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Suppressors of *glp-1*, a Gene Required for Cell Communication During Development in *Caenorhabditis elegans*, Define a Set of Interacting Genes

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ABSTRACT

The *glp-1* gene is essential for two cell interactions that control cell fate in *Caenorhabditis elegans*: induction of anterior pharynx in the embryo and induction of mitotic proliferation in the germ line. To identify other genes involved in these cell interactions, we have isolated suppressors of two temperature sensitive alleles of *glp-1*. Each of 14 recessive suppressors rescues both embryonic and germline *glp-1(ts)* defects. These suppressors are extragenic and define a set of six genes designated *sog*, for *s*uppressor *o*f ***g*lp-1**. Suppression of *glp-1* is the only obvious phenotype associated with *sog* mutations. Mutations in different *sog* genes show allele-specific intergenic noncomplementation, suggesting that the *sog* gene products may interact. In addition, we have analyzed a semidominant mutation that suppresses only the *glp-1* germline phenotype and has a conditional feminized phenotype of its own. None of the suppressors rescues a *glp-1* null mutation and therefore they do not bypass a requirement for *glp-1*. Distal tip cell function remains necessary for germline proliferation in suppressed animals. These suppressor mutations identify genes that may encode other components of the *glp-1* mediated cell-signaling pathway or regulate *glp-1* expression.

THE specification of certain cell fates in multicellular organisms depends on information that is received from the cellular environment. The development of a number of cell types in the nematode, *Caenorhabditis elegans*, depends on the presence of one or more neighboring cells (KIMBLE 1981; KIMBLE and WHITE 1981; SULSTON and WHITE 1980; SULSTON *et al.* 1983; STERNBERG and HORVITZ 1986; PRIESS and THOMSON 1987). The molecular mechanisms by which one cell influences the development of another cell, a process termed induction, are not understood. However, many genes have been identified that appear to mediate specific inductive events (reviewed in LAMBIE and KIMBLE 1991a).

The *glp-1* (for *g*erm *l*ine *p*roliferation defective) gene mediates at least two inductive cell-cell interactions during *C. elegans* development (AUSTIN and KIMBLE 1987; PRIESS, SCHNABEL and SCHNABEL 1987). One interaction occurs early in embryogenesis when descendants of one blastomere, P_1, induce descendants of another blastomere, AB, to produce pharyngeal muscle (PRIESS and THOMSON 1987). In the absence of P_1 or maternal *glp-1*, AB does not produce pharyngeal muscle (PRIESS and THOMSON 1987; PRIESS, SCHNABEL and SCHNABEL 1987). A second interaction occurs post-embryonically when two somatic cells, the distal tip cells, induce mitotic proliferation of the germ line (KIMBLE and WHITE 1981). In the absence of the distal tip cells or *glp-1*, germ cells do not proliferate and hermaphrodites are sterile (KIMBLE and WHITE 1981; AUSTIN and KIMBLE 1987). In addition, maternal *glp-1* function is essential for the formation of the embryonic hypodermis (PRIESS, SCHNABEL and SCHNABEL 1987). Finally, in the absence of *lin-12* (*lin*eage defective) gene product, *glp-1* is required for formation of several cells or structures required for larval viability (LAMBIE and KIMBLE 1991b).

To identify other genes involved in *glp-1*-mediated cell-signaling, we have isolated extragenic suppressors of two temperature sensitive (*ts*) *glp-1* mutations. Molecular analysis has shown that both *ts* alleles are missense mutations within the cytoplasmic portion of the predicted *glp-1* protein (KODOYIANNI, MAINE and KIMBLE 1992). We previously reported a group of extragenic *glp-1* suppressors with morphological defects, including mutations in three genes now known to encode collagen (MAINE and KIMBLE 1989). Here, we report the isolation and genetic characterization of 14 additional suppressors that have no obvious phenotype other than *glp-1* suppression. All 14 suppressors rescue both the embryonic and germline phenotypes of *glp-1*. We also describe a unique mutation that specifically suppresses the *glp-1* germline phenotype.

MATERIALS AND METHODS

Strains and culture methods: In general, 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) except where indicated. Nomenclature follows the guidelines of HORVITZ *et al.* (1979).

Mutations used in this study were [*bli* (*bli*ster), *dpy* (*dumpy*), *fog* (*f*eminization *o*f the *g*erm line), *glp* (*g*erm *l*ine *p*roliferation defective), *him* (*h*igh *i*ncidence of *m*ales), *lin* (abnormal *lin*eage), *lon* (*lon*g), *rol* (*rol*ler), *sel* (*s*uppressor and/or *e*nhancer of *lin-12*), *sma* (*sma*ll), *unc* (*unc*oordinated)]:

Linkage group *I* (LG *I*): *dpy-5(e61)*, *dpy-14(e188ts)*, *gld-1(q268)* (provided by T. SCHEDL), *lin-10(e1439)*, *lin-11(n566)*, *unc-13(e51, e1091)*, *nDf25*, *ozDf5* (provided by T. SCHEDL).

LG *II*: *rol-1(e91)*, *unc-4(e120)*.

LG *III*: *dpy-17(e164)*, *dpy-18(e364)*, *glp-1(q35, q50, q158, q224ts, q231ts)* (AUSTIN and KIMBLE 1987), *lin-12(q269)*, *sel-2(n655)* (provided by G. SEYDOUX and I. GREENWALD), *unc-32(e189)*, *unc-36(e251)*, *unc-79(e1068)*, *eT1*, *nDf11*.

LG *IV*: *dpy-13(e184)*, *dpy-20(e1282ts)*, *unc-5(e53)*, *unc-24(e138)*, *eDf18*, *eDf19*.

LG *V*: *dpy-11(e224)*, *him-5(e1467)*, *unc-42(e270)*, *sDf35*.

LG *X*: *lon-2(e678)*, *unc-1(e719)*, *unc-18(e81)*.

Isolation of recessive suppressors of *glp-1(ts)*: Fourth larval stage (L4) hermaphrodites of genotype *unc-32 glp-1(ts)* were raised at 15°, mutagenized, and returned to plates at 15°. Two strategies were used to isolate recessive suppressors. (1) To generate suppressors of both the germline and embryonic *glp-1* phenotypes, F_1 progeny of mutagenized animals were picked (three animals per plate) and grown at 15°. F_2 progeny were shifted to 20° as late embryos or L1 larvae. Plates were screened visually for viable F_3 progeny. In this way, three mutations, *q294*, *q295* and *q297*, were isolated from 2100 *glp-1(q224)* F_1 animals and nine mutations, *q298*, *q299*, *q300*, *q301*, *q303*, *q304*, *q305*, *q306* and *q308*, from 1900 *glp-1(q231)* F_1 animals. (2) In an attempt to isolate germline-specific suppressors, animals were treated as described above except that F_2 animals were shifted back to permissive temperature soon after reaching adulthood. One suppressor, *q345*, was isolated from 600 F_1 *glp-1(q224)* animals shifted to 20°; no suppressors were recovered from an additional 600 F_1 *glp-1(q224)* animals shifted to 25°. Upon testing, *q345* proved to suppress the embryonic as well as the germline phenotype of *glp-1*.

One recessive suppressor, *q309*, was recovered in an F_1 screen for dominant suppressors of *glp-1(q231)* that is reported in the accompanying paper by LISSEMORE *et al* (1993). Upon retesting (see below), it proved to be recessive.

Isolation of *q162*: *q162* was isolated separately in a non-complementation screen for new *glp-1* alleles (AUSTIN 1989). The allele used in the screen was *glp-1(q35)*. *q162* acts as a semidominant suppressor of the *glp-1(q35)* loss of function phenotype.

Recessivity tests: To remove extraneous mutations from the genome, suppressed lines were outcrossed to wild-type (N2) and fertile *unc-32 glp-1(ts)* animals were recovered in the F_2. Here, suppressors are designated *sup(x)*.

To test whether a given suppressor was strictly recessive, *unc-32 glp-1(ts); sup(x)* hermaphrodites were mated to N2 males (at 20°), heterozygous cross-progeny were isolated, and fertile Unc animals were recovered in the F_2. For all alleles except *q303*, 25% or fewer of the Unc F_2 were fertile, suggesting that (1) suppressed animals are homozygous for the *sog* mutation and (2) the *sog* mutations are unlinked to *glp-1* (see RESULTS). In most cases where fewer than 25% of the F_2 were suppressed, later tests indicated that the suppression phenotype was not completely penetrant (see RESULTS). As *q303* proved to be unlinked (see RESULTS), it was tested for semidominance by mating *glp-1(q231);him-5* males to *q303;unc-32 glp-1(q231)* hermaphrodites and examining the fertility and progeny viability of non-Unc cross progeny at 20°. The brood produced by 38 cross-progeny was 48 animals; on average two survived and produced viable progeny. Thus, *q303* is only very weakly semidominant.

Genetic mapping and complementation tests: Linkage and complementation were determined by standard tests (see Tables 2 and 3 in RESULTS). Complementation and mapping were done on the basis of suppression of *glp-1(ts)* in all cases except for the mapping of *q162*, which was done on the basis of its visible phenotype.

Mapping of suppressors of the germline and embryonic phenotypes: The recessive suppressors were mapped to a linkage group by one of two methods. (1) Strains containing *glp-1(q231)* and one marker mutation on each of three chromosomes were used to generate animals that were *dpy-5(e61/+);rol-1(e91/+);unc-32(e189/+) glp-1(ts);sog-?(+/−)* or *unc-5(e53/+);dpy-11 (e224/+);lon-2(e678/+);sog-?(+/−);glp-1(ts)*. From these heterozygous animals, progeny were isolated that were homozygous for one of the markers; they were tested at 20° for the presence of the suppressor. Fertile animals homozygous for a particular marker mutation were not recovered if the marker was located close to the suppressor. (2) *glp-1(ts)* males carrying a suppressor were mated to strains containing two markers on a single chromosome and cross-progeny were isolated. For example, *dpy-5 unc-13; glp-1(ts)* was used for mapping on LG *I*. From *dpy-5 unc-13/++;glp-1(ts);sog-?(−/+)* mothers, Dpy Unc [*glp-1(ts);dpy-5 unc-13*] hermaphrodites were recovered and tested for fertility. If the *sog* mutation in question is located on LG *I*, it should be difficult to recover fertile Dpy Unc animals.

Seven mutations, *q295*, *q298*, *q303*, *q305*, *q308*, *q309*, *q345*, mapped to LG *I*, and three-factor mapping with *dpy-5 unc-13* placed them in a common position close or to the right of *unc-13*; additional mapping of one allele, *q298*, with *dpy-14 unc-13* confirmed this location. All seven alleles fail to complement each other and are designated *sog-1*. More precise mapping of two alleles, *sog-1(q295)* and *sog-1(q298)*, was done with *unc-13 gld-1* and *unc-13 lin-10*. One mutation, *q299*, mapped to LG *II* and is designated *sog-2*; it was three-factor mapped with *unc-4 rol-1*. One mutation, *q294*, mapped to LG *IV* and is designated *sog-3*; it was three-factor mapped with *unc-5* and *dpy-20*. Two alleles, *q301* and *q304*, mapped to LG *V*; they fail to complement and are designated *sog-4*. They were three-factor mapped with *dpy-11* and *unc-42*. One allele, *q297*, mapped to LG *X* and is designated *sog-5*; it was three-factor mapped with *lon-2* and *unc-18*. Finally, two alleles, *q300* and *q306*, mapped to LG *IV*; they fail to complement and are designated *sog-6*. They were three-factor mapped with *unc-5* and *dpy-20* to a position distinct from *sog-3(q294)*.

Mapping of germline-specific suppressor: The germline-specific suppressor, *q162*, was three-factor mapped on the basis of its feminized germline (Fog) phenotype using *unc-93 dpy-17*: Dpy and Unc recombinants from *unc-93 dpy-17/q162* were picked at 20° and their progeny were examined at restrictive temperature (12°) for a Fog phenotype.

Determination of brood size and percent hatching: L4 hermaphrodites of genotype *sog;glp-1(ts)* were picked from stocks grown at restrictive temperature (20°), placed individually on Petri dishes and transferred every ~24 hr to a fresh plate. The total number of embryos produced by each hermaphrodite was counted; embryos were scored for viability ~36 hr after the hermaphrodite had been transferred. Hatched progeny were counted once they had achieved at least the L3 stage of development. As a control, brood sizes and percent hatching of *glp-1(q231)* and *glp-1(q224)* were determined at 20°. Heterozygous *q162* animals were tested

by crossing *unc-1(e719);q162 glp-1(q224)* hermaphrodites to *glp-1(q224);him-5* males. Brood sizes of the non-Unc cross-progeny were counted.

Temperature shift experiments: *Shift down:* Homozygous *q162* animals grown at 25° were picked to fresh plates preincubated at 25° to generate progeny for shifting to 15°. Progeny of the specified stages were picked and shifted to restrictive temperature (10°) on preincubated plates.

Shift up: Homozygous *q162* escaper hermaphrodites from a stock grown at 10° were picked to fresh plates preincubated at 10° to generate progeny for shifting up to 25°. Progeny of the specified stages were shifted to permissive temperature (25°) on plates preincubated at 25°.

Scoring shifted animals: Shifted animals were picked to individual plates and assayed for the production of self-progeny. In addition, they were examined by Nomarski optics if one gonad arm appeared to have a different phenotype than the other, or when sterile.

Dosage studies: For four genes, *sog-1*, *sog-4*, *sog-6* and *sog-10*, one or more deficiencies (*Df*) exist that are predicted to remove the gene based on its genetic map position. It should be noted that either one or both LG *I Df*s should delete *sog-1* whereas both LG *IV Df*s should delete *sog-6*. Deficiencies are maintained in a variety of ways: over a balancer chromosome, over a chromosome containing visible markers that flank or are included within the *Df*, or under a duplication. To test the visible phenotype of each *sog/Df* combination, *sog;glp-1;him* males were crossed to hermaphrodites carrying the *Df*. F_1 hermaphrodites were cloned out and their phenotypes examined. Those animals that segregated small, misshapen, dead embryos (*Df/Df*) and did not segregate the balancer or marked chromosome in the F_2 were presumed to be *sog/Df;glp-1/+*.

To determine whether the Glp phenotype is suppressed in *sog/Df;glp-1* animals, strains were constructed and tested as follows. In all cases, crosses were done at 15°, and progeny were shifted to 20° as embryos or L1 larvae. (1) For *sDf35*, a doubly balanced strain, *unc-32 glp-1/eT1;sDf35/eT1*, was constructed and crossed to *sog-4;glp-1;him* males. The fertility and progeny viability of *unc-32 glp-1/+ glp-1;sDf35/sog-4* cross-progeny was examined. (2) For deficiencies maintained over double marker chromosomes (*eDf19*, *nDf25*), homozygous *unc-32 glp-1* strains were constructed that carried the *Df* over the double marker chromosome; hermaphrodites were crossed to *sog;glp-1;him* males, and the fertility and progeny viability of non-Unc *sog/Df;unc-32 glp-1/+ glp-1* cross-progeny were examined. *eDf19/sog-6* animals were distinguished from *unc-24 dpy-20/sog-6* siblings by their small body size and low fertility. *nDf25/sog-1* were distinguished from *unc-13 lin-11/sog-1* siblings by their production of *Df/Df* F_2 embryos. (3) For *ozDf5*, an *ozDf5/ozDf5;nDp4;unc-32 glp-1* strain was constructed and hermaphrodites were crossed to *sog-1;glp-1;him-5* males. Embryo counts indicated that 15% (33/218) of progeny from *ozDf5/ozDf5;nDp4* hermaphrodites die, presumably from loss of *nDp4*. The fertility and progeny viability of non-Unc *ozDf5/sog-1;unc-32 glp-1/+glp-1* cross-progeny were tested; at least 15% of cross-progeny were assumed to have lost *nDp4*. (4) *nDf11* was not tested for suppression of *glp-1* since *sog-10/nDf11* animals could not be recovered.

Distal tip cell ablations: Ablation experiments were done by the method of SULSTON and WHITE (1980) using a laser microbeam system similar to that described by STERNBERG (1988). Prior to ablation, the number of germ cells was counted for later comparison. Animals were maintained at 20° both prior to and after ablation. The distal tip cell was identified by its location at the tip of the developing gonad arm and by its characteristic morphology (KIMBLE and WHITE 1981). Typically, ablations were done in L2 and/or L3 hermaphrodites. Cell death was verified ~2 hr after ablation. Animals were examined ~24 hr later to determine whether germ cells in the operated arm continued to divide. The unoperated arm was used as a control for proper germline growth.

RESULTS

Isolation of *glp-1(ts)* suppressors: We have isolated recessive suppressors of two temperature sensitive (*ts*) alleles of *glp-1*, *glp-1(q224)* and *glp-1(q231)*. *glp-1* is essential for germline proliferation and embryonic viability (AUSTIN and KIMBLE 1987; PRIESS, SCHNABEL and SCHNABEL 1987). Severe *glp-1* mutants produce four to seven germ cells rather than the normal 1000–2000 germ cells. Wild-type hermaphrodites first make ~300 sperm before switching to oogenesis; in contrast, *glp-1* hermaphrodites make only a few sperm because their germ lines are small and therefore they are sterile. In conditional or partial loss of function mutants, the germ line may proliferate and produce some embryos. However, these progeny of homozygous *glp-1* mothers die as embryos. The temperature-sensitive period for the embryonic lethality is from the 4- to 28-cell stage of early embryogenesis.

The molecular defects associated with *glp-1(q224)* and *glp-1(q231)* are now known to be amino acid substitutions within the cytoplasmic portion of the predicted protein (KODOYIANNI, MAINE and KIMBLE 1992). The cytoplasmic domain contains six copies of a sequence motif first described in two yeast genes, *cdc10* and *SWI6* (BREEDEN and NASMYTH 1987; YOCHEM and GREENWALD 1989) and subsequently identified in a number of proteins, including ankyrin (LUX, JOHN and BENNETT 1990). This domain has been shown to be involved in protein-protein interactions (DAVIS and BENNET 1990; DAVIS, OTTO and BENNET 1991; THOMPSON, BROWN and MCKNIGHT 1991; WULCZYN, NAUMANN and SCHEIDEREIT 1992). The *glp-1(q224)* and *glp-1(q231)* mutations are glycine to glutamic acid substitutions at sites 14 amino acids apart within the fourth *cdc10/SWI6* repeat (KODOYIANNI, MAINE and KIMBLE 1992).

Two mutagenesis schemes were used to isolate 13 of the suppressors reported here (Table 1 and see MATERIALS AND METHODS). The two remaining suppressors were isolated by other means (see below and MATERIALS AND METHODS). While *glp-1(ts)* mutants are sterile at both 20° and 25°, they make more germ cells at the lower temperature. The suppressors were selected at 20° in an attempt to isolate a wide constellation of suppressor types; we attempted unsuccessfully to select additional suppressors at 25° that could bypass the requirement for *glp-1* function altogether.

Twelve suppressors (*q294*, *q295*, *q297*, *q298*, *q299*, *q300*, *q301*, *q303*, *q304*, *q305*, *q306*, *q308*) were re-

TABLE 1

Recessive suppressors of *glp-1(ts)* selected at 20°

glp-1(ts)	*sog* allele	Mutation frequency[a]
glp-1(q224)	*sog-1(q295, q345), sog-3(q294), sog-5(q297)*	1/1350
glp-1(q231)	*sog-1(q298, q303, q305, q308, q309), sog-2(q299), sog-4(q301, q304), sog-6(q300, q306)*	1/420[b]

[a] Frequency is given per haploid genome.
[b] One mutation, *q309*, was recovered in a dominant screen (data not shown; to be reported elsewhere) and therefore not included in the frequency calculation.

covered in F_2 selections for fertile hermaphrodites and one more, *q345*, in an F_2 screen for germline-specific suppressors (all at 20°). Upon retesting, *q345* rescued both germline and embryonic phenotypes. An additional unlinked suppressor, *q309*, was recovered in a screen for dominant suppressors that will be reported elsewhere. In tests designed to separate these recessive suppressors from *glp-1(ts)* (see below), we have not been able to detect a phenotype other than suppression of *glp-1*. All 14 recessive suppressors are extragenic (see below); we have designated the genes identified by these mutations to be *sog* genes, for *s*uppressor *o*f ***g*lp-1**. In general, the *sog* mutations were shown to be recessive (see MATERIALS AND METHODS). However, one allele, *q303*, is very weakly semi-dominant at 20° (see MATERIALS AND METHODS).

***sog* mutations are extragenic and exhibit allele-specific interactions:** Both complementation analysis (Table 2A; data not shown) and mapping (Table 3; data not shown) were done by scoring suppression of the *glp-1(ts)* phenotype at 20°. The *sog* mutations fall into overlapping complementation groups, suggesting that alleles of different *sog* genes can interact to suppress *glp-1*. The *sog* mutations have been assigned to six loci based on map position: *sog-1* on LG *I* (seven alleles), *sog-2* on LG *II* (one allele), and *sog-3* on LG *IV* (one allele), *sog-4* on LG *V* (two alleles), *sog-5* on LG *X* (one allele), and *sog-6* on LG *IV* (two alleles) (Table 3; see MATERIALS AND METHODS). Their map positions are summarized in Figure 1.

Comparison of the mapping and complementation data indicates that some alleles of *sog-1* interact with one or more alleles of *sog-2*, *sog-3*, *sog-4*, *sog-5* and *sog-6* (Table 2A). Formally, these alleles act as dominant enhancers of each other. No interactions are seen between alleles of any of the other five *sog* genes (Table 2A). Intergenic noncomplementation may indicate that the *sog-1* product interacts with products of other *sog* genes.

Frequencies with which suppressors were isolated: Recessive suppressors of *glp-1(q224)* were recovered at a frequency of ~1/1,350 haploid genomes and recessive suppressors of *glp-1(q231)* were recovered at a frequency of ~1/420 haploid genomes (Table 1). The typical frequency for loss of function mutations in *C. elegans* under our conditions is 3–4 × 10^{-4} mutations/haploid genome. The relatively high frequency of isolation of *sog-4*, *sog-6* and particularly *sog-1* mutations suggests that simple loss of function may be sufficient for suppression of *glp-1(ts)*. The lower frequency of isolation of *sog-2*, *sog-3* and *sog-5* alleles

TABLE 2

Complementation analyses

A. Complementation analysis of *sog* mutations isolated in recessive screen.

sog-1							*sog-2*	*sog-3*	*sog-4*		*sog-5*	*sog-6*		
295	*298*	*303*	*305*	*308*	*309*	*345*	*299*	*294*	*301*	*304*	*297*	*300*	*306*	
–	–	–	–	–	–	–	+	+	+	–	+	–	+	*295*
	–	–	–	–	–	–	–	+	+	–	+	–	+	*298*
		–	–	–	–	–	+	+	+	+	+	–	+	*303*
			–	–	–	–	+	+	+	+	+	+	+	*305 sog-1*
				–	–	–	+	+	+	+	+	–	+	*308*
					–	–	+	–	–	–	–	–	–	*309*
						–	–	+	+	+	+	–	+	*345*
							–	+	+	+	+	+	+	*299 sog-2*
								–	+	+	+	+	+	*294 sog-3*
									–	–	+	+	+	*301 sog-4*
										–	+	+	+	*304*
											–	+	+	*297 sog-5*
												–	–	*300 sog-6*
													–	*306*

B. Complementation analysis of *sog-10(q162)* and other *sog* mutations.

	sog-1(q298)	*sog-1(q309)*	*sog-2(q299)*	*sog-3(q294)*	*sog-4(q304)*	*sog-5(q297)*	*sog-6(q306)*
q162	+	+	+	+	ND	+	+

"–", mutations failed to complement and *glp-1(q231)* was suppressed; "+", mutations complemented and *glp-1(q231)* was not suppressed. ND, not done.

TABLE 3

Three-factor mapping of *sog* mutations

Suppressor	Parental genotype	Recombinant phenotype	Recombinant genotype	No. of recombinants[a]
sog-1[b]	*dpy-5 unc-13/sog-1; glp-1*	Dpy	*dpy-5 sog-1; glp-1*	31/33
			dpy-5; glp-1	2/33
		Unc	*sog-1 unc-13; glp-1*	1/40
			unc-13; glp-1	39/40
sog-1[c]	*unc-13 gld-1/sog-1; glp-1*	Unc	*unc-13 sog-1; glp-1*	6/6
			unc-13; glp-1	0/6
	unc-13 lin-10/sog-1; glp-1	Unc	*unc-13 sog-1; glp-1*	2/7
			unc-13; glp-1	5/7
		Lin	*sog-1 lin-10; glp-1*	7/13
			lin-10; glp-1	6/13
sog-2	*unc-4 rol-1/sog-2; glp-1*	Unc	*unc-4 sog-2; glp-1*	0/4
			unc-4; glp-1	4/4
		Rol	*sog-2 rol-1; glp-1*	4/5
			rol-1; glp-1	1/5
sog-3	*unc-5 dpy-20/sog-3; glp-1*	Unc	*unc-5 sog-3; glp-1*	3/9
			unc-5; glp-1	6/9
		Dpy	*sog-3 dpy-20; glp-1*	6/10
			dpy-20; glp-1	4/10
sog-4[d]	*dpy-11 unc-42/sog-4; glp-1*	Dpy	*dpy-11 sog-4; glp-1*	18/27
			dpy-11; glp-1	9/27
		Unc	*sog-4 unc-42; glp-1*	3/15
			unc-42; glp-1	12/15
sog-5	*lon-2 unc-18/sog-5; glp-1*	Lon	*lon-2 sog-5; glp-1*	3/17
			lon-2; glp-1	14/17
		Unc	*sog-5 unc-18; glp-1*	8/11
			unc-18; glp-1	3/11
sog-6[e]	*unc-5 dpy-20/sog-6; glp-1*	Unc	*unc-5 sog-6; glp-1*	12/17
			unc-5; glp-1	5/17
		Dpy	*sog-6 dpy-20; glp-1*	4/19
			dpy-20; glp-1	15/19

[a] Number of recombinants of a particular class/total number of recombinants picked.
[b] Composite data for all seven *sog-1* alleles (*q295*, *q298*, *q303*, *q305*, *q308*, *q309*, *q345*).
[c] Composite data for two *sog-1* alleles (*q295*, *q298*).
[d] Composite data for both *sog-4* alleles (*q301*, *q304*).
[e] Composite data for both *sog-6* alleles (*q300*, *q306*).

suggests that they may not be simple loss of function mutations. In general, it appears that a greater variety of mutations is able to suppress the more weakly mutant allele, *glp-1(q231)*, than the stronger allele, *glp-1(q224)*. We think it unlikely that we have saturated the genome for *sog* genes because for three of them only one allele has been recovered.

Further characterization of *glp-1* suppression: We next examined the strength of each *sog* suppressor. To this end, we assayed the extent of germline proliferation by determining the average brood and percent fertility of *glp-1(ts);sog* hermaphrodites. In addition, we assayed the degree of embryonic viability by determining the percent of progeny that hatch and reach adulthood.

The range of strengths of suppression in the germ line: To facilitate comparisons between different *sog* alleles, each one was tested in a *glp-1(q231)* background (Table 4). In general, *glp-1(q231)* was more completely suppressed than was *glp-1(q224)* (data not shown). The ability of suppressors of *glp-1(q224)* to rescue *glp-1(q231)* indicates they are not allele-specific (although they do not suppress all *glp-1* mutations, see below). In general, *sog* mutations only partially suppress the *glp-1* germline defect. At best, proliferation is increased to give brood sizes that are ~60% of wild type (at 20°). For most *sog;glp-1(q231)* combinations, >90% of hermaphrodites make viable progeny (Table 4).

The range of strengths of suppression in the embryo: Progeny viability varies from 20–99% in different *sog; unc-32 glp-1(q231)* strains at 20° (Table 4). The ability of a given *sog* allele to suppress embryonic lethality does not correlate with its ability to restore germline proliferation. Most notably, *sog-6(q300)* rescues 24% of progeny although it does not measurably increase proliferation.

Tests for suppression of other *glp-1* alleles by *sog* mutations: One can ask a number of questions about the interactions between *sog* mutations and nontemperature sensitive alleles of *glp-1*. First, do *sog* mutations bypass the requirement for *glp-1* function? Sec-

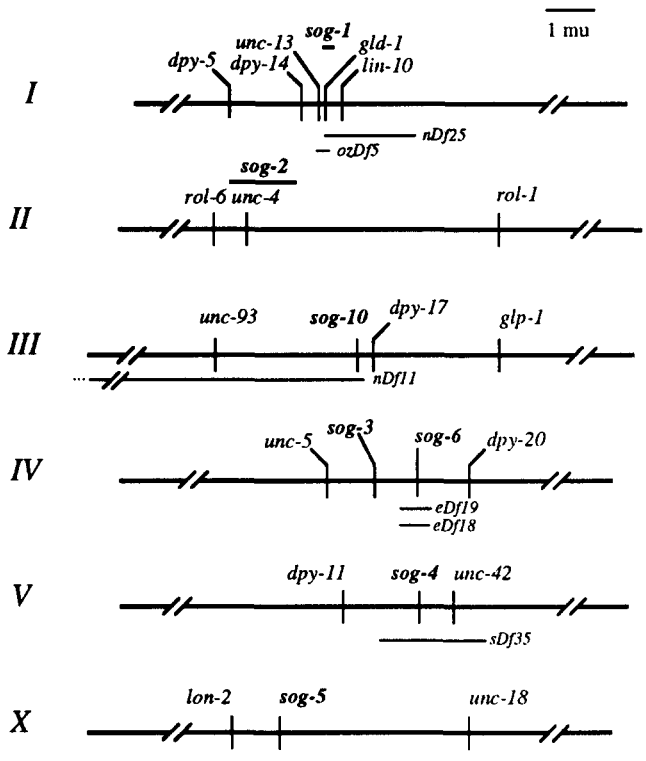

FIGURE 1.—Map positions of the *sog* genes, the marker genes used to map them, and *glp-1*.

TABLE 4

Suppression of *glp-1(q231)* by *sog* mutations at 20°

Suppressor	% ♀ producing viable progeny (*n*)[a]	Average no. embryos/♀ (*n*)[b]	% viable progeny[c]
—	0 (>200)	27 ± 4.5[d]	0
sog-1(q295)	>99 (257)	222 ± 14 (5)	56
sog-1(q298)	98 (61)	137 ± 15 (20)	49
sog-1(q303)	>99 (115)	145 ± 7 (3)	76
sog-1(q305)	>99 (74)	111 ± 10 (9)	20
sog-1(q308)	>99 (54)	182 ± 8 (10)	99
sog-1(q309)	>99 (91)	85 ± 25 (6)	95
sog-1(q345)	98 (65)	170 ± 10 (11)	93
sog-2(q299)	88 (82)	106 ± 6 (10)	36
sog-3(q294)	>99 (288)	171 ± 3 (5)	91
sog-4(q301)	84 (51)	41 ± 4 (10)	50
sog-4(q304)	98 (62)	122 ± 8 (9)	63
sog-5(q297)	>99 (332)	197 ± 7 (5)	75
sog-6(q300)	59 (59)	11 ± 6 (10)	24
sog-6(q306)	>99 (50)	78 ± 7 (10)	34

Genotype of all animals tested is *glp-1(q231); sog(x)*.

[a] Since ~98% of *glp-1(q231)* hermaphrodites make (inviable) progeny at 20°, a measure of *sog(x); glp-1(q231)* fertility at this temperature is not informative. Instead, we report the percentage of animals that actually make viable offspring.

[b] Standard errors are given. *n*, number of broods assayed to determine average number of embryos produced per hermaphrodite.

[c] Percentage of progeny to develop to at least L3.

[d] From MAINE and KIMBLE (1989).

ond, do they suppress partial loss of *glp-1* gene function in general or are they specific for *glp-1(q224)* and *glp-1(q231)*? Third, will they suppress only certain types of non-*ts* partial loss of function alleles?

TABLE 5

Tests for suppression of non-*ts* *glp-1* alleles by *sog* mutations

	glp-1(q50)[a] % fertile ♀ (*n*)		*glp-1(q35)*[b] % fertile ♀ (*n*)	
Suppressor	*sog(+)*	*sog(−)*	*sog(+)*	*sog(−)*
sog-1(q298)	15 (164)	11 (192)	55 (192)	100 (59)
sog-10(q162)	15 (164)	8 (118)	55 (192)	100 (81)

Tests were done at 20° unless otherwise noted. *sog-1(q298)* was marked with *dpy-14*; *sog-10* was marked with *dpy-17*.

[a] *n*, number of ovotestes scored. *glp-1(q50)* was marked with *unc-36(e873)*. *sog-1* test was done at 15° because the marked combination (*unc-36* and *dpy-14*) has a synthetic lethal phenotype at 20°. Individual ovotestes were scored (using DIC optics) because *glp-1(q50)* hermaphrodites often have one Glp and one wild type ovotestis. Progeny produced by *glp-1(q50); sog(x)* animals die at or before L1.

[b] *n*, number of germ lines scored. *glp-1(q35)* was marked with *unc-32*. Progeny produced by *glp-1(q35); sog(x)* animals die at or before L1.

A z-test [FREUND (1973)] indicates that both the *sog-1(q298); glp-1(q35)* and *sog-10(q162); glp-1(q35)* double mutants are significantly different from *glp-1(q35)* alone ($P < 0.05$), but the *sog-1(q298); glp-1(q50)* and *sog-10(q162); glp-1(q50)* double mutants are not significantly different from *glp-1(q50)* alone.

To address the first question, we tested representative alleles of several *sog* genes for suppression of an allele with a null phenotype, *glp-1(q158)* (AUSTIN and KIMBLE 1987). Suppression of *glp-1(q158)* was not seen in any case (data not shown).

To address the second and third questions, we focused on *sog-1*, using *sog-1(q298)* as a representative allele. We tested for suppression of *glp-1(q35)* and *glp-1(q50)* (AUSTIN and KIMBLE 1987; MANGO, MAINE and KIMBLE 1991) (see MATERIALS AND METHODS). Some germline proliferation can occur in each of these *glp-1* single mutants; however, their progeny always die as embryos. In *glp-1(q35)* mutants, germline proliferation is often intermediate between wild type and a severely mutant phenotype. *glp-1(q35)* contains a nonsense mutation within the cytoplasmic portion of the protein and is predicted to encode a slightly truncated protein (MANGO, MAINE and KIMBLE 1991). In *glp-1(q50)* mutants, proliferation is either wild type *or* severely mutant (AUSTIN and KIMBLE 1987; KODOYIANNI, MAINE and KIMBLE 1992). *glp-1(q50)* contains a missense mutation within the extracellular portion of the predicted protein (KODOYIANNI, MAINE and KIMBLE 1992). The *glp-1(q35)* germline defect was suppressed by *sog-1(q298)* (Table 5) but the embryonic phenotype was not. Germline proliferation is extensive enough to produce embryos in 100% of *glp-1(q35);sog-1(q298)* animals while only 55% of their *sog-1(q298/+)* or *sog-1(+)* siblings produce embryos. In contrast, *sog-1(q298)* has no significant suppressive effect on *glp-1(q50)* (Table 5).

Test for maternal suppression by *sog-1*: The embryonic lethality of *glp-1* is strictly maternal: progeny of *glp-1(−/−)* mothers die even if those progeny are

TABLE 6

Suppression of *glp-1* embryonic lethality by *sog-1* in the mother and/or zygote (20°)

Parental genotype	Average no. embryos/♀[a] (*n*)	% viable progeny[b]
sog-1(q298); glp-1(q231)	149 ± 8 (11)	56
sog-1(q298); glp-1(q231) × *sog-1(+); glp-1(q231)* ♂	113 (10)	46
sog1(q298/+); glp-1(q231)	25 ± 7 (29)	<1

[a] *n*, number of hermaphrodites whose progeny were counted. Standard errors are given for broods of self progeny. For cross progeny, the standard error was not calculated because matings were done *en masse*, and therefore individual brood sizes are not known.

[b] Percentage of progeny to develop to at least L3 stage.

glp-1(+/−) (AUSTIN and KIMBLE 1987; PRIESS, SCHNABEL and SCHNABEL 1987). We asked whether it is the maternal or embryonic *sog-1* genotype that rescues the *glp-1(q231)* embryonic lethality. Progeny of *sog-1; glp-1* mothers are rescued regardless of whether the embryos themselves are *sog-1(+/−)* or *sog-1(−/−)* (Table 6). In contrast, progeny of *sog-1(+/−); glp-1(ts)* mothers are not rescued, even when the progeny themselves are *sog-1(−/−)*. These results indicate that suppression of *glp-1(ts)* by *sog-1* depends on the genotype of the mother rather than that of the embryo.

A germline-specific suppressor of *glp-1* also has a feminized phenotype: One suppressor, *q162*, arose in a noncomplementation screen for new *glp-1* alleles (AUSTIN 1989 and see MATERIALS AND METHODS). In contrast to the *glp-1(q224)* and *glp-1(q231)* suppressors recovered in our screens, *q162* suppresses only the germline phenotype of *glp-1* (see below and Table 8). We have designated the gene identified by *q162* as *sog-10*. (*sog-7*, *sog-8* and *sog-9* have been identified and studied by J. PRIESS and A.-M. HOWELL; see DISCUSSION.)

sog-10(q162) has a cold-sensitive (*cs*) feminized germline (Fog) phenotype. At 10°, ~86% of *sog-10(q162)/sog-10(q162) XX* animals are female and ~14% are self-fertile hermaphrodites (Table 7A). At 15°, 50% of *XX* animals are female, and at 20° fewer than 1/200 animals are female (Table 7A). Feminization is weakly semidominant: if *sog-10(q162)* males are mated to hermaphrodites [marked with *unc-1(e719)* to allow detection of cross-progeny] and their offspring are raised at 10°, 21% of *sog-10(q162)/+; unc-1/+* animals are female (Table 7A). The cold sensitive period of *sog-10(q162)* falls during L1 and early L2 (Figure 2, and see MATERIALS AND METHODS), suggesting that the requirement for *sog-10* in hermaphrodite sperm production is during this time.

Germline-specific suppressor *sog-10(q162)* maps to LG *III*: *sog-10(q162)* lies ~0.3 m.u. to the left of *dpy-17* (Table 7B), close to a gene known to interact with *lin-12*, *sel-2* (suppressor and/or enhancer of *lin-12*) (G. SEYDOUX and I. GREENWALD, unpublished data). The *glp-1* and *lin-12* genes share overall structural organization and are ~60% identical at the amino acid level (YOCHEM and GREENWALD 1989; AUSTIN and KIMBLE 1989); in addition, they appear to be functionally redundant early in development (LAMBIE and KIMBLE 1991a). Complementation tests were done to determine whether *q162* might be an allele of *sel-2*. At both 15° and 20°, *sel-2(n655)* fully complements *q162* for the Fog phenotype (data not shown). Furthermore, *q162* does not enhance or suppress the phenotype of *lin-12(q269)* animals (as does *sel-2*): neither the egg-laying nor the sterile defects of *lin-12(q269)* were altered in *q162 dpy-17(e164) lin-12(q269)* animals (data not shown). Therefore, *q162* appears to be distinct from *sel-2*.

***sog-10(q162)* complements mutations in other *sog* genes:** We tested for functional interactions between *sog-10(q162)* and mutations in other *sog* genes by characterizing the *glp-1(ts)* phenotype in double heterozygous animals [*i.e.*, *sog-x/+;sog-10(q162) glp-1(ts)/+glp-1(ts)*]. One allele of each other *sog* gene (except *sog-4*) was tested, and in no case were viable, fertile offspring produced (Table 2B).

Characterization of suppression by *sog-10(q162)*: In contrast to the suppressors generated using a *glp-1(ts)* mutation, *sog-10(q162)* arose in a strain containing *glp-1(q35)* (AUSTIN 1989). We reexamined its suppression of *glp-1(q35)* as well as testing its ability to rescue other *glp-1* mutations, *glp-1(q50)*, *glp-1(q158)* and *glp-1(q224)* (Tables 5 and 8).

Germline proliferation and embryonic viability in q162 glp-1(q224) animals: For comparison with the germline and embryonic suppressors, we characterized the *sog-10(q162);glp-1(q224)* phenotype (Table 8). At 20°, *sog-10(q162)* is a recessive suppressor of the *glp-1(q224)* germline defect: *sog-10(q162) glp-1(q224)* animals produce an average brood of 118 embryos; at 25°, no embryos are produced (Table 8). The *glp-1* embryonic phenotype is not suppressed by *sog-10*: all offspring of *sog-10(q162) glp-1(q224)* hermaphrodites at 20° die as embryos (Table 8).

Does sog-10(q162) rescue a glp-1 null mutation? To address this question, we tested *sog-10(q162)* for suppression of a *glp-1* allele with a null phenotype, *glp-1(q158)* (AUSTIN and KIMBLE 1987). No suppression of *glp-1(q158)* was seen (data not shown).

What effect does sog-10(q162) have on partial loss function mutations in glp-1? We examined the interactions between *sog-10(q162)* and two partial loss of function alleles, *glp-1(q35)* and *glp-1(q50)* (described above; see MATERIALS AND METHODS). As originally seen when it was isolated (AUSTIN 1989), the germline phenotype of *glp-1(q35)* was suppressed by *sog-10(q162)*. At 20°, germline proliferation was restored sufficiently to allow oocyte production in 100% of the *sog-10(q162) glp-1(q35)* hermaphrodites examined

TABLE 7

Phenotypic analysis and mapping of germline-specific suppressor *q162*

A. Phenotypic analysis			*XX* progeny		
Parental genotype	Progeny genotype	Temp. (°C)	% Wt	% Fem	*N*
sog-10(q162)	*sog-10(q162)*	10	14.5	85.5	102
		15	50	50	884
		20	100	<0.5	>200
dpy-17; unc-1 × *sog-10(162)* ♂	*dpy-17/sog-10(q162); unc-1/+*	10	79	21	222
		15	99.6	0.4	120

B. Mapping			
Parental genotype	Recombinant phenotype	Recombinant genotype	No. of recombinants[a]
unc-93 dpy-17/sog-10(q162)	Unc	*unc-93 sog-10(q162)*	2/13
		unc-93	11/13
	Dpy	*dpy-17 sog-10(q162)*	1/18
		dpy-17	17/18

Fem, feminized phenotype; wt, wild type. *N*, number of animals examined.
[a] The number of recombinants of a particular class is indicated as a proportion of the total number of recombinants picked.

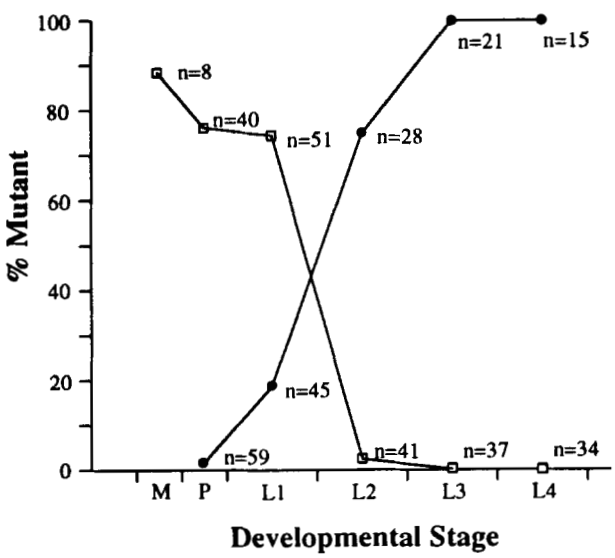

FIGURE 2.—Temperature-sensitive period of the *sog-10(q162)* Fog phenotype. The percent mutant animals is plotted as a function of the developmental stage when individuals were temperature shifted. Staged *sog-10(q162)* animals were shifted from 12° (restrictive temperature) up to 25° (permissive temperature) or from 25° down to 12°. Upshift, closed circles; downshift, open squares; n, number of individuals shifted and scored at a given developmental stage; M, midembryogenesis; P, pretzel stage; L1, first larval stage, L2, second larval stage; L3, third larval stage; L4, fourth larval stage.

(Table 5). In contrast, *glp-1(q50)* was not suppressed by *sog-10(q162)* (Table 5).

Analysis of *sog/Df* phenotype: To determine whether *sog* mutations cause a loss or gain of gene function, we examined their phenotype over a deficiency (see MATERIALS AND METHODS). We first looked for a novel visible phenotype in *sog/Df;glp-1(+)* animals. In addition, we examined the fertility and embryonic viability of *sog/Df;glp-1(q231)* hermaphrodites. If a *sog* mutation causes a reduction in gene function, then *sog/Df* should suppress *glp-1* at least as well as does *sog/sog*; in contrast, if a *sog* mutation causes a gain of function, then *sog/Df* should not suppress *glp-1* as well as *sog/sog*, if at all. We tested four genes, *sog-1*, *sog-4*, *sog-6*, and *sog-10*, for which deficiencies currently exist.

TABLE 8

Suppression of *glp-1(ts)* by germline-specific suppressor *sog-10(q162)*

Genotype	Temp. (°C)	No. of progeny/ ♀[a] (*n*)	% viable progeny
glp-1(q224)	25	0	NA
sog-10(q162) glp-1(q224)	25	0 (>100)	NA
glp-1(q224)	20	0	NA
sog-10(q162) glp-1(q224)	20	118 ± 31[b] (8)	0
sog-10(q162) glp-1(q224)/sog-10(+) glp-1(q224)	20	0 (30)	NA

NA, not applicable.
[a] *n*, number of hermaphrodites whose broods were counted. Standard error is given.
[b] One hermaphrodite was partially feminized, producing only six embryos and then oocytes.

Visible phenotypes of sog/Df: The *sog/Df* transheterozygotes differ in their phenotypes: the visible phenotypes of *sog-1* and *sog-4* are no more severe over a *Df* (*i.e.*, they have no obvious abnormality) whereas *sog-6/Df* and *sog-10/Df* animals have additional visible phenotypes not seen in *sog/sog* animals (Table 9A and see below). These results suggest that the *sog-6* and *sog-10* mutations are not null alleles of their respective genes whereas the *sog-1* and *sog-4* alleles may be null (but see below). *sog-6(q306)/eDf19* hermaphrodites are small and sterile or weakly fertile (brood size <20), often with underproliferative germ lines and morphologically abnormal oocytes; similarly, *sog-6(q306)/eDf18* and *sog-6(q300)/eDf18* hermaphrodites are sterile or have reduced brood sizes and are small, thin and die prematurely. *sog-10/nDf11* animals may be inviable, since we were unable to recover them. These

TABLE 9

Phenotypes of *sog/Df* transheterozygotes

sog gene[a]	Genotype	Phenotype[b]	*N*
A. Novel phenotype in a *glp-1(+)* background			
sog-1	*sog-1/ozDf5*	+	>10
	sog-1/ozDf5; nDp4	+	>10
	sog-1/nDf25	+	10
	sog-1/unc-13 lin-11	+	3
sog-4	*sog-4/sDf35*	+	4
	sog-4/eT1	+	10
sog-6	*sog-6/eDf19*	Semi-sterile	9
	sog-6/unc-24 dpy-20	+	23
	sog-6/eDf18	Semi-sterile	8
	sog-6/unc-24 dpy-20	+	21
sog-10	*sog-10/nDf11*	(Inviable)	0
	sog-10/unc-79 dpy-17	+	11
B. Suppression phenotype in a *glp-1(q231)* background			
sog-1	*sog-1/ozDf5; glp-1*	No suppression	6
	sog-1/nDf25; glp-1	No suppression	18
	nDf25/unc-13 lin-11; glp-1	No suppression	10
sog-4	*sog-4/sDf35; glp-1*	No suppression	15
	sDf35/+; glp-1	ND	
sog-6	*sog-6/eDf19; glp-1*	No suppression	6
	eDf19/dpy-20 unc-24; glp-1	No suppression	10

All tests were done at 20°. *N*, number of animals examined; ND, not done.

[a] *sog-1(q295)*, *sog-1(q298)*, *sog-4(q304)* and *sog-6(q306)* were used in all appropriate tests in parts A. and B; *sog-6(q300)* was only tested over *eDf18* in part A.

[b] See text for more complete description of phenotypes. +, wild type.

results suggest a vital function for both *sog-6* and *sog-10*. In each case, the *sog/Df* phenotype presumably results from a lack of *sog* gene activity, but it could also arise from intergenic interactions between the *sog* gene and either a haplo-insufficient locus that is also uncovered by the *Df* or a second mutation on the *sog* chromosome that is also uncovered by the *Df*. We think the latter possibility is unlikely, since the mutagenized chromosomes have been multiply crossed to wild type (N2).

Suppression phenotypes of sog/Df: For *sog-1*, *sog-4* and *sog-6*, the *Df/sog* heteroallelic combination does not suppress *glp-1(q231)* at 20° (Table 9B). This result is not surprising for *sog-6* given the *sog-6/Df* sterile phenotype in a *glp-1(+)* background. Neither *sog-1* nor *sog-6* appears to be haploinsufficient for suppression since a *Df/+* phenotype does not suppress *glp-1(q231)* (Table 9B). These results indicate that the *sog* mutations tested are not null (assuming that the deficiencies used are contiguous and indeed remove the expected *sog* gene). Instead, they appear to be recessive gain of function mutations. Because they must be present in two doses to be effective, they do not simply cause an elevated level of normal *sog* activity.

Do *glp-1* suppressors bypass the requirement for a distal tip cell? Mitosis in the wild-type germline depends on a somatic cell, the distal tip cell, located at the distal tip of the gonad (KIMBLE and WHITE 1981) (Figure 3). Genetic mosaic, molecular and immunocytochemical analyses of *glp-1* indicate that it encodes a membrane-associated protein in the distal germ line (AUSTIN and KIMBLE 1987, 1989; YOCHEM and GREENWALD 1989; S. CRITTENDEN, E. TROEMEL and J. KIMBLE, unpublished data). One simple model is that the distal tip cell signals the germ line, via *glp-1* protein, to continue mitosis (or prevent meiosis). Suppressors of *glp-1* loss of function mutations that act at the level of *glp-1* or downstream might render the putative distal tip cell signal unnecessary. To test whether any of the suppressors described above have this effect, the distal tip cell was ablated in one gonad arm of developing hermaphrodite larvae, and subsequent germline development was observed (see MATERIALS AND METHODS). The unoperated gonad arm in each animal served as an internal control.

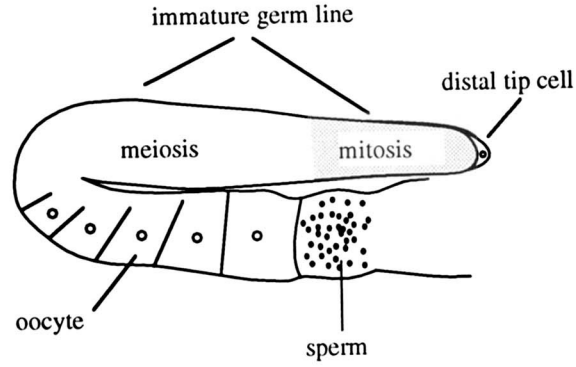

FIGURE 3.—Schematic drawing of one arm of the hermaphrodite gonad. Immature germ cells are located in the distal region: mitotic cells are present in the vicinity of the distal tip cell and meiotic cells are located more proximally. Cells in the loop region are visibly undergoing gametogenesis. Mature gametes are found in the most proximal region.

We ablated distal tip cells in animals homozygous for *sog-1(q298, q303, q305, q308, q309, q345)*, *sog-2(q299)*, *sog-4(q304)*, *sog-6(q306)* or *sog-10(q162)*. *sog-1(q298)* and *sog-10(q162)* were examined in *glp-1(+)* as well as *glp-1(−)* animals; other *sog* mutations were tested in only a *glp-1(−)* background (see MATERIALS AND METHODS). We reasoned that the *glp-1* background should be irrelevant in this experiment because we were testing whether a gene acting downstream of *glp-1* might be constitutively expressed. Germ cell proliferation was assayed by counting the number of germ cells before and after distal tip cell ablation. In each case, germline proliferation in the operated gonad arm stopped after ablation of its distal tip cell while proliferation continued in the intact gonad arm (data not shown). Hence, none of the tested suppressor mutations bypasses the requirement for a distal tip cell in the process of germline mitosis.

DISCUSSION

The *C. elegans glp-1* gene functions in formation of the pharynx and hypodermis in the early embryo and

in proliferation of the germ line (AUSTIN and KIMBLE 1987; PRIESS, SCHNABEL and SCHNABEL 1987). In this paper we describe recessive suppressors of both embryonic and germline defects of *glp-1* as well as one that appears to be specific for the germline defect. These recessive suppressors, designated *sog* mutations, are relatively weak and are extragenic. They define a group of seven genes, *sog-1* (LG *I*), *sog-2* (LG *II*), *sog-3* (LG *IV*), *sog-4* (LG *V*), *sog-5* (LG *X*), *sog-6* (LG *IV*) and *sog-10(q162)* (LG *III*). With the exception of *sog-10(q162)*, the *sog* mutations have no obvious visible phenotype of their own and show a complex pattern of intergenic interactions. *sog-10(q162)* is unique in that it appears to be germline-specific and has a Fog phenotype.

Our genetic analysis of the interactions between *glp-1* and the *sog* mutations (except for *sog-10*) allows us to draw a number of conclusions. First, each suppressor must affect a process that is common to *glp-1* germline and embryonic functions because both embryonic and germline *glp-1* defects are suppressed. Second, the suppressors do not bypass a requirement for *glp-1* gene function: *sog* mutations do not rescue a null allele of *glp-1*, and they are more effective at a moderate temperature (20°) than a more stringent temperature (25°). Instead, the suppressors most likely allow disabled *glp-1* product (from a *ts* or partial loss of function allele) to act more efficiently. Third, the suppressors do not bypass a requirement for distal tip cell function because they do not obviate the need for a distal tip cell in germline proliferation. Fourth, suppressor mutations in at least three genes, *sog-1*, *sog-4* and *sog-6*, appear to be recessive gain of function mutations. Therefore, these genes act (in a genetic sense) as positive regulators of *glp-1*. Consistent with this notion, it is very difficult to recover transposon-induced *sog* mutations; such mutations tend to eliminate gene function (E. MAINE, unpublished data).

It is intriguing that most *sog* mutations have no phenotype in the presence of *glp-1(+)*. Suppressors without a visible phenotype have been isolated for a number of other *C. elegans* genes, including *pha-1* (defective *pha*rynx development; SCHNABEL, BAUER and SCHNABEL 1991) and *lin-12* (SUNDARAM and GREENWALD 1993), as well as for various genes in yeast (BOTSTEIN and MAURER 1982; MOIR *et al.* 1982; NOVICK, OSMOND and BOTSTEIN 1989) and *Chlamydomonas* (DUTCHER, GIBBONS and INWOOD 1988; LUX and DUTCHER 1991). In addition, alleles of three genes (*sog-7*, *sog-8*, *sog-9*) that have been isolated as suppressors of *glp-1(e2142)*, a *glp-1(ts)* allele with only the embryonic mutant phenotype, have no visible phenotype of their own (A.-M. HOWELL and J. PRIESS, unpublished data). At least three explanations can be proposed for the lack of a visible phenotype. It is possible that *sog* mutations are weak or unusual alleles of genes with a visible null phenotype. Indeed, dosage data suggest that the mutations in at least three genes, *sog-1*, *sog-4* and *sog-6*, are not null alleles. Alternatively, some *sog* genes may share partial functional redundancy with each other or with other (unmutated) genes; a visible phenotype might be seen only in an animal that is mutant for two or more of these redundant genes. However, some double *sog* mutants have been examined (*e.g.*, *sog-1;sog-3*), and no novel phenotype has been seen (E. MAINE, unpublished data). Finally, some *sog* genes may have a wild-type null phenotype.

Some alleles of *sog-1* fail to complement specific alleles of *sog-2*, *sog-3*, *sog-4*, *sog-5* and *sog-6* for suppression of *glp-1(q231)*. This intergenic noncomplementation may be interpreted in at least two ways. First, the *sog* genes may act at different points in a regulatory or biochemical pathway. In a double heterozygote, the altered level of functional gene product at two points in the pathway may be sufficient to suppress *glp-1*. In this case, the allele-specificity of *sog* interactions would indicate that different alleles alter activity to differing degrees. Second, *sog* gene products may physically interact with each other. Studies in *Drosophila* (FULLER 1986; FULLER *et al.* 1989; HAYS *et al.* 1989) and yeast (STEARNS and BOTSTEIN 1988) have shown that some mutations in different (α and β) but interacting tubulins fail to complement each other.

The action of *sog-10(q162)* may be fundamentally different from that of the other suppressors: *sog-10(q162)* may affect a process that is specific to *glp-1* germline function and not involved in *glp-1* embryonic function. Consequently, *sog-10(q162)* may suppress *glp-1* by a different mechanism than that of mutations in the other *sog* genes. Alternatively, it is possible that suppression by *q162* is simply too weak to have any effect on embryonic viability. However, this possibility seems unlikely because many alleles of the other *sog* genes rescue germline proliferation less extensively than does *sog-10(q162)* at 20°, yet a substantial fraction of their progeny survive (compare Tables 4 and 8). Like the other *sog* mutations, *sog-10(q162)* bypasses neither a requirement for *glp-1* function nor for the distal tip cell.

The apparent inability of *sog-10/Df* animals to survive suggests that *sog-10* has a lethal null phenotype and therefore an essential function. Clearly, a careful characterization of the putative lethal phenotype is required before we can determine that function. Furthermore, the Fog phenotype of *sog-10(q162)* is suggestive of a connection in the germ line between sex determination and proliferation. Genetic analyses of *fog-1*, a gene required for sperm production, and *glp-1* have suggested that germ cells choose between spermatogenesis and oogenesis at approximately the time when they enter meiosis (BARTON and KIMBLE

1990). Indeed, one gene, *gld-1*, is known to be involved in both proliferation and sex determination in the germ line (T. SCHEDL, unpublished data). In addition, mutations in *fem-1* and *fem-2*, two genes required for production of sperm in hermaphrodites and males as well as a male soma, interact with mutations in *glp-1* (J. AUSTIN, T. EVANS, E. MAINE and J. KIMBLE, unpublished data). It is intriguing that both *glp-1* and *fem-1* contain the *cdc10/SWI6*/ankyrin protein-protein interaction motif. Perhaps both genes interact with a common regulatory factor. While coordinate regulation of sex determination and the mitotic/meiotic decision may seem surprising, it is reminiscent of the connection between mating type switching and the cell cycle in yeast (see reviews by HERSKOWITZ 1989; HORVITZ and HERSKOWITZ 1992).

Suppressors of *glp-1* were generated as a means of identifying other genes involved in the cell-signaling events mediated by *glp-1*. The *glp-1* gene has been shown by genetic, molecular and immunocytochemical analyses to encode a plasma membrane-associated protein in the germline (AUSTIN and KIMBLE 1987, 1989; YOCHEM and GREENWALD 1989; S. CRITTENDEN, E. TROEMEL and J. KIMBLE, unpublished data). Suppressors may act by altering the function and/or level of other components of the cell-signaling system or by altering the level and/or pattern of *glp-1* gene expression. In addition, they may be informational suppressors, of which two types have been characterized in *C. elegans*. Both types rescue specific alleles of many genes and they include *smg* (*s*uppressor with *m*orphological effect on *g*enitalia) mutations that apparently stabilize mutant mRNAs (HODGKIN *et al.* 1989) and suppressor tRNAs that allow readthrough of nonsense mutations (WILLS *et al.* 1983). We feel it unlikely that *sog* mutations are *smg* mutations because they do lack the requisite morphological defects. Furthermore, *smg-1* suppresses the embryonic phenotype of *glp-1(q35)* (MANGO, MAINE and KIMBLE 1991) while the *sog* mutations do not. In addition, *sog-1* complements *smg-1* (also located in the cluster on LG *I*) for the Smg phenotype (E. MAINE, unpublished data).

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