancers of *glp-1(bn18ts):* The mutagenesis scheme is outlined in Figure 1. Fourth larval stage (L4) *unc-32 glp-1(bn18ts)* hermaphrodites were mutagenized with EMS as described (BARTON and KIMBLE 1990) and returned to 15°. F_1 progeny were picked to individual plates and grown at 20°. F_2 progeny were screened visually for a substantial number of sterile animals. (The background of Glp-1 animals is ≤0.5% at 20°; see RESULTS.) DIC optics were used to identify steriles with a Glp-1 phenotype (see Figure 2). From screens of 30,000 haploid genomes, we isolated recessive enhancer mutations of varying activities (see RESULTS). Putative *ego* mutations were examined in a *glp-1(+)* background and then placed back into a *glp-1(bn18ts)* background to confirm that the identified mutation was indeed responsible for enhancement.

To determine whether an *ego* mutation was strictly recessive, we initially examined the progeny of *ego/+; unc-32 glp-1(bn18ts)/++* animals at 20°. In most cases ≤25% of the Unc animals had a Glp-1 sterile phenotype, suggesting that the *ego* mutation is unlinked to *glp-1* and that the Glp-1 animals are always homozygous for the *ego* mutation. In several cases, >99% of the Unc animals had a Glp-1 phenotype, suggesting that the sterile was either tightly linked (*unc-32 glp-1(bn18ts) ego/+++* or *unc-32 glp-1(bn18Ego)/++*) or dominant. The latter possibility is unlikely because a dominant sterile should not have been recovered in our mutagenesis. Once an *ego* mutation was marked, we determined the penetrance of both the *glp-1* enhancement phenotype and the *ego* phenotype in a *glp-1(+)* background (see below).

Genetic mapping and complementation tests: Linkage and complementation were determined by standard tests (Tables 1 and 2; data not shown; see Figure 3). For most genes, mapping and complementation tests were done on the basis of the visible *ego* phenotype; for alleles of *lag-1* and *ego-2*, mapping was done on the basis of *glp-1* enhancement (*i.e.*, in a *glp-1(bn18ts)* mutant background). Complementation tests were done to test for allelism of *ego* mutations at the same position and with mutations in previously known genes. Each mutation was assigned to a linkage group and subsequently mapped more precisely using combinations of three-factor (Table 1; data not shown) and deficiency (Table 2; data not shown) mapping. New genes were named *ego*; their names (*e.g., ego-1 vs. ego-2*) do not reflect anything about their relative phenotypes.

Three mutations on LGIR, *om14, om23,* and *om24,* were placed to the right of *unc-75* by three-factor mapping with *dpy-5 unc-75* (Table 1). They failed to complement each other for an oogenesis defective phenotype and each failed to complement *glp-4(bn2ts)* (Table 2). Additional three-factor mapping of *om14* with several doubles (*dpy-24 unc-101, let-202 unc-54, unc-75 unc-54, unc-101 unc-54*) confirmed the location of *om14* as approximately that of the previously mapped *glp-4(bn2ts)* (BEANAN and STROME 1992). Based on these data, we assign *om14, om23,* and *om24* to *glp-4.*

One mutation on LGIR, *om33,* was placed between *dpy-24* and *unc-101* by three-factor mapping with *dpy-5 unc-75, unc-75 unc-54,* and *dpy-24 unc-101* (Table 1); it appears to lie to the left of *unc-75.* Consistent with these results, it is uncovered by *hDf9.* We designate this gene *ego-2.*

Two mutations in the cluster on LGI, *om18* and *om71,* fail to complement for an oogenesis defective phenotype. Three-factor mapping of *om18* with several doubles (*unc-11 dpy-5, dpy-5 unc-29, dpy-14 unc-13, dpy-14 unc-29, unc-13 gld-1*) place it between *unc-13* and *gld-1. om18* is uncovered by two overlapping deficiencies (*Df*), *ozDf5* and *nDf25,* but not by *nDf24.* We designate this gene *ego-1.*

Six mutations on LGIV, *om13, om27, om79, om86, om104,* and *om108,* fail to complement each other for enhancement of *glp-1* and are balanced by *nT1[unc-?(n754) let-?].* Three-factor mapping of *om13* and *om27* with *dpy-13 unc-24* and *unc-5 dpy-20* placed them between *unc-5* and *unc-24.* Three-factor mapping of *om13* with *unc-5 bli-6* and *unc-44 bli-6* placed it to the right of *unc-44* at a position close to *lag-1. om13* fails to complement *lag-1(q385), lag-1(q476),* and *nDf41* for viability

TABLE 1

Three-factor map data for *ego* genes

ego gene[a]	Parental genotype	Recombinant phenotype	Recombinant genotype	*n*
ego-1	*unc-11 dpy-5/ego-1*	Unc	*unc-11 ego-1*	10/10
			unc-11	0/10
		Dpy	*dpy-5 ego-1*	0/21
			dpy-5	21/21
	dpy-5 unc-29/ego-1	Dpy	*dpy-5 ego-1*	14/30
			dpy-5	16/30
		Unc	*ego-1 unc-29*	6/16
			unc-29	10/16
	dpy-14 unc-13/ego-1	Dpy	*dpy-14 ego-1*	6/6
			dpy-14	0/6
		Unc	*ego-1 unc-13*	0/21
			unc-13	21/21
	dpy-14 unc-29/ego-1	Dpy	*dpy-14 ego-1*	2/5
			dpy-14	3/5
		Unc	*ego-1 unc-29*	16/30
			unc-29	14/30
	unc-13 gld-1/ego-1	Unc	*unc13 ego-1*	4/5
			unc-13	1/5
ego-2[b]	*dpy-5 unc-75/ego-2; glp-1*	Dpy	*dpy-5 ego-2; glp-1*	18/24
			dpy-5; glp-1	6/24
		Unc	*ego-2 unc-75; glp-1*	0/18
			unc-75; glp-1	18/18
	unc-75 unc-54/ego-2; glp-1	Unc-54	*ego-2 unc-54; glp-1*	18/18
			unc-54; glp-1	0/18
	dpy-24 unc-101/ego-2; glp-1	Unc	*ego-2 unc-101; glp-1*	19/23
			unc-101	4/23
ego-3	*dpy-11 unc-42/ego-3*	Dpy	*dpy-11 ego-3*	25/25
			dpy-11	0/25
		Unc	*ego-3 unc-42*	0/24
			unc-42	24/24
	sma-1 unc-76/ego-3	Sma	*sma-1 ego-3*	5/6
			sma-1	1/6
		Unc	*ego-3 unc-76*	1/6
			unc-76	5/6
	lon-3 unc-76/ego-3	Lon	*lon-3 ego-3*	7/8
			lon-3	1/8
		Unc	*ego-3 unc-76*	0/10
			unc-76	10/10
	sma-1 unc-61/ego-3	Sma	*sma-1 ego-3*	11/13
			sma-1	2/13
		Unc	*ego-3 unc-61*	0/9
			unc-61	9/9
	ego-3 unc-76/unc-61	Unc-76	*unc-61 unc-76*	10/10
			unc-76	0/10
	sdc-3 unc-76/ego-3	Unc	*ego-3 unc-76*	3/3
			unc-76	0/3
ego-4	*dpy-19unc-69/mar glp-1 ego-4*[c]	Dpy	*dpy-19 mar glp-1 ego-4*	2/24
			dpy-19 glp-1 ego-4	3/24
			dpy-19 ego-4	6/24
			dpy-19	13/24
	unc-69 dpy-18/sma-2 glp-1 ego-4	Unc	*unc-69 ego-4*	0/48
			unc-69	48/48
		Dpy	*dpy-18 ego-4glp-1sma-2*	20/20
			dpy-18 glp-1 sma-2	0/20
ego-5	*dpy-19unc-69/mar glp-1 ego-5*[c]	Dpy	*dpy-19 mar glp-1 ego-5*	0/26
			dpy-19 glp-1 ego-5	2/26
			dpy-19 ego-5	6/26
			dpy-19	18/26

TABLE 1

Continued

ego gene[a]	Parental genotype	Recombinant phenotype	Recombinant genotype	*n*
ego-5	*unc-69 dpy-18/sma-2glp-1 ego-5*	Unc	*unc-69 ego-5*	0/32
			unc-69	32/32
		Dpy	*dpy-18 ego-5glp-1sma-2*	18/18
			dpy-18 glp-1 sma-2	0/18
glp-4	*dpy-5 unc-75/glp-4*	Dpy	*dpy-5 glp-4*	22/26
			dpy-5	4/26
		Unc	*glp-4 unc-75*	1/30
			unc-75	29/30
	dpy-24 unc-101/glp-4	Unc	*glp-4 unc-101*	0/10
			unc-101	10/10
	let-202 unc-54/glp-4	Unc	*glp-4 unc-54*	8/8
			unc-54	0/8
	unc-75 unc-54/glp-4	Unc-54	*glp-4 unc-54*	10/18
			unc-54	8/18
	unc-101 unc-54/glp-4	Unc-101	*unc-101 glp-4*	0/2
			unc-101	2/2
		Unc-54	*glp-4 unc-54*	3/4
			unc-54	1/4

In mapping *ego-4* and *ego-5*, recombinants containing either the *glp-1* or *ego* allele alone or containing both mutants must be distinguished from each other. To do so, recombinants were scored at 20 and 25°. Recombinants containing *glp-1* but no *ego* should be fertile at 20° and Glp-1 sterile at 25°, whereas double mutants should be Glp-1 sterile at 20 and 25°. *ego-4* and *ego-5* alone are Mel and have reduced germline proliferation at 20 and 25°.

[a] Map position was confirmed for multiple alleles of each gene if available.

[b] *ego-2* was mapped based on its Ego phenotype.

[c] Marker mutation (*mar*) was either *sma-2* or *unc-32*.

and some L1 larval lethality (resembling a Lag phenotype) is associated with *om13* stocks. Therefore, we assign these six mutations to *lag-1*.

One mutation on LGV, *om40*, was three-factor mapped with several doubles (*dpy-11 unc-42, sma-1 unc-76, rol-4 unc-76, lon-3 unc-76, sma-1 unc-61, rol-4 unc-61*) to the region just to the left of *unc-61*. Consistent with these data, it is uncovered by *yDf8* but not uncovered by *nDf42*. Three-factor mapping of

TABLE 2

Complementation tests in a *glp-1*(+) background

LGI		*glp-4*			*ego-2*					
		om14	*om23*	*om24*	*om33*	*ozDf5*	*nDf25*	*nDf24*	*sog-1*	*gld-1*
ego-1	*om18*	+	ND	ND	+	−	−	+	+	+
glp-4	*bn2ts* (20°)	−(Oog)	−(Oog)	−(Oog)	+					
	bn2ts (25°)	−(Glp-4)	−(Glp-4)	−(Glp-4)	+					
	om14		−	−	+					
	om23			−	ND					
	hDf9				−					

LGIII		*ego-5*		*ego-4*						
		om31	*om62*	*om60*	*nDf40*	*ooc-4*		LGV	*nDf42*	*yDf8*
ego-4	*om30*	+	+	−	+	+		*ego-3*	+	−
ego-5	*om31*		−	+	+	+				

LGIV		*lag-1*								
		nDf41	*q385*	*q476*	*om27*	*om79*	*om86*	*om104*	*om108*	*sog-3*
lag-1	*om13*	−	−	−	−	−	−	−	−	+

All tests done at 20° unless otherwise noted. Oog, oogenesis defective; Glp-4, reduced germline proliferation. *sog-1*, *sog-3* mutations are recessive suppressors of *glp-1* that do not have obvious visible phenotypes of their own (MAINE and KIMBLE 1993); *ooc-4* mutants are sterile or produce oocytes that cannot support development (HODGKIN *et al.* 1988). See text for details.

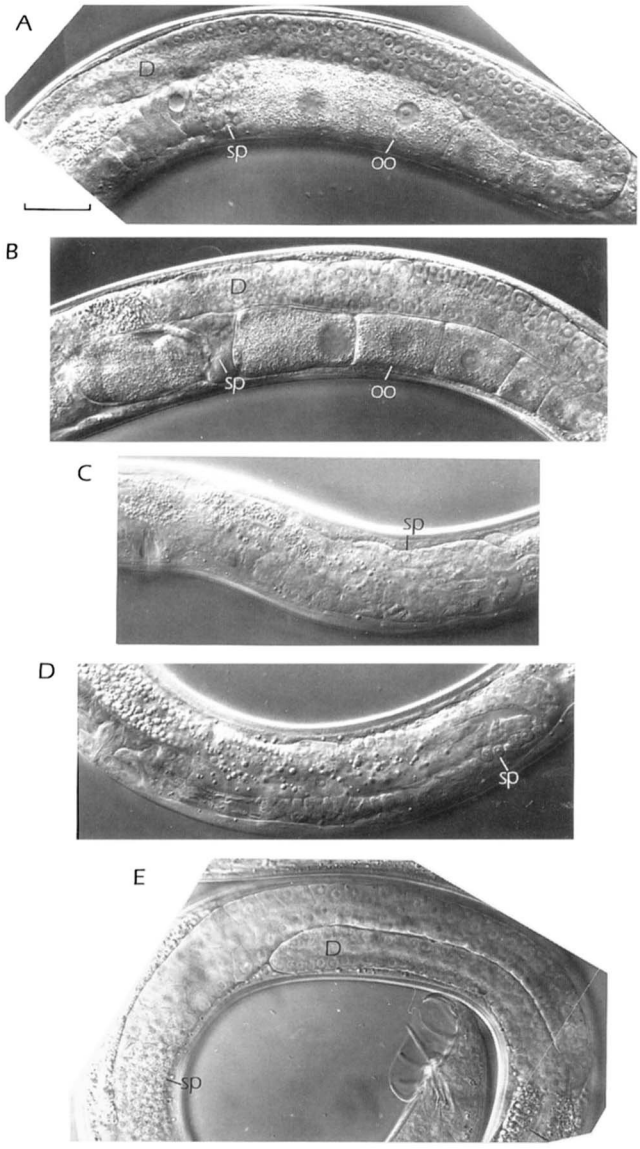

FIGURE 2.—Germline morphology in wild-type and Glp-1 mutant animals. DIC photomicrographs with distal proliferative nuclei (D), oocytes (oo) and sperm (sp) indicated. In hermaphrodites, one arm of the gonad is shown; sperm mark the proximal end of a proliferative germline. (A) Wild-type (N2) hermaphrodite at 20°, (B) *glp-1(bn18ts)* hermaphrodite at 20°, (C) *glp-1(bn18ts)* hermaphrodite at 25°, (D) *glp-1(bn18ts);ego-2(om33)* hermaphrodite at 20°. Note that C and D are similar: the germline comprises a few sperm scattered throughout the gonad and no proliferating cells. (E) A wild-type male gonad at late L4 stage. See text for details. Scale bar, 10 μm.

unc-61 relative to *ego-3 unc-76* confirm that *ego-3* maps very close to *unc-61*. Two-factor mapping places *ego-3* 0.7 map units (mu) from *unc-76*. Five *ego-3 unc-76/++* mothers produced a total of 1440 wild-type, 381 *ego-3 unc-76*, eight *unc-76* and five *ego-3* progeny.

Four mutations on LGIII, *om30, om31, om60* and *om62*, are separable from *glp-1(bn18ts)* and define two complementation groups based on their maternal effect lethal (Mel) and reduced germline proliferation phenotypes in a *glp-1(+)* background. Three-factor mapping with *sma-2 unc-69, dpy-19 unc-69, and unc-69 dpy-18* places these mutations between *glp-1* and *unc-69*. We assign *om30* and *om60* to *ego-4* and *om31* and *om62* to *ego-5*. Alleles of both genes complement *nDf40*. Other mutations on LGIII, *om32, om47*, and *om63*, were not separable from *glp-1*. We postulate that they are *glp-1* intragenic mutations that further reduce (prehaps eliminate) *glp-1* function.

Determination of germline proliferation: Intact animals were fixed for 20–30 min in methanol, stained for 20–30 min with DAPI (0.2 μg/ml), and mounted on either an agarose pad (4%) or in Vectashield mounting medium (Vector Laboratories, Inc., Burlingame, CA) for viewing under fluorescent light. The number of germ cells present in each animal was calculated as the number of undifferentiated germ cells and oocytes present plus 25% of the number of sperm. The presence of mitotic, meiotic (transition, pachytene and diakinesis), and sperm (hypercondensed) nuclei was noted. *glp-1(bn18ts)* and *glp-1(bn18ts);ego* hermaphrodites were compared at 20°. *glp-1(bn18ts)* and *glp-1(bn18ts);ego* males were compared at 15° because the former have a pronounced Glp-1 phenotype at 20°.

Phenotypic characterization: We characterized the phenotypes of representative alleles of each gene using DIC optics to examine cell morphology and DAPI staining to examine chromosome morphology. Wild-type adult hermaphrodites contain germline nuclei in the following distal to proximal pattern: mitotic, pachytene, diakinesis (oocytes), hypercondensed (sperm) (see Figures 2A and 4A). Developing gametes spend an extended period of time in pachytene phase of first meiotic prophase, and mature oocytes are arrested in diakinesis of first meiotic prophase until fertilization; spermatogenesis is complete by the time oogenesis is visible. Wild-type adult males contain germline nuclei in the following distal to proximal pattern: mitotic, pachytene, semicondensed (primary spermatocytes), hypercondensed (sperm) (see Figures 2E and 4B).

***ego-3* and wild-type developmental time course:** To describe the complex *ego-3* germline phenotype in some detail, synchronized animals were characterized in terms of germline nuclear morphology and, for all but the oldest adult timepoints, germline nuclei number. To do so, threefold stage embryos were picked to lightly seeded plates; the L1 larvae that hatched during a 2-hr interval were transferred to a fresh plate and allowed to develop for a defined period of time before methanol fixation and DAPI staining. Larvae and young adults were collected at 4-hr intervals beginning at 24 hr after hatching (*e.g.*, 24–26, 28–30, 32–34 hr, and so on) and older adults were collected at 12-hr intervals (*e.g.*, 72–74, 84–86 hr, and so on, after hatching).

Tests for interactions with other *glp-1* alleles: Double mutant strains were constructed by standard means. To examine whether or not *glp-1(e2142ts)* was enhanced by *lag-1(om13)*, we generated a *glp-1(e2142ts); unc-44 lag-1(om13)/++* stock at 15° and examined the *glp-1(e2142ts); unc-44 lag-1(om13)* progeny. To examine *glp-1(oz112gf);ego* double mutants, we balanced the *glp-1(oz112gf)* allele with *glp-1(q224ts)*, an allele that produces a severe *glp-1* loss-of-function phenotype at 20°; the *glp-1(oz112gf)* allele was marked with *unc-32*. We generated *ego; unc-32glp-1(gf)/glp-1(ts)* strains in two steps. First, we generated *unc-32 glp-1(oz112gf)/glp-1(q224ts)* strains that were homozygous for one of a number of unlinked balancer chromosomes or marker mutations. At 20°, these animals segregated Glp-1 steriles [*glp-1(q224ts)*], Unc-32 animals that did produce viable progeny and had extensive germline overproliferation [*unc-32 glp-1(oz112gf)*], and non-Unc-32 animals that produced a few viable progeny and had extensive germline overproliferation [*unc-32 glp-1(oz112gf)/glp-1(q224ts)*]. Next, we crossed in males heterozygous for *glp-1(q224ts)* and a marked

TABLE 3

Enhancement of *glp-1(bn18ts)* in hermaphrodites

ego gene	Genotype	No. germ cells[a]	Range	*n*	Percentage of control proliferation[b]
—	N2 (wild-type)	641 ± 44	454–701	5	100
—	*unc-32 glp-1(bn18ts)*[c]	289 ± 15	255–351	7	45
lag-1	*lag-1(om13)*	453 ± 19	348–530	11	71
	lag-1(om13); glp-1	16 ± 2	10–24	8	3
—	*unc-54*[d]	509 ± 23	461–561	5	100
glp-4	*glp-4(om14)unc-54*	557 ± 11	494–625	11	109
	glp-4(om14) unc-54; glp-1	71 ± 9	44–113	8	14
	glp-4(bn2ts)	287 ± 12	210–357	12	—
	glp-4(bn2ts); glp-1	115 ± 3	91–138	14	—
ego-1	*ego-1(om18) unc-29*	327 ± 35	196–531	10	51
	ego-1(om18); unc-32 glp-1	105 ± 14	58–154	11	16
ego-3	*ego-3(om40)*	318 ± 32	167–577	14	50
	ego-3(om40); glp-1	54 ± 18	1–202	14	12
—	*dpy-19(e1259ts)*[e]	752 ± 36	682–802	3	100
ego-4	*dpy-19 ego-4(om30)*	203 ± 18	122–317	10	27
	unc-32 glp-1 ego-4(om30)	11 ± 4	2–42	11	2
ego-5	*dpy-19 ego-5(om31)*	234 ± 10	182–353	10	31
	unc-32 glp-1 ego-5(om31)	27 ± 12	3–129	12	4

n, number of animals scored. All tests done at 20° except that *glp-4(bn2ts)* and *glp-4(bn2ts); glp-1(bn18ts)* were tested at 18°. All animals were examined at young adult stage (just after the molt from L4 to adult) except that *glp-4* mutants (which could not be marked well) and *unc-54* controls were examined at ~12 hr after the molt to adulthood when their germline phenotype was apparent. At 12 hr past young adult stage, *glp-1(bn18ts)* controls had 338 ± 17 germ cells (range 251–429, $n = 12$).

[a] Values are means ± SE.

[b] The control is wild-type (N2) in all cases except for stocks marked with *unc-54*, for which *unc-54(e1301ts)* was the control, and stocks marked with *dpy-19*, for which *dpy-19(e1259ts)* was the control. To help illustrate the amount of enhancement, we also calculated the percent proliferation in *ego; glp-1* double mutants relative to *ego* and *glp-1* single mutants. Proliferation in *lag-1(om13); glp-1(bn18ts)* animals was 3.5% of *lag-1(om13)* and 5.5% of *glp-1(bn18ts)*. Proliferation in *glp-4(om14); glp-1(bn18ts)* animals was 13% of *glp-4(om14)* and 21% of *glp-1(bn18ts)*. Proliferation in *ego-1(om18); glp-1(bn18ts)* animals was 33% of *ego-1(om18)* and 36% of *glp-1(bn18ts)*. Proliferation in *ego-3(om40); glp-1(bn18ts)* was 17% of *ego-3(om40)* and 19% of *glp-1(bn18ts)*. Proliferation in *ego-4(om30); glp-1(bn18ts)* animals was 5% of *ego-4(om30)* and 4% of *glp-1(bn18ts)*. Proliferation in *ego-5(om31); glp-1(bn18ts)* was 12% of *ego-5(om31)* and 9% of *glp-1(bn18ts)*.

[c] *glp-1(bn18ts)* used in all stocks. The average brood size at 20° was 262 ± 22 ($n = 5$ broods).

[d] *glp-4(om14)* was marked with *unc-54(e1301ts)*.

[e] *ego-4* and *ego-5* were marked with *dpy-19(e1259ts)*. Proliferation was quantified in Dpy progeny of *dpy-19(e1259/+)* mothers.

ego mutation and recovered an *ego(-)marker(-)/balancer; unc-32 glp-1(ox112gf)/glp-1(q224ts)* strain.

Most *ego* homozygotes are infertile; they were maintained in the following strains in either a *glp-1(+)* or *glp-1(bn18ts)* background. *lag-1*: (1) *lag-1/nT1[unc-?(n754) let-?]*, (2) *unc-44 lag-1/nT1[unc-?(n754) let-?]*, (3) *lag-1 bli-6/nT1[unc-?(n754) let-?]*, (4) *lag-1* or (5) *lag-1; him-5*. *glp-4*: (1) *glp-4 unc-54(ts)/ hIn(1) [unc-54]*, (2) *glp-4/ hIn(1) [unc-54]*, or (3) *glp-4 −/+); him-5*. *ego-1*: (1) *ego-1 unc-29/hT2* or (2) *ego-1/spe-4 lin-10; him-5*. *ego-3*: *ego-3/nT1[unc-?(n754) let-?]*. To generate *ego-3* males, *ego-3/+* males were crossed to *ego-3/nT1[unc-?(n754) let-?]* hermaphrodites or a *him-5(−/−) ego-3(−/+)* strain was used. *ego-4* and *ego-5*: (1) *dpy-19 ego/sma-2 unc-69; him-5*, (2) *unc-32 glp-1 ego/sma-2 unc-69; him-5*, or (3) *unc-32 glp-1 ego/eT1*. *ego-2* can be maintained as a homozygote in either a *glp-1(+)* or *glp-1(bn18ts)* background at 15°.

RESULTS

We recovered enhancers of a weak *glp-1* mutation, *glp-1(bn18ts)*, as a means of identifying other components of the *glp-1*-mediated signaling pathway. Like *glp-1*, such genes might be involved in cell signaling in more than one tissue. Thus, homozygous null animals might be inviable, and we would not be able to examine their effects on germline development. Therefore, we designed a mutagenesis strategy to allow us to recover partial loss-of-function mutations in these genes. To do so, we screened for enhancers of *glp-1* in animals with a borderline amount of *glp-1(+)* activity (see below). We reasoned that a partial decrease in activity of another signaling pathway component might have a large effect on pathway activity overall. Components of a number of other biochemical pathways have been identified successfully in screens for enhancers of mutations that have just sufficient levels of gene product activity for normal development (*e.g.*, SIMON *et al.* 1991).

The temperature sensitive (*ts*) *glp-1(bn18ts)* allele has a strong Glp-1 phenotype at 25° (Figure 2C) that results from a single base pair substitution within the fourth *cdc10*/ankyrin repeat in the cytoplasmic portion of the protein (KODOYIANNI *et al.* 1992). At permissive temperature (20°), we found that >99.5% of hermaphrodites have extensive germline proliferation (Table 3, Figure 2B) with brood sizes of 262 ± 22 ($n = 5$) in which 58%

of the progeny are inviable because of the requirement for maternal *glp-1* expression (see below). Although proliferation at the young adult stage is ~50% of wild-type, we observe mitotic germline nuclei in all stages of adulthood examined. However, at 21°, the frequency of Glp animals increases to ~1% and the number of inviable progeny also increases. We chose to screen for enhancers at 20° to minimize the background level of animals with proliferation defects caused by *glp-1(bn18ts)* alone.

Putative recessive enhancers of *glp-1(bn18ts)* were isolated as described (Figure 1; MATERIALS AND METHODS) and examined in a *glp-1(+)* background. A bona fide *ego* mutant was required to have extensive germline proliferation when compared with the *ego;glp-1* double mutant. An enhancer was categorized as "strong" if it had little or no proliferation defect of its own such that its interaction with *glp-1* is synergistic. In contrast, an enhancer was categorized as "moderate" or "weak" if it had a fairly pronounced proliferation defect of its own such that its interaction with *glp-1* presumably is additive. Synergistic effects may indicate that an *ego* mutation decreases activity of the *glp-1* pathway, whereas additive effects may indicate that an *ego* mutation alters another (perhaps parallel) process in the germline. To understand the *glp-1* pathway, we focused on a subset of the "strong" enhancers. Other enhancers will be described in detail elsewhere.

Extragenic *ego* mutations identify seven genes: Strong *ego* mutations can be assigned to two previously known genes, *lag-1* on IV (*om13, om27, om79, om86, om104, om108*) and *glp-4* on IR (*om14, om23, om24*), and five new genes, *ego-1* on IC (*om18, om71*), *ego-2* on IR (*om33*), *ego-3* on VC (*om40*), *ego-4* on IIIRC (*om30, om60*) and *ego-5* on IIIRC (*om31, om62*) (Figure 3; Tables 1 and 2 and data not shown; see MATERIALS AND METHODS). Seven additional *ego* mutations map between *unc-13* and *dpy-24* on LGI and show a complex pattern of interactions with each other, *ego-1*, and *glp-4* (J. SPOERKE, L. QIAO, S. STACEY and E. MAINE, unpublished data); they have not been assigned to specific genes and will be described elsewhere.

Most enhancer mutations produce visible phenotypes when placed into a *glp-1(+)* background. Our screens were designed to allow isolation of non-null mutations in genes that might function in several tissues or cells within the body. Null mutations in these genes are likely to be lethal (as is known to be the case for *lag-1* and *lag-2*) (LAMBIE and KIMBLE 1991). Consistent with our scheme, comparison of *ego(−/−)* and *ego(−/Df)* phenotypes shows that *ego* mutations in several genes are associated with a partial loss of function but do not appear to be null (see below). In general, *ego* mutants have mildly underproliferative germlines. Furthermore, *glp-4(Ego), ego-1, ego-3, ego-4,* and *ego-5* mutants have other germline defects. In contrast, some *lag-1(Ego)* and the single *ego-2* mutants do not have obvious visible defects.

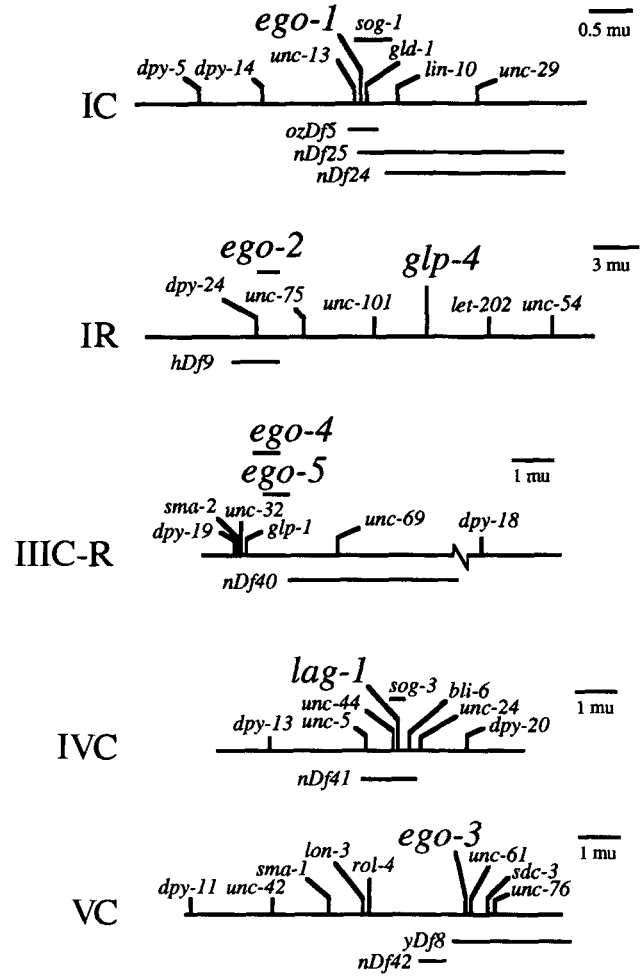

FIGURE 3.—Map positions of *ego* genes, *glp-1*, deficiencies and marker genes used for mapping experiments and known genes tested for failure to complement *ego* mutations. Note difference in scale for IR, IC, and the other linkage groups.

Other *lag-1(Ego)* alleles have somatic defects. These visible phenotypes, as well as interactions between *glp-1* and *ego* mutations, are described in more detail below for each gene except *ego-2*. We describe the interactions between *ego-2* and *glp-1* elsewhere.

Characteristics of *lag-1(Ego)* alleles: Previous genetic analysis of *lag-1* had identified it as a possible component of the *glp-1* signaling pathway (LAMBIE and KIMBLE 1991), and recent molecular data strongly support this hypothesis (S. CHRISTENSON and J. KIMBLE, personal communication) (see DISCUSSION). Therefore, recovery of weak *lag-1* alleles (referred to here as *lag-1(Ego)* alleles) suggests that we are indeed able to identify components of the *glp-1* signaling pathway using our screen. *lag-1(Ego)* alleles are relatively weak but vary in strength relative to one another. The strongest allele, *om13*, is lethal when placed over a deficiency (*nDf41*) or *lag-1* null allele (*q385* or *q476*) (Table 2).

Enhancement of glp-1(lf) in the germline and embryo: We quantified the interactions between *lag-1* and *glp-1* using a strong *lag-1(Ego)* allele, *lag-1(om13)*. Germline prolifer-

TABLE 4

Tests for enhancement of *glp-1(bn18ts)* in late L4 males

ego gene	Genotype	No. germ cells[a]	Range	*n*
—	N2 (wild-type)	256 ± 12	213–280	5
—	*glp-1(bn18ts); him-5*[b]	107 ± 16	6–213	18
	unc-44	144 ± 21	92–184	4
lag-1	*unc-44 lag-1(om13)*	154 ± 15	120–205	6
	unc-44 lag-1(om13); glp-1	68 ± 11	17–97	7
ego-1	*ego-1(om18) unc-29; him-5*	120 ± 7	87–145	11
	ego-1(om18); unc-32glp-1; him-5	92 ± 21	16–144	5
ego-3	*ego-3(om40)*	151 ± 11	83–229	15
	ego-3(om40); glp-1	74 ± 11	5–217	29
ego-4	*dpy-19 ego-4(om30); him-5*	238 ± 21	180–300	6
	unc-32 glp-1 ego-4(om30); him-5	148 ± 16	39–229	11
ego-5	*dpy-19 ego-5(om31); him-5*	198 ± 20	139–257	6
	unc-32 glp-1 ego-5(om31); him-5	134 ± 12	79–184	8

glp-1(+) stocks raised at 20°; *glp-1(bn18ts)* stocks raised at 15° (see text). *n*, number of animals scored.
[a] Values are means ± SE.
[b] Nineteen of 29 animals had proliferating germ cells and no sperm; 10 of 29 animals had only sperm.

ation is reduced severely in *lag-1(om13);glp-1(bn18ts)* hermaphrodites when compared with *lag-1(om13)* and *glp-1(bn18ts)* hermaphrodites (Table 3). Double mutants have 3.5% of the number of germ cells found in *lag-1(om13)* single mutants and 5.5% of the number of germ cells found in *glp-1(bn18ts)* single mutants (Table 3). Based on comparison of *ego(−)* and *ego(−);glp-1(−)* germlines, *lag-1(om13)* is one of the two strongest enhancers reported here (Table 3). We were not able to test whether *glp-1(bn18ts)* is enhanced in *lag-1(om13)/nDf41* animals because they are not viable. However, based on the previously characterized phenotype of *lag-1* (LAMBIE and KIMBLE 1991), we believe the enhancing activity results from a loss (rather than gain) of gene function.

glp-1 expression is regulated differently in the two sexes (CRITTENDEN *et al.* 1994), and therefore we were interested in knowing whether *glp-1* enhancers that had been identified in hermaphrodites could also enhance the *glp-1* phenotype in male germlines. The germline phenotype of *glp-1(bn18ts)* in males at any given temperature is more severe than its phenotype in hermaphrodites. After examining several temperatures, we chose to characterize the effect of *ego* mutations at 15° where average germline proliferation is moderate although highly variable (Table 4). *lag-1(om13)* appears to be a moderate enhancer of the *glp-1(bn18ts)* phenotype in males.

It would be particularly interesting to know whether mutations that enhance *glp-1* in the germline have the same effect in other tissues. Maternal *glp-1* expression is required for several cell–cell interactions during embryogenesis (PRIESS *et al.* 1987; LAMBIE and KIMBLE 1991; HUTTER and SCHNABEL 1994; MANGO *et al.* 1994; MELLO *et al.* 1994; MOSKOWITZ *et al.* 1994; reviewed by PRIESS 1994). We used *glp-1(e2142ts)*, a mutation that causes maternal effect lethality but no germline proliferation defect (PRIESS *et al.* 1987; KODOYIANNI *et al.* 1992), to examine whether *lag-1(Ego)* mutations enhance the *glp-1* maternal effect lethality. At 25°, progeny of *glp-1(e2142ts)* mothers die as embryos or L1 larvae because they fail to execute one or more embryonic cell–cell interactions (PRIESS *et al.* 1987; KODOYIANNI *et al.* 1992). In contrast, at 15°, the progeny of *glp-1(e2142ts)* mothers are 100% viable (PRIESS *et al.* 1987; KODOYIANNI *et al.* 1992). *glp-1(e2142ts)* contains a single amino acid substitution in the first of 10 EGF-like (epidermal growth factor-like) repeats in the extracellular portion of the gene and therefore may be defective in ligand binding (KODOYIANNI *et al.* 1992).

lag-1(om13) strongly enhances the maternal effect lethality of *glp-1(e2142ts)* at the normally permissive temperature of 15° such that <0.1% of the progeny of *lag-1(om13); glp-1(e2142ts)* mothers were viable (Table 5). Most progeny die as embryos rather than L1 larvae. Enhancement apparently depends on the maternal *ego* genotype because *lag-1(om13);glp-1(e2142ts)* animals segregating from *lag-1(om13/+);glp-1(e2142ts)* mothers are viable and self-fertile (at 15°) but produce only dead progeny. If the zygotic *lag-1* genotype were responsible for enhancing *glp-1* embryonic lethality, then we would

TABLE 5

Tests for enhancement of *glp-1* maternal effect at 15°

Genotype	Viable progeny (%)	*n*
glp-1(e2142ts)	100[a]	—
lag-1(om13)	88.5[b]	5
lag-1(om13); glp-1(e2142ts)	<0.05	20

n, number of broods scored.
[a] From KODOYIANNI *et al.* (1992).
[b] Some progeny die as embryos because of the *lag-1(om13)* maternal effect; see text.

expect the *lag-1(om13);glp-1(e2142ts)* progeny of *lag-1(om13/+);glp-1(e2142ts)* mothers to be inviable.

Germline and somatic defects associated with lag-1(Ego) mutations in a glp-1(+) background: lag-1(om13) mutants have *ts* germline and somatic defects. As described above, the germline defect is a mild reduction in proliferation (29% in *lag-1(om13)* hermaphrodites; Table 3). This germline phenotype appears to be mildly *ts*; young adult hermaphrodites raised at 25° have 19% fewer germ cells than do those raised at 20° (average 366 ± 19 germ cells at 25°, $n = 7$). We found a milder temperature effect on N2 animals; germ cell number was reduced 8% at the young adult stage in N2 animals raised at 25° (average 589 ± 20, $n = 11$). Therefore, the *lag-1(om13)* proliferation defect appears to be truly temperature sensitive.

Embryonic and larval lethality associated with *lag-1(om13)* depend on maternal and zygotic defects, respectively. The larval lethality is zygotic and appears to be an incompletely penetrant Lag phenotype. At either 20 or 25°, 40–55% of the expected *lag-1(om13)* progeny from *lag-1(om13/+)* mothers survive to adulthood (when *unc-44* is used as a marker), whereas other animals die as young larvae (10 broods counted); in contrast, at 15°, virtually no dead larvae are produced (five broods counted).

The embryonic lethality depends on the maternal genotype. Forty percent of the progeny of *lag-1(om13)* mothers die as embryos at 25 or 20°, whereas 11.5% of them die as embryos at 15° (five broods counted at each temperature; see Table 5). This maternal effect lethality can be largely rescued by a paternal wild-type copy of *lag-1*; when wild-type males are crossed to *lag-1(om13)* mothers at 25°, 88.5% of the progeny (presumably *lag-1(om13/+)* cross-progeny) survive to become fertile adults (five broods counted).

A maternal effect is not characteristic of previously recovered hypomorphic alleles of *lag-1* (LAMBIE and KIMBLE 1991). As a result, we were concerned that a linked mutation in another gene might be causing the maternal effect lethality. The different *ts* profiles of the maternal effect Let, zygotic Let, and Glp phenotypes are consistent with the idea that more than one mutation may be responsible for these various phenotypes. However, in our mapping experiments, we were unable to separate the maternal effect lethality from the other phenotypes; if a linked maternal effect lethal is present, it must lie within the 0.39 map unit interval between *unc-44* and *bli-6*.

Characteristics of *glp-4(Ego)* alleles: The previously defined *glp-4* phenotype is quite distinct from that of *glp-1*. At restrictive temperature, animals mutant for the original *glp-4* allele, *glp-4(bn2ts)*, have severely underproliferative germlines that slowly undergo mitosis and do not differentiate (BEANAN and STROME 1992). Based on this phenotype, *glp-4* appears to promote progression of germ cells through the mitotic cell cycle and, perhaps, has nothing to do with the mitotic/meiotic choice. In addition, when *glp-4(bn2ts)* hermaphrodites are shifted to restrictive temperature as adults, they produce defective oocytes (BEANAN and STROME 1992).

Enhancement of glp-1(lf) in the germline: We isolated three *glp-4(Ego)* mutations with phenotypes somewhat different from those of *bn2ts* (see below). We characterized the interaction of a representative allele, *glp-4(om14)*, with *glp-1(bn18ts)* (Table 3). Germline proliferation is reduced substantially in *glp-4(om14);glp-1(bn18ts)* double mutants when compared with *glp-4(om14)* and *glp-1(bn18ts)* single mutants (Table 3). Double mutants have 13% of the number of germ cells found in *glp-4(om14)* single mutants and 24% of the number of germ cells found in *glp-1(bn18ts)* single mutants (Table 3). We were not able to examine whether *glp-4(Ego)* mutations enhance the *glp-1* maternal effect because they are oogenesis defective (see below). Enhancement in males has not been quantified because of the absence of a tightly linked marker mutation.

We also examined *glp-4(bn2ts);glp-1(bn18ts)* animals at various temperatures to determine whether *glp-4(bn2ts)* might enhance *glp-1(bn18ts)*. Double mutants have approximately normal germline proliferation at 15° and a moderate Glp-4 (reduced proliferation without gametogenesis) phenotype at 20°. However, we found evidence of enhancement of *glp-1(bn18ts)* at the intermediate temperature of 18°. Young adult *glp-4(bn2ts);glp-1(bn18ts)* hermaphrodites have a moderate Glp-1 phenotype with 115 germ cells (Table 3), most of which are in pachytene or undergoing spermatogenesis; mature sperm are also present. In contrast, *glp-4(bn2ts)* young adults have 287 germ cells (Table 3) arranged in the normal fashion with mitotic and pachytene germ cells, spermatocytes, and sperm.

glp-4(Ego) mutations have multiple hermaphrodite germline phenotypes in a glp-1(+) background: glp-4(Ego) mutants differ from *glp-4(bn2ts)* mutants in that the number of germline nuclei is only slightly reduced (Table 3) but resemble *glp-4(bn2ts)* mutants in that they produce abnormal oocytes. We define an oogenesis defective (Oog) phenotype as the production of grossly abnormal oocytes (typically small and irregularly sized) that fail to support normal embryonic development. [This phenotype is distinct from a Mel phenotype where oocytes are morphologically normal and simply lack a specific maternal gene product; they are usually fertilized. Oog is also distinct from the previously defined defective oocyte phenotype (Ooc) (WOOD 1988) where oocytes often look normal.] Each *glp-4(Ego)* allele (*om14, om23, om24*) fails to complement *glp-4(bn2ts)* for the Glp-4 proliferation phenotype at 25° and an Oog phenotype at 20° (Table 3 and data not shown). In general, the phenotypes of heteroallelic *glp-4(Ego/bn2ts)* mutants are intermediate between the phenotypes of animals homozygous for either of the two alleles. *glp-4* mutants can be placed in the following series based on their

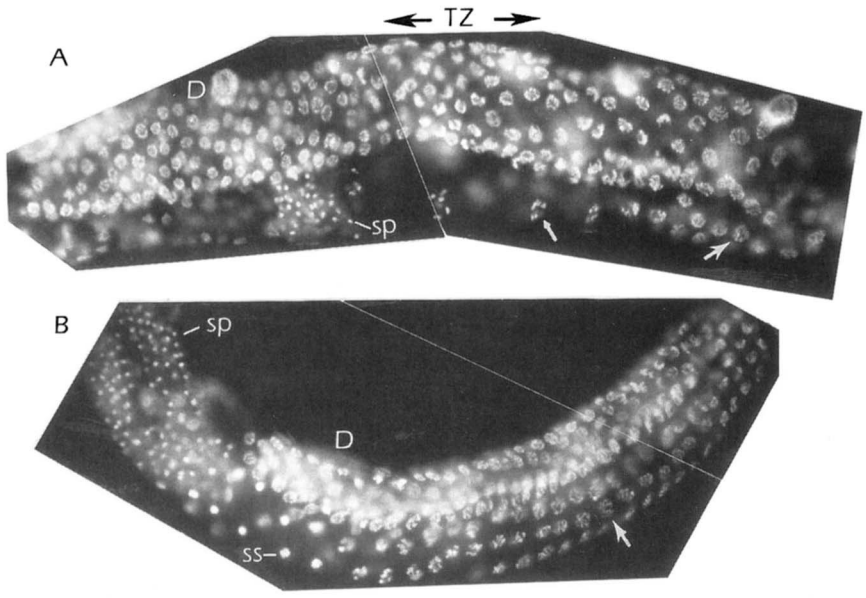

FIGURE 4.—Chromosome morphology in wild-type germlines. Photomicrographs of DAPI-stained animals with distal mitotic region (D), pachytene nuclei (large arrow), oocyte diakinesis nuclei (small arrow), primary spermatocytes (ss) and hypercondensed sperm nuclei at the proximal end (sp). (A) Young adult hermaphrodite; (B) late L4 stage male grown at 20°. In A, the mitosis-to-meiosis transition zone (TZ) is indicated.

proliferation defects: *bn2* > *bn2/om14* > *bn2/om24* > *bn2/om23* (data not shown). Of the three *glp-4(Ego)* alleles, *om23* has the least severe Oog phenotype.

We examined the germline phenotype of *glp-4(om14)* mutants in detail. Young adult hermaphrodites contain several germline abnormalities that can be seen using a combination of DIC microscopy and DAPI staining (see MATERIALS AND METHODS) (Figure 5). These phenotypes are penetrant to different extents, as indicated. First, a few large abnormal nuclei are visible in the mitotic region and mitotic/meiotic transition zone in 70% of animals examined (Figure 5B). Second, in all animals the transition zone is very large and the mitotic and pachytene regions are small relative to wild-type animals (Figure 5A and data not shown). Third, all animals produce small unevenly sized oocytes that accumulate and become crowded together as animals age (Figure 5, C and D). Fourth, the chromosome morphology of some oocyte nuclei changes as animals age; the time of onset of this phenotype is variable. Chromosomes in some proximal oocyte nuclei take on an elongated appearance and homologues appear to dissociate; eventually, many oocyte nuclei become cloudy as if they have become polyploid (Figure 5D). Thus, oocytes appear to undergo endomitosis (multiple rounds of DNA replication in the absence of cell division). This phenotype is reminiscent of the endomitosis phenotype described by J. MCCARTER, K. IWASAKI and T. SCHEDL (personal communication). The later phenotypes (abnormal oogenesis, endomitosis) may be secondary abnormalities that arise as a result of earlier germline defects. Although *glp-4(om14)* oocytes are often fertilized, their progeny are never viable.

In the absence of a tightly linked marker, it was not possible to assess carefully the *glp-4(om14)* phenotype in males. However, *glp-4(om14)* males appear to have fully proliferative germlines because male progeny of *glp-4(om14/+); him-5* mothers have wild-type numbers of germline nuclei (data not shown). In addition, 25% of the males from *glp-4(om14/+); him-5* mothers have disorganized germlines; we believe these animals are likely to be *glp-4(om14)* mutants.

Characteristics of *ego-3* mutants: In addition to its interactions with *glp-1*, the single *ego-3* mutation causes a variety of germline and somatic phenotypes. Below, we describe the interactions between *ego-3(om40)* and *glp-1(lf)* and *glp-1(gf)* mutations. In addition, we describe the *ego-3(om40)* phenotype and investigate the cause of one aspect of this phenotype in particular.

Interactions between ego-3 and glp-1(lf) in the germline: Germline proliferation is reduced substantially in *ego-3(om40); glp-1(bn18ts)* hermaphrodites when compared with *ego-3(om40)* and *glp-1(bn18ts)* single mutants (Table 3). At young adult stage, double mutants have 17% of the number of germ cells found in *ego-3(om40)* single mutants and 19% of the number of germ cells found in *glp-1(bn18ts)* single mutants (Table 3). *ego-3; glp-1* males are moderately enhanced, containing 50% of the number of germ cells found in *glp-1(bn18ts)* single mutant males at late L4 stage (Table 4). Using a deficiency for the *ego-3* region, we were able to examine germline proliferation in *ego-3/yDf8; glp-1(bn18ts)* hermaphrodites. At 20°, these animals resemble *ego-3; glp-1* mutants. Therefore, the enhancement of *glp-1* results from a loss of *ego-3* gene function. We were not able to ask whether *ego-3* enhances the *glp-1* maternal effect because *ego-3* mutants are oogenesis defective.

Loss of ego-3 gene function is associated with germline and somatic phenotypes in a glp-1(+) background: ego-3(om40) mutants develop slowly relative to N2 (Figure 7) and have distinct early and late germline phenotypes in both hermaphrodites and males (Figures 6–9, and data not shown). The early germline phenotype is seen in L3 and L4 stage larvae. In wild-type hermaphrodites, meiosis is

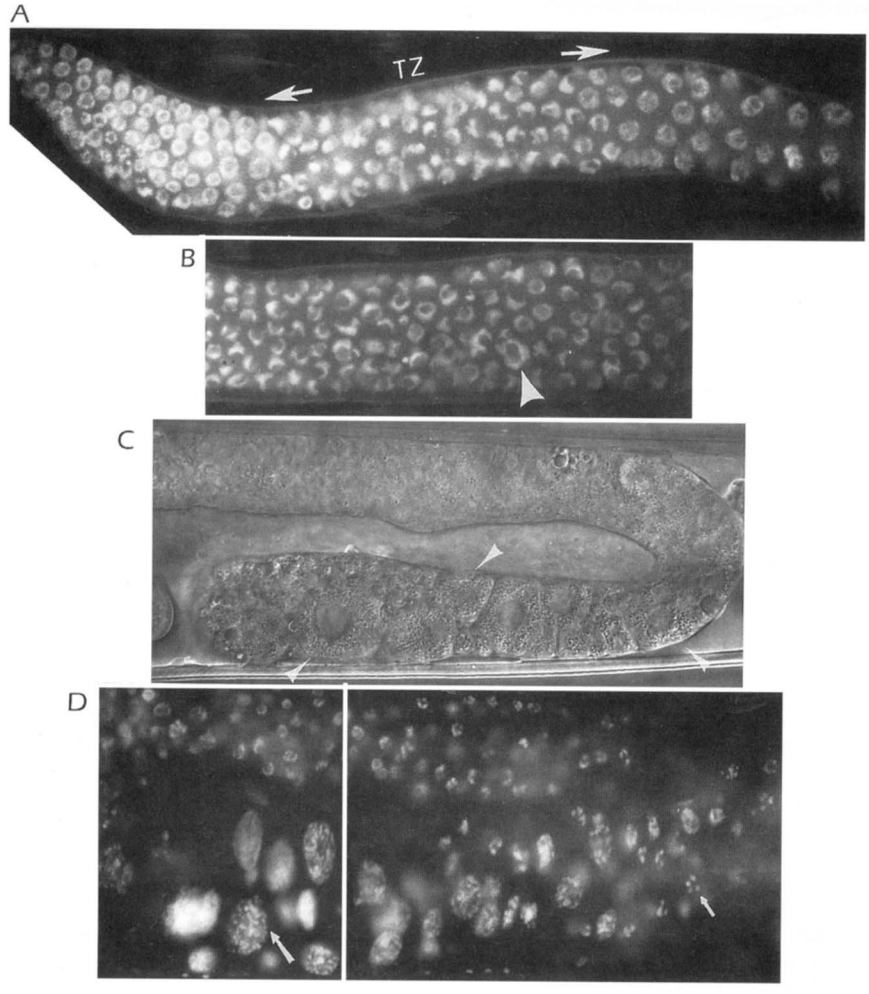

FIGURE 5.—*glp-4(om14)* hermaphrodite germline defects. (A) Photomicrograph of gonad stained with DAPI; note the large mitosis-to-meiosis transition zone (TZ). (B) Photomicrograph of gonad stained with DAPI; note the occasional large abnormal nuclei (arrowhead). Compare with wild-type in Figure 4A. (C) DIC photomicrograph showing abnormal oocytes (arrowhead) in an adult *glp-4(om14)* hermaphrodite. (D) Oocyte nuclei in a slightly older *glp-4(om14)* hermaphrodite stained with DAPI. Many nuclei are arrested in diakinesis (small arrow) both proximal and distal to the loop. Diakinesis arrest appears to have failed in many proximal nuclei, leading to endomitosis (large arrow). See text for details. The images in A and B look slightly different from those in D and in all other figures in this report because the gonads were dissected before staining with DAPI; all other animals pictured were intact when stained (as described in MATERIALS AND METHODS).

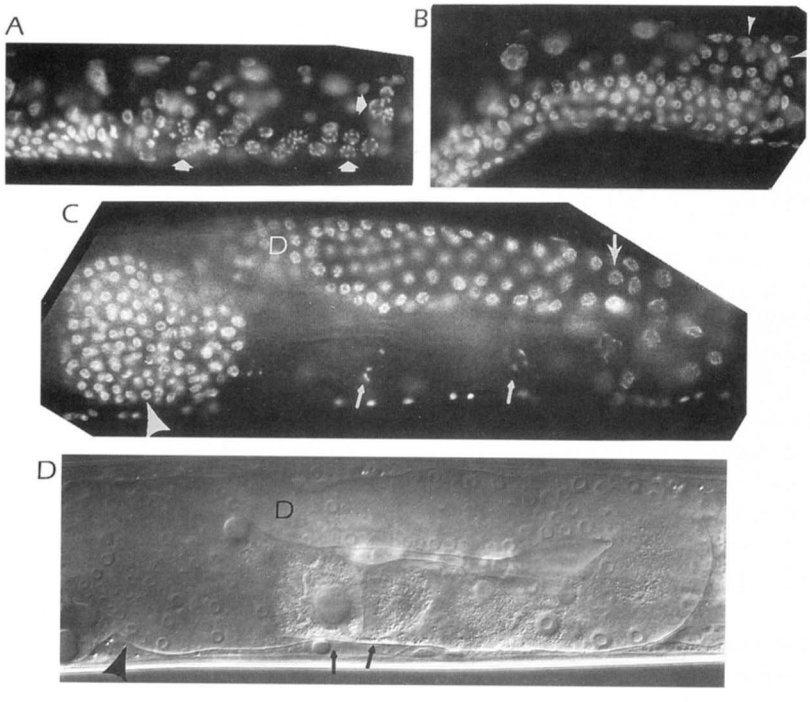

FIGURE 6.—Germline phenotype of *ego-3* hermaphrodites. One arm of the gonad is shown. (A–C) photomicrographs of animals stained with DAPI; (D) a DIC photomicrograph. (A) Larva at L3/L4 lethargus; note large germline nuclei (arrow). (B) Larva at early L4 stage; most germline nuclei are mitotic (small arrowhead). (C and D) A 72-hr adult; note proximal proliferative region (large arrowhead), oocytes (small arrow), pachytene (large arrow), and distal proliferative region (D). Proximal meiosis is not yet visible in these individuals. See text for details.

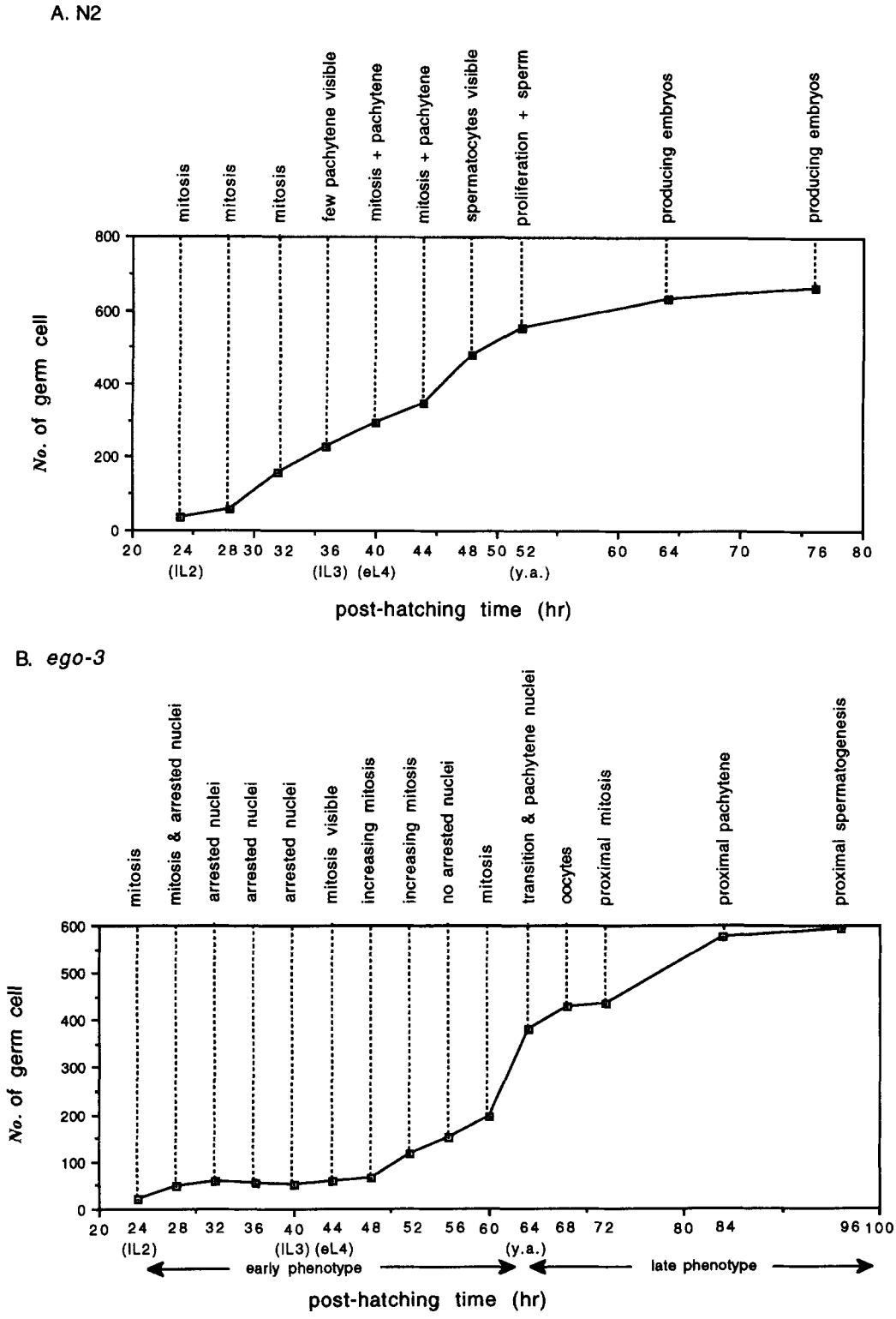

FIGURE 7.—Time course of germline development in *ego-3* and wild-type hermaphrodites. Germline development is described for (A) wild-type (N2) controls and (B) *ego-3* mutants. Time points indicate hours after hatching as L1 larvae. For each time point, animals were collected over a 2-hr period beginning with the time indicated (*e.g.*, animals at the "24"-hr time point were 24–26 hr posthatching). The average germ cell number at each time point is plotted. Nuclear morphologies at each time point are indicated above the graph; in addition, distal mitosis and meiosis are visible at each of the adult timepoints. lL2, late L2 larval stage; eL3, early L3 larval stage; eL4, early L4 stage; y.a., young adult (newly molted) stage. See text for details of phenotypes. The following numbers of animals were characterized. For each *ego-3* timepoint from 24 to 96 hr, 10–15 animals were examined and germline nuclei in ≥10 animals were counted. For 108–132 hr, 15–20 animals were examined, but germline nuclei were not counted; these time points are not included on the graph but are referred to in text. For each N2 timepoint, 12–25 animals were examined, and germline nuclei in 10–15 animals were counted for all but the 76-hr time point. Five animals were counted for the 76–78-hr timepoint.

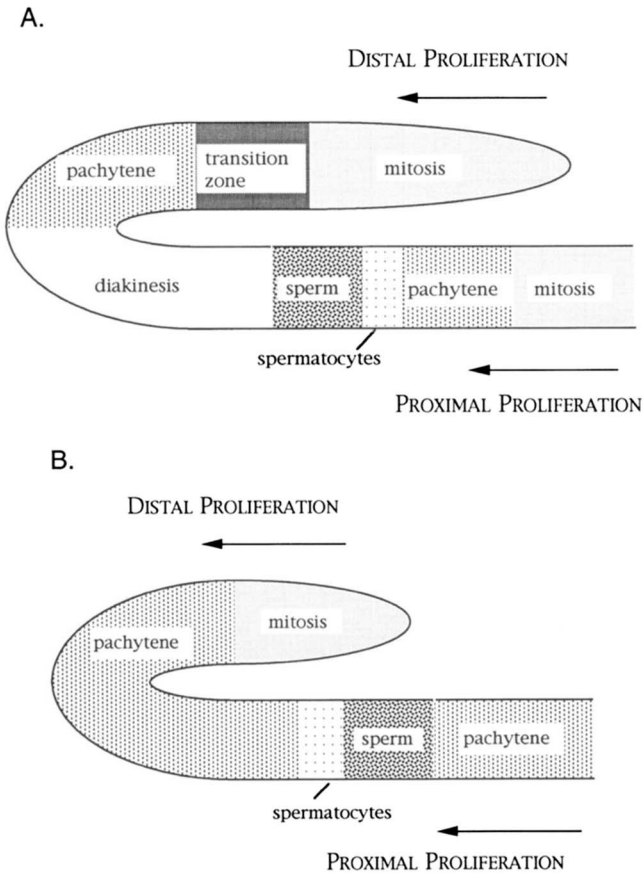

FIGURE 8.—Schematic representation of the *ego-3* germline in (A) a hermaphrodite at >108 hr after hatching and (B) a male at 24–48 hr after late L4 stage. See text for description of phenotypes.

first visible at approximately the L3 to L4 molt. During L4 stage, meiosis gradually increases giving rise to sperm during late L4 and to mature oocytes beginning at young adult stage. In contrast, germline proliferation arrests in 100% of *ego-3* hermaphrodites examined during L3 stage (Figure 7, 32–40 hr); all germ nuclei exhibit the large size characteristic of meiosis (Figure 6A). However, they do not have a characteristic transition stage or pachytene chromosome morphology. Thus, their cell cycle state is difficult to interpret. They may have arrested in mitosis or entered meiosis and arrested at a very early stage of meiotic prophase. Consistent with either hypothesis, the number of germ cells does not increase during this time (Figure 7). In 100% of animals examined at early to mid L4 stage (44–48-hr timepoints), mitotic nuclei become visible at random locations within the gonad, and the germ cell number begins to increase (Figure 7, ≥48 hr; Figure 6B). Neither transition nor pachytene nuclei become visible in *ego-3* hermaphrodites until adulthood (Figure 7).

The late *ego-3* germline phenotype is seen in adults and is more variable than the early phenotype (Figures 6, C and D, and 7, 64–96 hr, and data not shown). In wild-type hermaphrodites, spermatogenesis occurs during the latter part of L4 stage and mature oocytes are first produced at the time of the molt to adulthood (Figure 7). In contrast, 100% of young adult *ego-3* hermaphrodites contain neither sperm nor oocytes (Figure 7). During the first 48 hr of adulthood, oogenesis becomes visible before spermatogenesis in 82% of animals examined, whereas spermatogenesis preceeds oogenesis in 13% of animals (Figure 7 and data not shown, $n > 90$). At this time, 5% of animals have produced no gametes. In those animals where oocytes form before sperm, nuclei in diakinesis become visible at 72 hr after hatching (~8 hr into adulthood; Figure 7), and small irregular oocytes form (Figure 6D). At approximately the same time, from 72 to 74 hr, a small region of mitotic cells becomes visible in the *proximal* gonad of 15% of animals examined (Figure 7, 72 hr and data not shown). By 84 hr, 60% of animals have proximal proliferation (Figure 7 and data not shown); in some animals this proliferation is extensive (*e.g.*, Figure 6, C and D). The remaining worms without proximal proliferation appear to have excessive distal mitosis because their mitotic regions are larger than those of N2 animals (data not shown). In 50% of the 108-hr adults examined, some proximal nuclei have entered meiosis; by

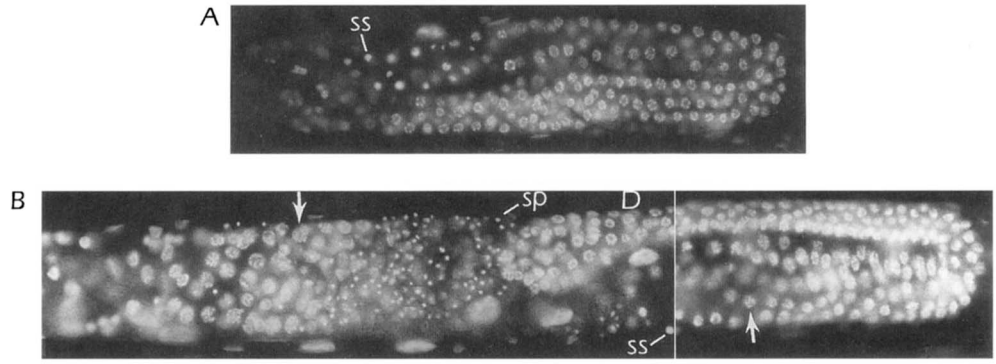

FIGURE 9.—Germline phenotype of *ego-3* males. Photomicrographs show animals stained with DAPI. (A) Male gonad from a late L4 larva. Note that spermatogenesis is just beginning; a few primary spermatocytes (ss) are visible. Compare with Figure 4B. (B) Adult male gonad; note proximal proliferative germ cells now in pachytene (large arrow), sperm (sp), spermatocytes (ss) and distal proliferative region (D).

132 hr, proximal meiosis is visible in 80% of animals examined. Subsequently, sperm are at least sometimes produced from the proximal germline; it is possible that proximal meiosis always gives rise to sperm given enough time. The germline of such an *ego-3* hermaphrodite is diagramed in Figure 8A. In those few animals (13%) where spermatogenesis preceeds oogenesis, sperm form from the distal population of germ cells as they would in wild-type. Oogenesis and proximal proliferation then proceed in a manner similar to that described above. Fertilization can occur in *ego-3* hermaphrodites, but development of the embryo is always abnormal.

ego-3 mutants also are severely Unc (***unc***oordinated) during larval stages. Surprisingly, the Unc phenotype nearly disappears during adulthood. It is unclear whether this somatic phenotype is caused by the same mutation that causes the germline defects or an associated mutation in another gene. We have not been able to separate the two phenotypes, suggesting that they map within 0.35 mu of each other.

The early and late germline phenotypes are also seen in *ego-3* males. During the L3 stage, germline proliferation arrests and nuclei assume a morphology similar to that found in *ego-3* hermaphrodites so that 100% of late L3 to early L4 males examined had the arrested phenotype (n = 20). Mitosis resumes during mid to late L4 stage (n = 16). In a wild-type male gonad, sperm are first visible in mid to late L4 animals. In contrast, mature sperm are not yet visible in 100% of late L4 *ego-3* males examined, although pachytene nuclei are present in all of them ($n > 20$; Figure 9A). In 100% of animals examined, sperm production starts within the next 24 hr (n = 20). In 69% of animals examined at 24–48 hr after late L4, a region of meiosis is visible proximal to the mature sperm as if a brief period of proximal mitosis had occured (n = 33; Figure 9B). Sperm are produced by the distal population of germ cells and sometimes appear to be produced by proximal germ cells as well. The germline of an older *ego-3* male is diagramed in Figure 8B. *ego-3* males do not produce cross-progeny, probably because they are Unc and/or proximal germline proliferation prevents sperm transfer during mating.

ego-3/yDf8 hermaphrodites resemble *ego-3* animals with respect to the Unc and early germline phenotypes. However, the late germline phenotype is somewhat less severe in *ego-3/yDf8* animals. Spermatogenesis begins earlier in *ego-3/yDf8* than in *ego-3* animals (although still later than in wild-type) and oocytes are more often fertilized. Thus, the *om40* mutation may be an unusual allele with both loss and gain of function character; the Unc and early germline phenotypes may result from a loss of gene function, whereas the late germline phenotype may result from, at least in part, a gain of gene function. Similarly, the late germline phenotype is milder in *ego-3/yDf8* males than it is in *ego-3* males. Only a small proportion of animals have proximal proliferation, and spermatogenesis is less delayed than it is in *ego-3* males. Therefore, the late germline phenotype in *ego-3* males may arise in part from a gain of gene function, as well.

TABLE 6

Tests for suppression of *glp-1(gf)* in the germline

Genotype	Percentage overproliferators	Percentage *ego-3* early germline phenotype
glp-1(gf)	100	0
glp-1(gf/lf)	100	0
ego-1	0	NA
ego-1; glp-1(gf)	100[a]	NA
ego-1; glp-1(gf/lf)	31	NA
ego-3	100 (proximal)	100
ego-3; glp-1(gf)	100	50
ego-3; glp-1(gf/lf)	100	100

See text for details of phenotypes; >30 animals of each genotype examined. NA, not applicable; *gf*, gain of function; *lf*, loss of function.

[a] These animals are partially suppressed; see text.

Interactions between ego-3 and glp-1(gf): To determine whether *ego-3* acts upstream or downstream of the GLP-1 receptor, we asked whether loss of *ego-3* gene function could suppress a *glp-1* gain-of-function (*gf*) phenotype. The semidominant *glp-1(oz112gf)* mutation causes overproliferation of the germline and embryonic lethality; a complete description of *glp-1(oz112)* will be published elsewhere by L. WILSON BERRY and T. SCHEDL (personal communication). If *ego-3* were upstream of *glp-1*, then we would expect *glp-1(gf)* to be epistatic to *ego-3*. Alternatively, if *ego-3* were downstream of *glp-1*, then we would expect *ego-3* to be epistatic to *glp-1(gf)* and suppress the *gf* phenotype. Because *glp-1(gf/lf)* animals have slightly lower GLP-1 activity than do *glp-1(gf/gf)* animals (L. WILSON BERRY and T. SCHEDL, personal communication), we tested whether *ego-3(−)* suppressed overproliferation in *glp-1(gf)* and/or *glp-1(gf/lf)* animals (Table 6). The early germline phenotype of *ego-3* is epistatic to *glp-1(oz112gf/lf)* and, 50% of the time, to *glp-1(gf)*. In L3 double mutants, germline proliferation arrests; during L4 stage, proliferation resumes. However, it is difficult to determine whether or not all aspects of the late germline phenotype are epistatic to *glp-1(gf)*. In double mutant adults, the germline is highly proliferative, as it is in *glp-1(oz112gf)* and to a lesser extent *ego-3* animals alone. Gametogenesis is delayed and oocytes are often small, as is characteristic of *ego-3* animals. Because the early *ego-3* phenotype is epistatic to *glp-1(gf/lf)* and, often times, *glp-1(gf/gf)*, *ego-3* appears to act downstream of *glp-1* at the L3/L4 stage. However, it is difficult to order *glp-1* and *ego-3* activity at later stages.

What is the origin of proximal mitosis in ego-3 mutants? We investigated two possible sources for the proximal proliferation in *ego-3* germlines. First, proxi-

mal proliferation might depend on the activation of an aberrant signaling system; it has been shown that a somatic cell in the proximal gonad, the anchor cell (AC), will signal germline proliferation under certain conditions (SEYDOUX *et al.* 1990). Second, germ cells determined to undergo spermatogenesis might be unable to proceed with meiosis (until >72 hr into adulthood) and instead undergo a prolonged period of mitosis. (A caveat is that the spermatogenesis block in some way must depend on *glp-1(+)* activity, because *ego-3;glp-1* double mutants do not have proximal proliferation and are not delayed in spermatogenesis.)

We carried out two different experiments to investigate these possibilities. First, we examined whether proximal proliferation occurs when the AC is eliminated by a *lin-12(gf)* mutation, *lin-12(n302)* (GREENWALD *et al.* 1983). Proximal germline proliferation occurs in *lin-12(gf);ego-3(om40)* double mutants, suggesting that the AC does not signal ectopic proliferation (data not shown). Second, we eliminated spermatogenesis in the *ego-3* germline using a feminizing *fem-1(ts)* mutation (DONIACH and HODGKIN 1984) at restrictive temperature and observed germline proliferation. If proximal mitosis arises from germ cells determined to execute spermatogenesis, then we would expect proximal mitosis to be absent in *fem-1;ego-3* double mutants. In contrast, if germ cells proliferate ectopically regardless of sexual identity, then we would expect to see proximal mitosis in *fem-1;ego-3* mutants. In fact, proximal mitosis is extensive in *fem-1(hc17ts);ego-3(om40)* hermaphrodites at restrictive temperature (25°) (data not shown). Thus, proximal proliferation does not appear to depend on germ cell sexual identity. As an aside, we noted that older oocytes in *fem-1;ego-3* animals usually contained 12 regions of DAPI staining, rather than the usual six, as if homologues had separated from each other (data not shown). This phenotype is seen in a very few nuclei in old *ego-3* adults. A few such nuclei are present in 50% of animals examined at 108 hr after hatching ($n = 18$) and 64% of animals at 132 hr ($n = 11$).

Characteristics of *ego-1* mutants: In addition to their interactions with *glp-1*, mutations in *ego-1* cause mild germline underproliferation, delayed meiosis and gametogenesis, and abnormal oogenesis (see below). We used *ego-1(om18)* as a representative allele to characterize interactions with *glp-1(lf)* and *glp-1(gf)* as well as to describe the *ego-1* mutant phenotype.

Interactions between ego-1 and glp-1 in the germline: ego-1(−/Df) and *ego-1(−/−)* animals resemble each other, suggesting that the *ego-1* germline phenotypes are associated with a loss of gene function. This result is true for both *ego-1(om18)* and *ego-1(om71).* (See below for more details of the phenotype.) Although *glp-1(bn18ts)* at 20° and *ego-1(om18)* each reduce germline proliferation by 50%, germline proliferation in *glp-1(bn18ts);ego-1(om18)* double mutant hermaphrodites is reduced to 16% of wild-type (Table 3). Although *ego-1(om18)* is the weakest enhancer mutation reported here, the interactions between it and *glp-1* are nevertheless synergistic, suggesting that the two genes affect a common process. Unfortunately, we were not able to determine whether *ego-1* enhances the *glp-1* maternal effect because *ego-1* mutants produce abnormal oocytes.

At 20°, *ego-1(om18/Df);glp-1(−)* hermaphrodites have a Glp-1 phenotype resembling that of *ego-1(om18);glp-1(−)* animals. The simplest interpretation of this result is that the Ego phenotype results from a loss of *ego-1* gene function. If so, then the wild-type function of *ego-1* is to promote proliferation in the germline.

To determine whether *ego-1* acts upstream or downstream of the GLP-1 receptor, we asked whether loss of *ego-1* gene function could suppress *glp-1(oz112gf)* (see above). If *ego-1* acts downstream of *glp-1*, then it should suppress the overproliferation associated with *glp-1(gf).* Alternatively, if *ego-1* acts upstream of *glp-1*, then it should not alter the overproliferation phenotype. We examined whether *ego-1(om18)* can suppress germline overproliferation in *glp-1(gf)* and/or *glp-1(gf/lf)* animals. Germline overproliferation is fully suppressed in 69% of *glp-1(gf/lf);ego-1(om18/om18)* animals (Table 6). These animals have the normal germline organization of distal mitosis followed by meiosis and gametogenesis. In contrast, *glp-1(gf);ego-1(om18)* animals always have overproliferative germlines in that mitotic nuclei can be found throughout the gonad; however, the level of proliferation is partially decreased (data not shown). Taking these results together, *ego-1* mutations appear to suppress a moderate *glp-1* gain of function phenotype in the germline, suggesting that *ego-1* acts downstream of *glp-1* to modulate activity of the signaling pathway.

Germline defects associated with ego-1 mutations: The onset of meiosis seems to be slightly delayed in *ego-1(om18)* hermaphrodites relative to wild-type. Pachytene nuclei can be seen as early as late L3 stage and always are present in early L4 stage N2 hermaphrodites ($n = 10$–20 animals at each stage). In contrast, we never found pachytene nuclei in *ego-1* mutants at late L3 stage ($n =$ 15 animals); early L4 stage mutants had many fewer pachytene nuclei than were found in wild-type animals of the same age ($n = 20$ animals). The delay in meiosis onset is reminiscent of that reported by KIMBLE and WHITE (1981) in animals where one of the two germ cell precursors, Z3, was laser ablated. These operated animals, like *ego-1* mutants (Table 3), had germlines of about half wild-type size.

Spermatogenesis is subsequently delayed in *ego-1* hermaphrodites so that young adults contain pachytene nuclei and primary spermatocytes, but only 55% of ovotestes have actually produced some mature sperm ($n > 24$) (Figure 10A). By 24 hr after young adult stage, sperm are present in >97% of ovotestes ($n > 98$). No obvious germline defects are found in *ego-1(om18)* males; neither meiosis nor spermatogenesis is delayed and no strong proliferation defect is present (Table 4).

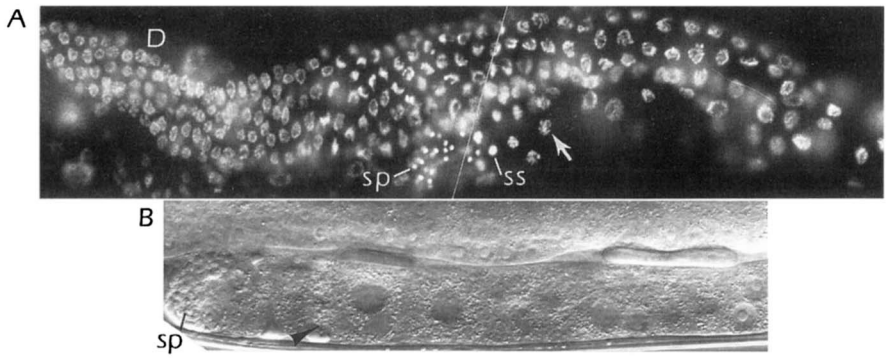

FIGURE 10.—Germline phenotypes of *ego-1* mutant hermaphrodites. (A) One arm of the gonad in a young adult *ego-1(om18)* hermaphrodite that has been stained with DAPI. Spermatogenesis has not yet been completed; in contrast, spermatogenesis is complete and oogenesis has begun in the wild-type animal shown in Figure 4A. Sperm (sp), spermatocytes (ss), pachytene nuclei (arrow) and distal region of germline (D) are indicated. (B) DIC photomicrograph showing abnormal oocytes (arrowhead) in an older *ego-1(om18)* adult; sperm (sp) are also indicated. See text for details.

ego-1 mutant hermaphrodites are Oog. They often produce small and/or irregularly sized oocytes (61%, $n > 100$; Figure 10B). Some animals lack oocytes altogether (based on both cell and chromosome morphology), but the grainy cytoplasm typical of oocytes is always present at the loop region of the gonad arm. Eighteen percent of gonad arms ($n > 100$) produces some embryos; they are always inviable. Although we have not characterized them in detail, morphological abnormalities in embryogenesis are often obvious within the first few cell divisions (data not shown).

As mentioned above, *ego-1* mutations appear to cause a loss of gene function because *ego-1/ozDf5*, *ego-1/nDf25*, and *ego-1* animals resemble each other. However, *ego-1(om18)/ozDf5* animals have a more severe Oog phenotype on average than do *ego-1(om18)* or *ego-1(om18)/nDf25* animals; in particular, *ego-1(om18)/ozDf5* animals have never been observed to produce embryos ($n = 76$). Therefore, *ego-1(om18)* may not represent a complete loss of gene function and *nDf25* may not fully delete *ego-1* gene activity. Alternatively, *ozDf5* may enhance the Ego-1 phenotype by deleting a gene to the left of *nDf25*.

Characteristics of *ego-4* and *ego-5* mutants: The phenotypes of *ego-4* and *ego-5* mutants are similar. We characterized these genes using *ego-4(om30)* and *ego-5(om31)* as representative alleles. Mutations in each gene cause defects in germline proliferation and oogenesis. Underproliferation of the *ego-4* and *ego-5* mutant germlines is more substantial than for any other enhancer gene mutants (Table 3). This defect is variable, with a low percentage of hermaphrodites producing so few germ cells that they do not form oocytes (data not shown). Males appear normal and are fertile.

Most (96–97%) *ego-4(om30)* and *ego-5(om31)* hermaphrodites produce abnormal variably sized oocytes, many of which become fertilized; embryos are always inviable and cannot be paternally rescued. We consider this a Mel rather than Oog phenotype because many oocytes appear wild-type in morphology and the fertilization rates are high. We have not characterized the embryonic lethal defects in detail; however, we made some observations. Development in embryos from *ego-5(om31)* mothers often appears to have failed at the time of elongation to form a worm; twitching (muscle) cells are often present. In contrast, embryos from *ego-4(om30)* mothers typically appear as a jumbled ball of cells, without signs of elongation or functional muscle cells.

ego-4 and *ego-5* are also similar in their interactions with *glp-1*. *glp-1 ego-4* and *glp-1 ego-5* hermaphrodites have germline proliferation phenotypes similar to strong *glp-1* loss-of-function alleles (Table 3). Based on comparison of *ego(−)* and *ego(−);glp-1(−)* germlines, *ego-4(om30)* is one of the two strongest enhancers reported here (Table 3). *ego-5(om31)* is a more variable enhancer and therefore slightly weaker (Table 3). In contrast, neither *ego-4* nor *ego-5* reduces proliferation in *glp-1* males (Table 4). Because no deficiency is available for these genes, we cannot tell whether their mutant phenotypes are associated with a loss of gene function. Therefore, we cannot test whether *ego-4* and *ego-5* act upstream or downstream of *glp-1*. Unfortunately, their Mel defects prevented us from examining whether *ego-4* or *ego-5* mutations could enhance the *glp-1* maternal effect.

DISCUSSION

Signaling pathways mediated by *glp-1*, *lin-12*, and *Notch*—as well as their vertebrate counterparts—have generated a great deal of interest, especially as their importance for a variety of inductive cues in different cells or tissues during development has become clear. Previous studies in *C. elegans* have recovered suppressors of a *glp-1* and/or *lin-12* phenotype in one or more tissues (*e.g.*, MAINE and KIMBLE 1989, 1993; SUNDARAM and GREENWALD, 1993). Here, we report the identification of mutations in seven genes that act as enhancers of a weak *glp-1* mutation in the hermaphrodite germline and describe their interactions with *glp-1*. We recovered very weak alleles of *lag-1*, a gene previously known to be essential for the formation of many cells or structures (LAMBIE and KIMBLE 1991). We also recovered new alleles of *glp-4*, a gene previously defined by a single conditional allele as functioning in germline mitosis and

oogenesis (BEANAN and STROME 1992); our results are consistent with a role for *glp-4* in oogenesis. Mutations in two new genes, *ego-1* and *ego-3*, also disrupt the process of oogenesis. In addition, *ego-1* mutants have moderately reduced germline proliferation and *ego-3* mutants have a complex germline proliferation phenotype. *ego-4* and *ego-5* mutants appear to be Mel because they never produce viable progeny, although their oocytes typically appear more or less normal. Based on these pleiotropic phenotypes, all seven genes appear to function in multiple aspects of development, as does *glp-1* itself.

Weak mutations in *lag-1* enhance a weak Glp-1 phenotype in the germline: We recovered weak alleles of *lag-1*, a gene identified in other studies as a candidate member of the *glp-1* mediated pathway in several tissues (LAMBIE and KIMBLE 1991; S. CHRISTENSON and J. KIMBLE, personal communication). This result indicates that our screening method identifies *glp-1* pathway components. Recent data from Drosophila as well as *C. elegans* has shed light on the role of *lag-1* in the signaling process. In Drosophila, the *Suppressor of Hairless* [*Su(H)*] gene product physically interacts with the cytoplasmic portion of *Notch* protein (FORTINI and ARTAVANIS-TSAKONAS 1994); upon signal binding to Notch, Su(H) moves into the nucleus where it presumably regulates gene expression (FORTINI and ARTAVANIS-TSAKONAS 1994). The *lag-1* and *Su(H)* genes are related in sequence (S. CHRISTENSON and J. KIMBLE, personal communication); therefore, *lag-1* is likely to be the *C. elegans* equivalent of *Su(H)* and may be a transcriptional regulator. In keeping with these molecular data, L. WILSON BERRY and T. SCHEDL have shown that previously isolated *lag-1* alleles suppress *glp-1(gf)* activity in the germline (personal communication). Similarly, we found that *lag-1(om13)* suppresses *glp-1(gf)* (E. MAINE, unpublished data).

Mutations in *lag-1* enhance both embryonic and germline phenotypes of *glp-1* and therefore must affect a process that is common to these functions. Because most progeny die as embryos rather than as L1 larvae, *lag-1* enhances an early embryonic *glp-1* function. Enhancement of the *glp-1* maternal effect depends on the maternal genotype with respect to *lag-1*. That is, *lag-1(−/−);glp-1(−/−)* animals from *lag-1(−/+);glp-1(−/−)* mothers are viable but cannot produce viable offspring. Consistent with these results, we found a maternal effect lethality associated with the *lag-1(om13)* allele; a paternally supplied *lag-1(+)* gene can partially rescue this lethality, suggesting that zygotic expression of *lag-1(+)* can in part compensate for the absent maternal expression. This maternal effect is not characteristic of previously reported *lag-1* alleles (LAMBIE and KIMBLE 1991). It is unlikely to be caused by a linked mutation in another gene as we have been unsuccessful at separating it from other aspects of the *lag-1* phenotype (enhancement of *glp-1* and larval lethality).

***glp-4* may play multiple roles in germline development:** Based on the analysis of a single conditional allele, *glp-4* was previously described as essential for the progression of germ cells through mitosis and for oogenesis (BEANAN and STROME 1992). The three *glp-4(Ego)* alleles described here cause oogenesis defects but have little effect on germline proliferation unless placed in a *glp-1* mutant background. In the absence of a deficiency for the gene, it is difficult to say which of these *glp-4* defects result from a loss of function. It is possible that *glp-4* activity is regulated by the DTC-to-germline signaling pathway and promotes germline mitosis.

Because it is associated with each of the four existing mutant alleles, we believe that an oogenesis defect may result from a loss of *glp-4* gene activity. Therefore, *glp-4* activity may promote oogenesis. Oogenesis is abnormal in at least two ways in *glp-4* mutants. First, oocytes are small and irregularly sized; although sometimes fertilized, they are incapable of supporting normal development. Second, some oocytes in older animals do not maintain the usual meiotic arrest at diakinesis and instead appear to become endomitotic. Because oocyte arrest may be accomplished via a cell-signaling system, it is possible that *glp-4* function is critical for the activity of two signaling pathways.

***ego-1*, *ego-3*, *ego-4* and *ego-5* may play multiple roles in germline development:** Mutations in the newly identified *ego* genes are associated with various germline and somatic defects. Subsets of *ego* genes with similar mutant phenotypes may be involved in common processes. For example, *ego-4* and *ego-5* mutants have moderately reduced germlines and are Mel. In contrast, *ego-1* and *ego-3* mutants are Oog, producing oocytes that are usually quite small. However, *ego-1* and *ego-3* mutants have unique and perhaps opposite germline defects. Loss of *ego-3* gene function may cause a premature onset of meiosis, whereas loss of *ego-1* gene function causes a delay in the onset of meiosis. Thus, *ego-1* and *ego-3* may be unrelated in function to each other or to *ego-4* and *ego-5*.

How do *ego-1* and *ego-3* mutations decrease activity of the *glp-1* pathway? An *ego* gene might encode a component of the *glp-1* signaling pathway or regulate the expression of one of those components in either the DTC or germline. To investigate whether *ego-1* and *ego-3* act upstream or downstream of *glp-1*, we tested whether *ego-1* or *ego-3* mutations could suppress a gain of *glp-1* gene function. (We did not test *ego-4* and *ego-5* because we do not know whether these mutations cause a loss or gain of gene function.) *ego-1* appears to act downstream of *glp-1* because it partially suppresses the germline overproliferation associated with *glp-1(oz112gf)* (see RESULTS). The situation with *ego-3* is more complex. However, *ego-3* is epistatic to *glp-1(gf)* in L3/L4 larvae, suggesting that it acts downstream at this time. If LAG-1 is immediately downstream of GLP-1 in the signaling

pathway, then both *ego-1* and *ego-3* might act downstream of *lag-1*. Regardless of their relative positions in the pathway, we would expect *ego-1* and *ego-3* mutations to enhance a weak *lag-1* phenotype in the germline. In fact, preliminary results indicate that this is the case for *ego-3*: the germlines of *ego-3(om40); lag-1(om13)* double mutants have a moderate to severe Glp-1 phenotype (E. MAINE, unpublished data).

ego-3: In *ego-3* mutants, all germ cells take on an abnormal nuclear morphology during L3 stage. These cells may enter meiosis prematurely (a phenotype consistent with a role for *ego-3* in the mitotic/meiotic choice) or may arrest in mitosis. In the latter case, arrest may occur because the germline is depleted of an *ego-3(+)* maternal product. Proliferation may not be able to resume until a sufficient amount of the zygotic, partially defective *ego-3(om40)* product is synthesized to support continued mitosis. Because the arrest phenotype seems to be associated with a loss of gene function, wild-type *ego-3* activity might promote germline mitosis. Consistent with this hypothesis, enhancement of *glp-1* also seems to be caused by a loss of *ego-3* gene function. A combined decrease in both the *glp-1* and *ego-3* mitosis-promoting activities might be sufficient to cause the inappropriate entry of all germ cells into meiosis. If so, then the later germline phenotype, which may result in part from a gain of function, may be extraneous to enhancement. Previously described examples of mutations with both loss and gain of function character include certain alleles of the *C. elegans* genes *gld-1* (***g***erm ***l***ine ***d***efective) (FRANCIS *et al.* 1995) and *tra-1* (***tra***nsformer) (SCHEDL *et al.* 1989). Future analysis of *ego-3* at the molecular level will help resolve these issues.

Proliferation in the proximal germline of *ego-3* mutants may result from either an aberrant cell-signaling system or signal independence (which in turn might result from the inability of proximal germ cells to undergo meiosis). As has been shown previously, the proximal germline is capable of responding to a proliferative signal from the AC (SEYDOUX *et al.* 1989). However, we have shown that an AC is not necessary for proximal proliferation in *ego-3* germlines. This result suggests that proximal proliferation occurs independent of an external signal as no other cell in the proximal gonad is known to be capable of signaling germline proliferation. However, we cannot rule out that one or more somatic gonadal cell (besides the AC) produces a proliferative signal in *ego-3* mutants. As an alternative explanation, ectopic proliferation may arise because cells can undergo mitosis independent of an external signal. This phenomenon could occur in at least two ways. For example, if the early *ego-3* phenotype results from mitotic arrest (perhaps reflecting a need for zygotic EGO-3 activity), then proximal mitosis may be signal-independent proliferation that likewise can occur in the absence of sufficient EGO-3 activity. Alternatively, if the early *ego-3* phenotype reflects a meiotic arrest, then proximal mitosis might be the type of signal independent proliferation that follows unsuccessful meiosis in *C. elegans gld-1* mutants (FRANCIS *et al.* 1995). As has been shown in yeast and in *C. elegans*, entry into meiosis is not irreversible (HONIGBERG *et al.* 1992; HONIGBERG and ESPOSITO 1994; FRANCIS *et al.* 1995). However, this explanation seems unlikely because *ego-3* appears only to promote meiosis rather than to be essential for it. Meiosis is eventually executed in old *ego-3* animals, and a decrease in *glp-1* activity allows *ego-3* germ cells to complete meiosis during larval development. Clearly, the *ego-3* null phenotype must be examined to decide these points.

ego-1: In *ego-1* mutants, meiosis appears to be delayed slightly and the germline is moderately reduced. Enhancement of *glp-1* may be caused by whatever mechanism causes the reduced germline; if so, then a common function may regulate the rate of proliferation and the timing of meiosis onset. The meiosis delay also may be caused by the reduced size of the germline itself. As suggested by KIMBLE and WHITE (1981), perhaps the germline has to reach a certain size before proximal nuclei are free of the distal tip cell signal.

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