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Identification of genes that interact with *glp-1*, a gene required for inductive cell interactions in *Caenorhabditis elegans*

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Summary

The *glp-1* gene functions in two inductive cellular interactions and in development of the embryonic hypodermis of *C. elegans*. We have isolated six mutations as recessive suppressors of temperature-sensitive (*ts*) mutations of *glp-1*. By mapping and complementation tests, we found that these suppressors are mutations of known *dumpy* (*dpy*) genes; *dpy* genes are required for development of normal body shape. Based on this result, we asked whether mutations previously isolated in screens for mutants defective in body shape could also suppress *glp-1(ts)*. From these tests, we learned that unselected mutations of eight genes required for normal *C. elegans* morphogenesis, including the four already identified, suppress *glp-1(ts)*. All of these suppressors rescue all three mutant phenotypes of *glp-1(ts)* (defects in embryonic induction of pharyngeal tissue, in embryonic hypodermis development, and in induction of germline proliferation). However, they do not rescue putative *glp-1* null mutants and therefore do not bypass the requirement for *glp-1* in development. In the light of current ideas about the molecular nature of the *glp-1* and suppressor gene products, we propose an interaction between the *glp-1* protein and components of the extracellular matrix and speculate that this interaction may impose spatial constraints on the decision between mitosis and meiosis in the germline.

Key words: *Caenorhabditis elegans*, extragenic suppressor *glp-1*, cell interaction, temperature-sensitive mutant.

Introduction

Communication between cells is crucial in the development of multicellular organisms. Inductive interactions, in which cells of one tissue regulate cell fate in a second tissue, are particularly important to the development of whole structures and tissues (e.g. Spemann & Mangold, 1924). However, the molecular mechanism(s) by which inductive interactions are mediated remain unknown. Recently, important clues have been obtained using both biochemical and genetic approaches. In *Xenopus laevis*, specific growth factors that influence embryonic induction have been identified in *in vitro* assays (Kimmelman & Kirshner, 1987; Smith, 1987; Slack *et al.* 1987). And in the nematode *Caenorhabditis elegans*, a gene required for two inductive events has been identified by mutation (Austin & Kimble, 1987; Priess *et al.* 1987); this gene is called *glp-1* (for *g*erm*l*ine *p*roliferation defective).

During *C. elegans* development, *glp-1* functions in two distinct inductive interactions. In embryogenesis, descendants of one blastomere (P_1) induce descendants of a different blastomere (AB) to generate pharyngeal tissue (Priess & Thompson, 1987) by a mechanism that requires maternal *glp-1* gene function (Priess *et al.* 1987; Austin & Kimble, 1987). Similarly, in postembryonic development, two somatic cells, the distal tip cells, stimulate germ cells to proliferate (Kimble & White, 1981) by a mechanism that requires *glp-1* gene function (Austin & Kimble, 1987; Priess *et al.* 1987). In the absence of either the relevant cell interaction or *glp-1* gene function, AB does not produce pharyngeal tissue and the germline is virtually nonexistent. In the interaction between distal tip cell and germline, an analysis of genetic mosaic animals indicates that *glp-1* is required in the induced tissue and therefore may mediate the response to the inductive signal (Austin & Kimble, 1987). Besides its role in known inductive events, *glp-1* is required during embryogenesis for hypodermal development (Priess *et al.* 1987). The morphogenesis of an embryo from an aggregate of cells into a worm depends on the hypodermis (Priess & Hirsh, 1986). However, no inductive interactions are known to influence hypodermal development or embryonic morphogenesis (Priess & Thompson, 1987).

To identify other genes necessary for inductive interactions, we generated extragenic suppressors of *glp-1* mutations. Surprisingly, several suppressors were defective in larval morphogenesis and had an altered body shape. Mutations in numerous genes affect body shape

(Brenner, 1974; Higgins & Hirsh, 1977; Cox *et al.* 1980; Rose & Baillie, 1980; Hodgkin, 1983; Meneely & Wood, 1984). These suppressors have a 'dumpy' mutant phenotype; animals are shorter than normal but have the same diameter. Previous studies of 25 *dumpy* (*dpy*) genes indicated that six are involved in the process of dosage compensation (Hodgkin, 1983; Meneely & Wood, 1984; Wood *et al.* 1985; Meyer & Casson, 1986) but that the remaining nineteen, which include those discussed here, do not affect dosage compensation. These other *dpy* genes are thought to be required for larval morphogenesis. Other genes that affect body morphology include *squat* (*sqt*), *small* (*sma*), *long* (*lon*), and *roller* (*rol*) genes. Here we show that mutations in eight of these genes (seven *dpy* and one *sqt*) suppress both germline and embryonic defects of *glp-1(ts)* mutations.

Materials and methods

Strains and culture methods

Worms were maintained on agar-filled Petri dishes seeded with *E. coli* as described (Brenner, 1974). The wild-type strain *C. elegans* var. Bristol, (strain N2) and mutant strains used in this study were obtained either from the Cambridge collection (Brenner, 1974) or from the *Caenorhabditis* Genetics Center. All mutants are described in Hodgkin *et al.* (1988) except where indicated.

Our nomenclature follows the guidelines of Horvitz *et al.* (1979). Mutant phenotypes are designated by a capitalized abbreviation (e.g. Dpy for a dumpy phenotype) and genes are designated by a lower case, italicized abbreviation (e.g. *dpy* for *dumpy* genes). Where appropriate, abbreviations are placed after an allele number to indicate that it is temperature sensitive (ts), amber (am), or semidominant (sd). For each gene, one null or putative null allele has been designated as the 'reference' allele (see Hodgkin *et al.* 1988); in general, we used reference alleles in our suppression tests.

Fig. 1 is a partial genetic map showing the loci used in this paper. LG*I*: *dpy-5(e61)*, *dpy-14(e188ts)*, *dpy-24(s71)*. LG*II*: *dpy-2(e8)*, *dpy-10(e128)*, *dpy-25(e817sd)*, *rol-1(e91)*, *rol-6(e187)*, *rol-8(sc15)* (Kusch & Edgar, 1986), *sma-6(e1482)*, *sqt-1(sc1, sc13, sc100, e1350)*, *sqt-2(sc3)*, *mnDf30*, *mnC1*. LG*III*: *dpy-1(e1)*, *dpy-17(e164)*, *dpy-18(e364am)*, *dpy-19(e1259ts)*, *glp-1(q35, q50, q158, q224ts, q231ts)*, *lon-1(e185)*, *sma-2(e502)*, *sma-3(e491)*, *sma-4(e729)*, *unc-32(e189)*, *unc-36(e873)*. LG*IV*: *dpy-4(e1166)*, *dpy-9(e12)*, *dpy-13(e184sd)*, *dpy-20(e2017am)*. LG*V*: *dpy-11(e224)*, *dpy-21(e428)*, *him-5(e1490)*, *rol-3(e754)*, *sma-1(e30)*, *sqt-3(sc63ts)*. LG*X*: *dpy-3(e27, e182)* (originally called *dpy-12(e182)* but later shown to be allelic to *dpy-3*; see Meneely & Wood, 1987), *dpy-6(e14)*, *dpy-7(e88)*, *dpy-8(e130)*, *lon-2(e678)*, *sma-5(n678)*, *unc-6(e78)*, *unc-18(e81)*, *vab-3(e648)*.

Isolation of extragenic suppressors of glp-1

L4 hermaphrodites homozygous for *glp-1(ts)* and a closely linked marker mutation, *unc-32(e189)*, were raised at permissive temperature [12°C for *glp-1(q224)* and 15°C for *glp-*

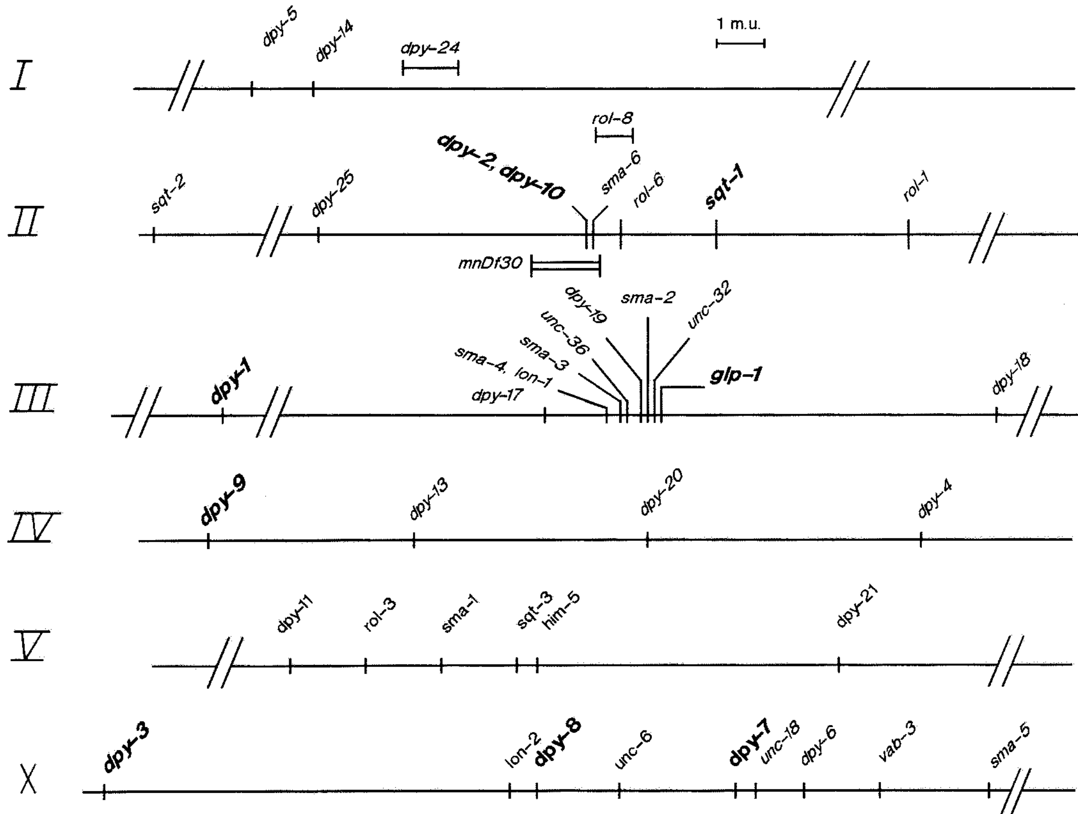

Fig. 1. Partial genetic map of *C. elegans* indicating the relative positions of genes used in this study. The six linkage groups are represented by a heavy horizontal line with well-mapped genes placed on the line and less precisely mapped genes indicated by bars placed above the line. Deficiencies are represented by an open bar placed below the line. *glp-1* and genes that interact with *glp-1* are indicated in bold-faced type.

1(q231)], mutagenized with ethyl methane sulphonate (Brenner, 1974), and returned to plates at permissive temperature. To isolate recessive suppressors, F_1 progeny were picked (3 animals/plate) and grown at permissive temperature. F_2 progeny were shifted to restrictive temperature (20°C) as late embryos or L1 larvae. Suppressors were sought by screening plates visually for production of an F_3 generation. Among the suppressed *glp-1* animals isolated, six contained a mutation with a Dumpy phenotype: two from 2100 *glp-1(q224)* F_1 and four from 1900 *glp-1(q231)* F_1. Other suppressors will be described elsewhere.

Genetic mapping and complementation tests

Linkage and complementation were determined by standard tests (see Tables 1 and 2 in Results). *dpy* mutations on LG*X* were roughly positioned by three-factor mapping: Lon and Unc recombinants from *lon-2 unc-6/dpy* and *lon-2 unc-18/dpy* heterozygotes were examined. *q288* was positioned more precisely by additional mapping: two-factor mapping established the distance between *unc-18* and *q288* (Table 1). Three-factor mapping established the position of *q288* relative to *unc-18*: Unc and Dpy recombinants were recovered from a strain that was *unc-18 q288/vab-3* and examined for a *vab-3* phenotype. *dpy* mutations *q291* and *q292* were initially linked to *unc-4(e120)* and then mapped to the region deleted by *mnDf30* (Sigurdson *et al.* 1984). *mnDf30* deletes both *dpy-2* and *dpy-10*; *mnC1*, a balancer for half of LG*II* including the *mnDf30* region, carries a *dpy-10* mutation. *q291* and *q292* were placed over *mnDf30* by mating *q291/+* or *q292/+* males to *mnDf30/mnC1* hermaphrodites.

Construction of double mutants

Double mutants with *glp-1(ts)* and one of several mutations that change body shape were constructed in one of two ways. 1) Males heterozygous or hemizygous for the mutation altering body shape were mated to *unc-32 glp-1(ts)* hermaphrodites at permissive temperature and non-Unc F_1 cross-progeny picked. Double mutants (e.g. *unc;dpy*) were isolated from the F_2. 2) *unc-32 glp-1(ts)/++* males were mated with hermaphrodites homozygous for the mutation that altered body shape and cross-progeny picked. Again, double mutants were isolated from the F_2. This second scheme was used with *sqt* mutations, as their heterozygous Rol phenotype effectively prohibits males from mating. In some cases, a Dpy phenotype was so extreme that animals could barely move and, therefore, Unc Dpy animals could not be distinguished from Dpy animals; in such cases, Unc non-Dpy F_2 animals were picked to isolate Unc Dpy animals from the F_3. For two genes, *rol-3* and *sma-5*, we constructed and tested stocks that did not contain *unc-32(e189)*.

Tests of glp-1(ts) *suppression*

In the initial test of *glp-1* suppression by mutations that affect body shape, (*dpy*, *sqt*, *rol*, *lon*, or *sma*), >200 embryos homozygous for *unc-32 glp-1(ts)* and the mutation to be tested were shifted to 20°C after the embryonic temperature-sensitive period for *glp-1* (4–28 cells). These embryos were allowed to develop and then examined for their ability to produce embryos; any embryos made were in turn examined for hatching, development to adulthood, and ability of these progeny to produce offspring.

Later suppression tests were done using stocks without *unc-32*. To remove *unc-32*, *glp-1(q231)*; *him-5(e1490)* males were mated with hermaphrodites homozygous for *unc-32 glp-1(q231)* and the suppressor mutation. Non-Unc heterozygous cross-progeny were isolated, F_2 individuals picked to individual plates, and a stock homozygous for *glp-1* and the suppressor obtained.

DAPI staining

Chromosome morphology was visualized by staining with diamidinophenolindole (DAPI) as described by Austin & Kimble (1987). From a 20°C stock, adults were picked just after moulting and before the onset of embryo production; these adults were maintained for 24, 48, 72, 96, 120, or 144 h at 20°C before fixation. For each timepoint, 4–10 animals (8–20 ovotestes) were examined. These animals are designated as 1-, 2-, 3-, 4-, 5-, or 6-day adults in Table 5.

Determination of brood sizes and per cent hatching

L4 hermaphrodites homozygous for *glp-1(q231)* and a suppressor mutation were placed individually on Petri dishes at 20°C and transferred to a fresh plate every 24 h. The total number of embryos produced by each hermaphrodite was counted; eggs were scored for hatching ~36 h after the hermaphrodite had been transferred. Hatched progeny were observed until at least L3 to assess their development. As a control, brood sizes and per cent hatching of *glp-1(q231)* were determined in a similar fashion at both 20°C (to determine brood size) and 17°C (to determine viability on successive days). At 17°C, we counted the brood sizes of eleven animals: they made an average of 175 embryos; 21 % of those progeny hatched and developed normally. No decrease in per cent hatching was observed as animals aged.

Tests for suppression of other glp-1 *alleles*

Double mutants possessing one of several *glp-1* alleles that are not temperature sensitive and a suppressor of *glp-1(ts)* were constructed. The partial loss-of-function *glp-1(q35)* and putative null *glp-1(q158)* alleles each were marked with *unc-32(e189)*; the partial loss-of-function allele *glp-1(q50)* originally was induced on an *eT1* chromosome and hence was marked with *unc-36(e873)*. The germline phenotype of *glp-1(q35)* shows some cold sensitivity (J. Austin, personal communication). Therefore, all experiments using *glp-1(q35)* were done at an intermediate temperature of 20°C and Unc, non-Dpy siblings were assayed in each experiment as a control for minor fluctuations in incubator temperature. Suppression of *glp-1(q35)* was retested in the absence of *unc-32(e189)* to eliminate the possibility of a marker effect. *unc-32(e189)* was removed by mating *glp-1(q35)/eT1*; *him-5(e1490)* males to suppressor hermaphrodites. F_1 cross-progeny segregating Glp (and no Unc) animals in the F_2 were obtained. These were heterozygous both for *glp-1(q35)* and the suppressor. F_2 animals either with or without the suppressor were picked (as L3 or L4 animals) and scored as producing (1) no eggs, (2) dead eggs only, or (3) viable F_3 progeny (and no dead eggs). All animals producing no eggs or only dead eggs were presumed to be *glp-1(q35)/glp-1(q35)*.

Tests for maternal effects on the suppression of glp-1(ts)

To examine *dpy/+* progeny of *dpy* homozygous mothers, *dpy-10(e128)*; *glp-1(q231)* hermaphrodites were mated to *glp-1(q231)*; *him-5(e1490)* males and examined for production of non-*dpy* [*dpy-10(e128)/+*; *glp-1/glp-1*; *him-5(e1490)/+*] cross-progeny at 20°C. These matings were done *en masse* in an effort to increase mating efficiency of the *glp-1* males. Unfortunately, 5 of the 14 mated hermaphrodites died prematurely, and therefore the average brood size in this experiment is lower than expected; normally, mated hermaphrodites have larger broods than unmated ones, yet the control broods averaged 147 embryos and the mated broods 122

embryos. To look at *dpy* homozygous progeny of *dpy*/+ mothers, *dpy-10(e128)*/ + ;*glp-1(q231)*/*glp-1(q231)*; *him-5(e1490)*/+ hermaphrodites were examined for production of *dpy* [*dpy-10(e128)*;*glp-1(q231)*] progeny at 20°C. As a control in this experiment, the survival of *dpy* progeny from *dpy* mothers was examined using *dpy-10(e128)*; *glp-1(q231)* at 20°C.

Results

Isolation and initial characterization of glp-1(ts) *suppressors with a dumpy left roller phenotype*

We have isolated recessive suppressors of two temperature-sensitive alleles of *glp-1*, *glp-1(q224ts)* and *glp-1(q231ts)*. Loss of *glp-1* gene function causes germ cells that normally would be in mitosis to enter meiosis instead. [Although the *C. elegans* germline is syncytial (Hirsh *et al.* 1976), for simplicity we refer to germline nuclei as germ 'cells'.] As a result of the *glp-1* effect on germline mitoses, only 4–7 germ cells are produced in severe *glp-1* mutants rather than the 1000–2000 made in wild-type animals. Because hermaphrodites normally produce ~300 sperm before switching to oogenesis, the few gametes produced by *glp-1* mutant hermaphrodites are sperm and the animals are sterile. If *glp-1(ts)* is shifted to restrictive temperature at any time during postembryonic development, germ cells at the distal end of the gonad that would normally be mitotic enter meiosis; therefore the *glp-1* product must function throughout development for continued mitoses. A second *glp-1* mutant phenotype is seen in conditional or leaky *glp-1* mutants which can make enough germ cells to produce some embryos. Progeny of a homozygous *glp-1* mutant mother inevitably die during embryogenesis; a *glp-1(+)* allele introduced from the father cannot rescue the lethal phenotype. The temperature-sensitive period for lethality is early in embryogenesis (4–28 cells).

The scheme used to isolate suppressors of *glp-1(ts)* is outlined in Materials and methods. In brief, after EMS mutagenesis of *glp-1*(ts), F_2 animals were tested for production of live progeny that developed into fertile adults at the restrictive temperature of 20°C. At 20°C, *glp-1(ts)* animals are sterile, but make more germ cells than at the more stringent restrictive temperature of 25°C. This intermediate temperature was used with the idea that some functional *glp-1* product might be present and that a bypass of *glp-1* function would not be required to achieve suppression. Among the suppressors isolated were six with a dumpy left roller phenotype, termed simply dumpy (Dpy). The six suppressors include *q287* and *q291*, suppressors of *glp-1(q224)*, and *q288*, *q289*, *q290*, and *q292*, suppressors of *glp-1(q231)*. Other recessive suppressors isolated will be described elsewhere.

To confirm that the *dpy* mutations (rather than a second mutation with a more subtle phenotype) were indeed suppressing *glp-1*, each *dpy* was separated from *glp-1(ts)* by multiple crosses with wild-type (N2) animals. Then, *glp-1(ts)*; *dpy* double mutants were reconstructed. To retest each double mutant, we shifted embryos with more than 28 cells to 20°C and looked both for development into fertile adults and for continued production of fertile animals over several generations. In every case, the germline and embryonic defects associated with *glp-1* were suppressed (see below). The *dpy* mutations were isolated as recessive suppressors of *glp-1* and remained recessive upon retesting: *dpy*/+; *glp-1*/*glp-1* animals were not suppressed at 20°C (see below and Table 6). Moreover, no suppression of the dumpy left roller phenotype by *glp-1(ts)* was observed. Each *dpy* suppressor was further tested at the more stringent restrictive temperature of 25°C. At this temperature, suppression of *glp-1* embryonic lethality was not observed. However, the germline phenotype was partially suppressed: some *glp-1(q231)*; *dpy* animals produced many more germ cells than the 6–25 typical of *glp-1(q231)* alone (data not shown).

To test for allele specificity, each suppressor of *glp-1(q231)* was tested for suppression of *glp-1(q224)* and *vice versa*. In every case, the suppressor rescued both *glp-1* alleles. (However, they do not suppress all *glp-1* mutations, see below and Table 4). Because suppression is not specific for either *glp-1(ts)* allele, many of the tests described below were done using only *glp-1(q231)*.

glp-1 *suppressors are alleles of known* dpy *genes*

Numerous *dpy* genes have been identified (Brenner, 1974; Cox *et al.* 1980; Rose & Baillie, 1980; Hodgkin, 1983; Meneely & Wood, 1984). The *glp-1* suppressors might identify a novel class of *dpy* genes or they might be new alleles of known *dpy* genes. We have done both complementation tests (Table 1) and mapping experiments (Table 2) to distinguish between these two possibilities.

Table 1. *Complementation analysis of* glp-1(ts) *suppressors with a Dpy phenotype*

							LG*X*				LG*II*	
Allele (LG)	*q287*	*q288*	*q289*	*q290*	*q291*	*q292*	*dpy-3(e27)*	*dpy-6(e14)*	*dpy-7(e88)*	*dpy-8(e130)*	*dpy-2(e8)*	*dpy-10(e128)*
q287 (X)	Dpy	+	Dpy*	+	+	+	+	+	+	Dpy		
q288 (X)		Dpy	+	Dpy	+	+	+	+	+	+		
q289 (X)			Dpy	+	+	+	+	+	+	Dpy		
q290 (X)				Dpy	+	+	+	+	Dpy	+		
q291 (II)					Dpy	+					+	Dpy
q292 (II)						Dpy					Dpy	+

* Slightly Dpy.

Table 2. (A) *Three-factor mapping*

Suppressor	Parental genotype	Recombinant phenotype	Recombinant genotype	Number
q287	*lon-2 unc-6/q287*	Lon	*lon-2 unc-6/lon-2 q287*	4
			lon-2 unc-6/lon-2	21
		Unc	*lon-2 unc-6/q287 unc-6*	11
			lon-2 unc-6/unc-6	5
	lon-2 unc-18/q287	Lon	*lon-2 unc-18/lon-2 q287*	9
			lon-2 unc-18/lon-2	32
q288	*lon-2 unc-6/q288*	Lon	*lon-2 unc-6/lon-2 q288*	13
			lon-2 unc-6/lon-2	0
	lon-2 unc-18/q288	Lon	*lon-2 unc-18/lon-2 q288*	42
			lon-2 unc-18/lon-2	0
	unc-18 q288/vab-3	Unc	*unc-18 q288/unc-18*	5
			unc-18 q288/unc-18 vab-3	0
		Dpy	*unc-18 q288/q288*	0
			unc-18 q288/q288 vab-3	8
q289	*lon-2 unc-6/q289*	Lon	*lon-2 unc-6/lon-2 q289*	1
			lon-2 unc-6/lon-2	19
		Unc	*lon-2 unc-6/q289 unc-6*	13
			lon-2 unc-6/unc-6	1
	lon-2 unc-18/q289	Lon	*lon-2 unc-18/lon-2 q289*	4
			lon-2 unc-18/lon-2	62
q290	*lon-2 unc-6/q290*	Lon	*lon-2 unc-6/lon-2 q290*	49
			lon-2 unc-6/lon-2	0
	lon-2 unc-18/q290	Lon	*lon-2 unc-18/lon-2 q290*	38
			lon-2 unc-18/lon-2	5
		Unc	*lon-2 unc-18/q290 unc-18*	4
			lon-2 unc-18/unc-18	30

(B) *Two-factor mapping*

Suppressor	Heterozygous parent	Segregants	Map distance
q288	*unc-18 q288/++*	2684 wildtype 721 Dpy Unc 3 Dpy 5 Unc	0·23 %

(C) *Deficiency mapping*

Suppressor	Heterozygous phenotype	Heterozygous genotype	Number
q291	Dpy	*q291/mnDf30* or *q291/mnC1*	26
	non-Dpy	*+/mnDf30* or *+/mnC1*	17
q292	Dpy	*q292/mnDf30*	6
	non-Dpy	*q292/mnC1* or *+/mnC1* or *+/mnDf30*	24

Four suppressors, *q287*, *q288*, *q289*, and *q290*, map to LG*X*. Three *dpy* genes on LG*X*, *dpy-3*, *dpy-7*, and *dpy-8*, have numerous alleles, many of which are left rollers as well as dumpy. A fourth *dpy* gene on LG*X*, *dpy-6*, is represented by only two, non-roller alleles. Complementation tests and mapping of *q287* and *q289* suggest that both are alleles of *dpy-8*. The *q287/q289* heterozygote is slightly Dpy, and both mutations fail to complement *dpy-8(e130)* and complement other *dpy* genes on LG*X* (Table 1). Although both map between *lon-2* and *unc-6* (Table 2A), *q287* maps precisely to *dpy-8* whereas *q289* maps ~0·5 map units to the left of *dpy-8*. The discrepancy in *q289* map position may result from statistical error or may indicate an association with a small DNA rearrangement that changes the apparent map position of *q289* by suppressing recombination. The other two *X*-linked suppressors, *q288* and *q290*, fail to complement *dpy-7(e88)* and do complement other *X*-linked *dpy* genes (Table 1). Although *q288* and *q290* complement each other (Table 1), both suppressors map to *dpy-7* between *unc-6* and *unc-18* (Table 2A and B). These two mutations are therefore assigned to *dpy-7*.

The remaining two suppressors, *q291* and *q292*, map to LG*II*. Similarly, *dpy-2* and *dpy-10* map to LG*II* and have some left roller alleles. *q291* fails to complement *dpy-10(e128)*, whereas *q292* fails to complement *dpy-2(e8)* (Table 1). Furthermore, *dpy-2*, *dpy-10*, *q291*, and *q292* all map to a deficiency on LG*II*, *mnDf30* (Sigurdson *et al.* 1984; this paper: Table 2C). On the basis of this complementation and map data, we assign *q291* to *dpy-10* and *q292* to *dpy-2*.

Unselected alleles of dpy-2, dpy-7, dpy-8, *and* dpy-10 *also suppress* glp-1(ts)

All six *glp-1* suppressors are mutations of previously identified *dpy* genes. It is possible that only specific rare alterations in these genes lead to *glp-1* suppression. We tested alleles obtained in a screen for visible mutants to ask whether these unselected alleles would also suppress *glp-1(ts)*. Double mutants containing a reference

Table 3. *Tests for suppression of* glp-1(q231) *by unselected alleles of* dpy, sqt, sma, rol *and* lon *mutations*

Gene(allele)	Suppression* 20 °C	25 °C
dpy-1(e1)	+	−
dpy-2(e8)	+	−
dpy-3(e27, e182)	+	−
dpy-4(e1166sd)	−	nd
dpy-5(e61sd)	−	nd
dpy-6(e14)	−	nd
dpy-7(e88)	+	−
dpy-8(e130)	+	−
dpy-9(e12)	+	−
dpy-10(e128)	+	−
dpy-11(e224)	−	nd
dpy-13(e184sd)	−	nd
dpy-14(e188ts)	−	nd
dpy-17(e164)	−	nd
dpy-18(e364am)	−	nd
dpy-19(e1259ts)	−	nd
dpy-20(e2017am)	−	nd
dpy-21(e428)	−	nd
dpy-24(s71)	−	nd
dpy-25(e817sd)	−	nd
sqt-1(e1350)	+	−
(sc1, sc13, sc100)	−	nd
sqt-2(sc3)	−	nd
sqt-3(sc63ts)	−	−
sma-1(e30)	−	nd
sma-2(e502)	−	nd
sma-3(e491)	−	nd
sma-4(e729)	−	nd
sma-5(n678)	−	nd
sma-6(e1482)	−	−
rol-1(e91)	−	nd
rol-3(e754)	−	nd
rol-6(e187)	−	nd
rol-8(sc15)	−	nd
lon-1(e185)	−	nd
lon-2(e678)	−	nd

* Suppression was determined by continuous production of fertile progeny at 20 °C or 25 °C. See text.

allele (see Materials and methods) of *dpy-2*, *dpy-7*, *dpy-8*, or *dpy-10* plus *glp-1(ts)* were shifted to 20 °C as embryos of more than 28 cells and examined for continued production of fertile animals over several generations (Table 3). Unselected alleles of all four *dpy* genes suppressed both germline and embryonic abnormalities of both *glp-1(q224)* and *glp-1(q231)* at 20 °C. As found with the selected suppressors, these reference alleles partially suppressed the germline defect, but did not suppress the embryonic defect, at 25 °C. We conclude that the selected suppressors are not unusual mutations of their respective genes.

Tests for glp-1 *suppression by other mutations that alter body shape*

Given the result that unselected mutations in four *dpy* genes suppress *glp-1(ts)*, we next tested mutations in other genes required for normal body morphogenesis to learn whether these too might affect the phenotype of *glp-1(ts)*. We tested mutations in essentially all *dumpy* (*dpy*), *roller* (*rol*), *squat* (*sqt*), *small* (*sma*), and *long* (*lon*) genes. Only one of seven *dpy* genes implicated in control of dosage compensation, *dpy-21*, was tested. *rol* mutants roll helically as they move forward or backward (Brenner, 1974; Higgins & Hirsh, 1977; Cox *et al.* 1980). Most *sqt* mutants roll as heterozygotes and are dumpy as homozygotes (Cox *et al.* 1980); they were therefore tested for suppression as heterozygotes as well as homozygotes. Several phenotypically different alleles of *sqt-1* were tested (see below). *sma* mutants are proportionally smaller (i.e. both shorter and thinner) and *lon* mutants are longer than wild type (Brenner, 1974). When available, an amber mutation was used because such alleles are probably null.

Mutants homozygous for *unc-32 glp-1(ts)* plus a mutation in one of the genes described above were assayed for suppression of *glp-1*(ts) at restrictive temperature (Table 3). We found that mutations in three additional *dpy* genes, *dpy-1*, *dpy-3* and *dpy-9*, and one *sqt* gene, *sqt-1*, were recessive suppressors of *glp-1(q231)* at 20 °C. At 25 °C, these suppressors prolonged germline proliferation but did not prevent embryonic lethality. Mutations in other genes neither suppressed nor enhanced the mutant phenotypes of *glp-1(ts)* (enhancement data not shown).

There is no correlation between severity of dumpy phenotype (i.e. length of the animal) and degree of suppression. Both *dpy-1* and *dpy-5* are extremely dumpy, for example, but *dpy-1* suppresses *glp-1(ts)* and *dpy-5* does not. Conversely, both *dpy-3* and *dpy-18* have a less severe dumpy phenotype, and *dpy-3* suppresses and *dpy-18* does not. Suppression by *sqt-1* is unique in that only one *sqt-1* allele suppresses *glp-1(ts)*. Although *sqt-1(sc1)* and *sqt-1(e1350)* have similar phenotypes (dominant Rol and recessive Dpy), only the latter suppresses *glp-1(q231)*. Further, neither the recessive Rol allele *sqt-1(sc15)* nor the phenotypically wild type, putative null allele *sqt-1(sc100)* is a suppressor.

Tests for suppression of other glp-1 *alleles*

The greater suppression of *glp-1(ts)* at 20 °C than at 25 °C suggested that the *dpy* mutations might suppress partial loss-of-function but not null alleles of *glp-1*. We therefore tested each suppressor with a putative null allele, *glp-1(q158)*, and two partial loss-of-function alleles, *glp-1(q35)* and *glp-1(q50)*. Whereas *glp-1(q158)* mutants produce only 4–7 germ cells, *glp-1(q35)* mutants produce a variable number of germ cells (47–198) and *glp-1(q50)* mutants produce either 5–8 or about 800 germ cells (Austin & Kimble, 1987). Double mutants homozgyous for one *glp-1* allele, *glp-1(x)* and a suppressor were compared to single mutants of *glp-1(x)* (Table 4). For *glp-1(q158)*, the germline defect was not suppressed and the embryonic defect could not be tested because too few germ cells are generated to produce embryos. For *glp-1(q50)* neither the germline nor embryonic defect was suppressed. In contrast, though the embryonic defect of *glp-1(q35)* was not suppressed, the germline defect was partially suppressed. Three suppressors, *dpy-7(q288)*, *dpy-8(q287)*, and *dpy-10(q291)*, were retested with *glp-1(q35)* in the

Table 4. *Tests for suppression of non-*ts glp-1 *alleles*

	*glp-1(q158)** % Glp§ (*n*)		*glp-1(q50)*† % Glp§ ovotestes (*n*)		*glp-1(q35)*‡ % Glp§ (*n*)	
Suppressor	*dpy* or *sqt*(−)	*dpy* or *sqt*(+)¶	*dpy* or *sqt*(−)	*dpy* or *sqt*(+)	*dpy* or *sqt*(−)	*dpy* or *sqt*(+)
dpy-1(e1)	100 (41)	100 (44)	nd	nd	53 (166)	83 (188)
dpy-2(q292)	100 (38)	100 (124)	nd	nd	nd	nd
dpy-3(e27)	100 (57)	100 (126)	nd	nd	nd	nd
dpy-7(q288)	100 (37)	100 (90)	68 (77)	74 (349)	2 (65)	41 (204)
dpy-8(q287)	100 (19)	100 (118)	79 (86)	88 (260)	14 (76)	78 (399)
dpy-9(e12)	100 (28)	100 (67)	nd	nd	16 (37)	88 (176)
dpy-10(q291)	100 (53)	100 (140)	nd	nd	9 (23)	77 (79)
sqt-1(e1350)	100 (25)	100 (55)	78 (18)	77 (64)	11 (36)	82 (125)

* Experiments were done at 22–23°C with *unc-32(e189)* as a marker for *glp-1(q158)*. n = number of animals.
† Experiments were done at 20°C or 22–23°C with *eT1(e873)* as a marker for *glp-1(q50)*. Each ovotestis was examined for its ability to produce embryos. A z-test (Freund, 1973) indicates that each double mutant is not significantly different from *glp-1(q50)* alone. n = number of ovotestes.
‡ Experiments were done at 20°C with *unc-32(e189)* as a marker for *glp-1(q35)*. A z-test indicates that each double mutant is significantly different from *glp-1(q35)* alone ($P < 0{\cdot}05$). n = number of animals.
§ "Glp" indicates a sterile phenotype; no oocytes are produced.
¶ A wild-type allele is designated "+", and a mutant (suppressor) allele is designated "−".

absence of *unc-32(e189)* to eliminate the possibility of a marker effect. Similar germline suppression was observed in all three cases (data not shown).

Further characterization of glp-1 *suppression*

Our initial test of suppression relied on continued production of progeny after shifts of animals homozygous for *unc-32(e189) glp-1(ts)* and a suppressor to 20°C. For subsequent experiments, we removed *unc-32* to eliminate possible marker effects.

Suppression of the glp-1(q231) *germline proliferation defect (Table 5, Fig. 2)*

In wild-type adults, meiotic nuclei are not observed in the most distal portion of the gonad (Klass *et al.* 1976; Kimble & White, 1981) and mitotic figures are still visible six days after the moult to adulthood (Table 5). In contrast, in *glp-1(q231)* animals raised at 20°C, all germline nuclei have entered meiosis two days after this final moult (Table 5, Fig. 2A). To examine the suppression of the *glp-1(ts)* germline phenotype, we compared the state of distal germline nuclei in double mutants homozygous for *glp-1(ts)* plus a suppressor to single mutants carrying the suppressor alone. Germline chromosomes were examined at 24 hour intervals starting with newly moulted adults. In contrast to *glp-1(q231)* animals, mitotic nuclei were still observed in the distal gonad after two days of adulthood in the double mutant (Table 5, Fig. 2B). However, by four or five days after the last moult, all germline nuclei in the distal gonad of the double mutant were in pachytene (Table 5). Therefore, the suppressors delay the abnormal entry into meiosis caused by *glp-1(q231)* but do not prevent it. In control animals, mitotic nuclei were present in the distal gonad of single mutants homozygous for the suppressor alone even after six days of adulthood (Table 5). Hence, the suppressor mutations themselves do not block germline mitoses. Consistent with this finding that a suppressed *glp-1(ts)* germline continues mitoses longer than does a *glp-1(ts)* germline, the number of progeny produced by the suppressed *glp-1(ts)* hermaphrodite is greater than that of the *glp-1(ts)* hermaphrodite (Table 5).

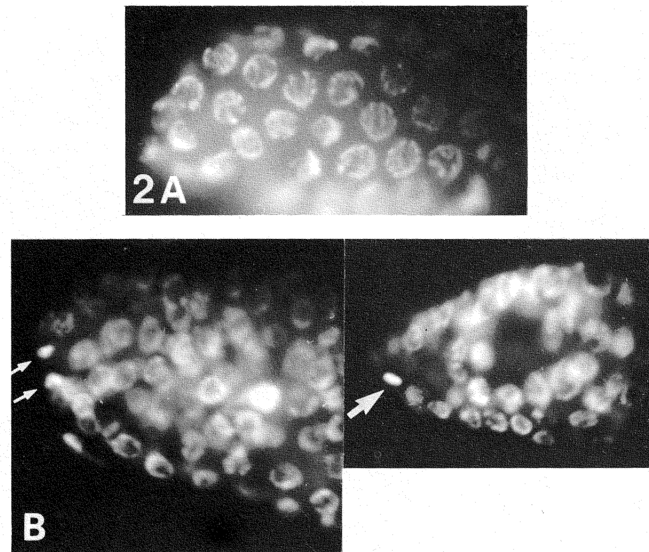

Fig. 2. Suppression of *glp-1* germline phenotype (20°C). The distal region of a single ovotestis is shown in each picture with the distal tip to the left; virtually only germline nuclei are shown. Animals were stained with DAPI after 2 days of adulthood (see Materials and methods). (A) *glp-1(q231)*: all germline nuclei are in pachytene; (B) *glp-1(q231);dpy(q288)*: mitotic germline nuclei are present. A metaphase plate (right) and a pair of late anaphase nuclei (left) are indicated by arrows.

Suppression of glp-1(q231) *embryonic lethality (Table 5, Fig. 2)*

Almost all wild-type embryos hatch (>99 %) (Hodgkin *et al.* 1979), but only an occasional *glp-1(q231)* embryo produced at 20°C hatches and none develops to adult-

Table 5. *Suppression of the germline and embryonic phenotypes of* glp-1 *at 20°C*

	Germline phenotype*			Total number embryos/⚥†		% Hatching‡	
Suppressor	*glp-1(+)* 6d	*glp-1(q231)* 2d	*glp-1(q231)* 4–5d	*glp-1(+)*	*glp-1(q231)*	*glp-1(+)*	*glp-1(q231)*
–	mitotic $n=5$	meiotic $n=3$	nd	330§	27 ± 4·5 $n=12$	>99§	0
dpy-1(e1)	mitotic $n=5$	mitotic $n=3$	meiotic $n=5$	254 ± 20 $n=5$	189 ± 15 $n=11$	94	15
dpy-2(q292)	mitotic $n=6$	mitotic $n=4$	meiotic $n=4$	240 ± 29 $n=2$	190 ± 29 $n=9$	100	15
dpy-3(e27)	mitotic $n=4$	mitotic $n=6$	meiotic $n=5$	284 ± 1 $n=2$	122 ± 27 $n=10$	99	38
dpy-3(e182)	mitotic $n=4$	mitotic $n=5$	meiotic $n=3$	278 ± 9 $n=3$	198 ± 9 $n=12$	100	29
dpy-7(q288)	mitotic $n=5$	mitotic $n=10$	meiotic $n=10$	263 ± 9 $n=3$	166 ± 12 $n=11$	100	12
dpy-7(q290)	mitotic $n=4$	mitotic $n=3$	meiotic $n=6$	271 ± 12 $n=3$	146 ± 20 $n=12$	100	13
dpy-8(q287)	mitotic $n=5$	mitotic $n=10$	meiotic $n=10$	216 ± 7 $n=3$	136 ± 22 $n=12$	100	19
dpy-8(q289)	mitotic $n=3$	mitotic $n=10$	meiotic $n=10$	249 ± 17 $n=3$	78 ± 29 $n=8$	100	4
dpy-9(e12)	mitotic $n=4$	mitotic $n=4$	meiotic $n=4$	255 ± 5 $n=2$	116 ± 20 $n=11$	98	11
dpy-10(q291)	mitotic $n=6$	mitotic $n=3$	meiotic $n=5$	281 ± 26 $n=3$	102 ± 24 $n=11$	100	15
sqt-1(e1350)	mitotic $n=3$	mitotic $n=8$	meiotic $n=4$	213 ± 12 $n=5$	128 ± 17 $n=13$	99	2

* n = number of animals examined by DAPI staining. See text.

† Both live progeny and dead eggs were counted to determine the total number of embryos produced by each hermaphrodite. n = number of hermaphrodites.

‡ Per cent hatching was calculated by dividing the number of hatchlings by the total number of hatchlings and dead eggs.

§ From Hodgkin, Horvitz & Brenner (1979).

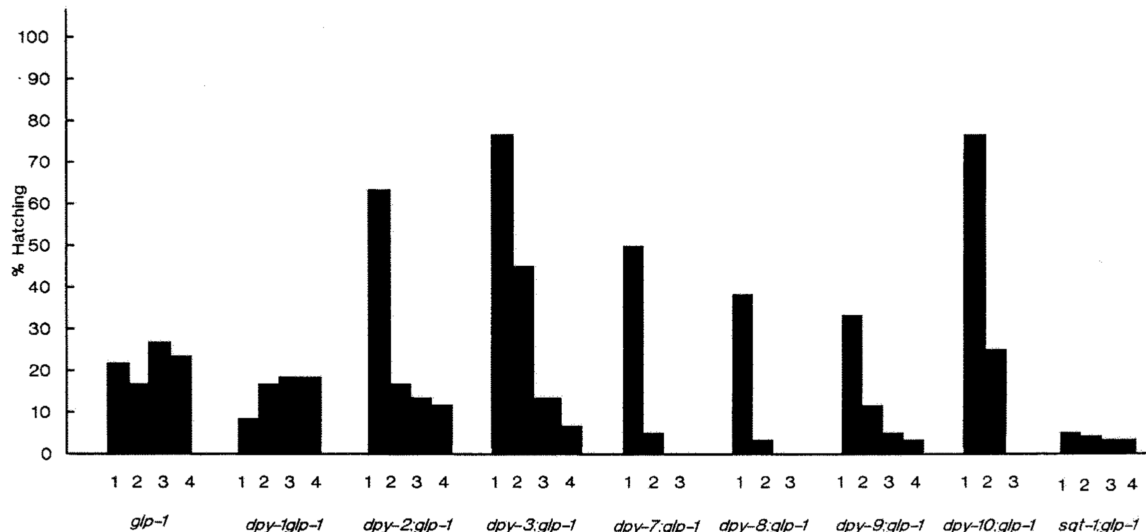

Fig. 3. Suppression of *glp-1* embryonic phenotype decreases with maternal age. Histograms indicate the percentage of embryos that hatch; the number below each bar represents the maternal age in days after the moult to adulthood. A *glp-1(q231)* control shows essentially no change in per cent hatching with maternal age. In contrast, seven of the eight double mutants with *glp-1* plus a suppressor show a drastic decrease in progeny viability with maternal age. Note that the *glp-1(q231)* control was raised at 17°C, an intermediate temperature at which some hatching could be observed, while the double mutants were raised at the restrictive temperature of 20°C.

hood (Table 5). When the percentages of embryos that hatch and grow to adulthood were compared among double mutants homozygous for *glp-1(q231)* and one of the suppressors, we found that the suppressors vary in strength (2 % to 38 %; Table 5). We also observed in this experiment that the percentage of embryos that hatch and develop to adulthood decreases as mothers age (Fig. 3). This decrease might have been associated with the phenotype of either single mutant, i.e. *glp-1(q231)*, or the suppressor, rather than reflecting a

Table 6. *Suppression of* glp-1 *embryonic lethality by* dpy-10 *in mother and/or zygote (20°C)*

Parental genotype	Total number embryos/⚥		% Hatching
*dpy-10(−/−);glp-1(−/−)**	154 ± 15	*n* = 10	17
dpy-10(−/−);glp-1(−/−) × glp-1(−/−);him-5(−/−)† ♂	122	*n* = 14	16
*dpy-10(−/+);glp-1(−/−)**	29 ± 6	*n* = 25	0

* *n* = number of hermaphrodites for which an entire brood was counted. Both live and dead progeny were counted to determine the total brood size.
† *n* = number of mated hermaphrodites for which an entire brood was counted. 25 % of the surviving offspring were non-Dpy cross-progeny from the mating and 75 % were Dpy self-progeny. Because the matings were done *en masse*, no standard deviation was calculated. The average brood size in this experiment is smaller than in the control because five hermaphrodites died prematurely and therefore did not produce their full complement of progeny.

property of suppression. When we examined *glp-1(q231)* at 17°C, where approximately 21 % of the embryos hatch and develop to adulthood, we found no decrease in per cent hatching with maternal age (Fig. 3). Similarly, when we counted the per cent of embryos that hatch on successive days from mothers homozygous for each suppressor, we found viability to be high (Table 5) with no decrease over maternal age (data not shown). The drastic drop in per cent hatching observed in suppressed *glp-1*(ts) embryos must therefore be associated with suppression.

The maternal dpy *genotype is crucial for suppression of* glp-1(ts) *embryonic lethality*

The embryonic lethality of *glp-1* is dependent on the maternal genotype: progeny of homozygous *glp-1* mothers die whether heterozygous or homozygous for *glp-1* (Austin & Kimble, 1987; Priess *et al.* 1987). We asked if suppression of the *glp-1* embryonic lethality is also dependent on the maternal genotype. We chose *dpy-10(e128)* for this study because it is most likely to be a null mutation based on dosage studies (Sigurdson *et al.* 1984). Progeny of mothers homozygous for both *glp-1(q231)* and *dpy-10(e128)* are rescued. This rescue is independent of whether the progeny themselves are heterozygous or homozygous for the suppressor (Table 6). Conversely, progeny of mothers homozygous for *glp-1(q231)* but heterozygous for *dpy-10(e128)* are never rescued. This lack of rescue is again independent of whether the progeny themselves are heterozygous or homozygous for the suppressor (Table 6). These results indicate that suppression of *glp-1(ts)* depends on the genotype of the mother and not that of the progeny.

Discussion

The *glp-1* gene functions in two inductive interactions and in development of the embryonic hypodermis of *C. elegans* (Austin & Kimble, 1987; Priess *et al.* 1987). In this paper, we show that the mutant phenotypes of two temperature-sensitive (*ts*) alleles of *glp-1* are suppressed by mutations in eight genes required for normal morphogenesis during larval and adult stages. Further, one of the mutant phenotypes of a third, partial loss-of-function allele is suppressed. These suppressor genes include seven *dpy* (*dpy-1*, *dpy-2*, *dpy-3*, *dpy-7*, *dpy-8*, *dpy-9*, and *dpy-10*) and one *sqt* (*sqt-1*) gene. The *dpy* mutants are shorter than normal and many, but not all, roll helically as they move (Brenner, 1974); the *sqt* mutant is shorter than normal as a homozygote and rolls as a heterozygote (Cox *et al.* 1980). However, neither a shorter body nor the ability to roll *per se* is sufficient to suppress *glp-1*. Mutations in most *dpy*, *sma*, *rol*, and *sqt* genes do not suppress *glp-1*. In addition, there is no correlation between body length and ability to suppress. Certain severe *dpy* mutants do not rescue *glp-1(ts)*, and *lon* mutants, which have an increased body length, neither enhance nor suppress *glp-1*.

From our genetic characterization of the interactions between *glp-1* mutations and the suppressor mutations that alter larval body shape, we draw three main conclusions. First, the suppressors must affect a process that is common to all three functions of *glp-1* (induction of the pharynx, induction of the germline, and development of the embryonic hypodermis), because all three *glp-1* defects are suppressed. Second, the suppressors do not bypass the requirement for *glp-1* since they do not rescue a putative null mutant of *glp-1*, and since they are better suppressors at 20°C, a moderate restrictive temperature, than at 25°C, a stringent restrictive temperature. Instead, the suppressors probably allow disabled *glp-1* product to function more efficiently. Third, the interaction of these genes with *glp-1* suggests that the suppressor genes constitute a functional subclass distinct from other genes affecting body morphology.

In addition, our results show that these suppressors are not unusual mutations of their respective loci. Suppression of *glp-1(ts)* is obtained not only by mutations isolated as suppressors but also by mutations isolated in visual screens for phenotypes altering body shape. However, we cannot conclude that these mutations are due to complete loss of the suppressor gene product. The null phenotype of *sqt-1* is wild-type (Kusch & Edgar, 1986; Kramer *et al.* 1988). In contrast, the suppressor *sqt-1* mutation has a visible phenotype indicating that its product is probably present but defective. Although the null phenotypes of the *dpy* suppressor genes are not known, two lines of evidence support the idea that the *dpy* suppressors are at least partial loss-of-function mutations: they are fully recessive and they arise at a frequency similar to loss-of-function alleles in genes understood at the molecular level.

Suppression of *glp-1* by mutations in seven *dpy* genes

and *sqt-1* indicates that these genes are not only required for body morphogenesis, but that they are also involved at some level in the decision between mitosis and meiosis. Because the *glp-1* mutations do not suppress (or enhance) the Dpy or Sqt phenotypes, we do not propose a role of *glp-1* in larval morphogenesis. However, an interaction between genes required for larval morphogenesis and *glp-1* is not inconsistent with known *glp-1* functions: the hypodermis of *glp-1* mutant embryos develops incorrectly resulting in defective embryonic morphogenesis (Priess *et al.* 1987).

How might the *glp-1* and suppressor gene products interact? An understanding of the molecular nature of these gene products provides insight into this problem. The *glp-1* gene has now been isolated and its DNA sequence obtained (Austin & Kimble, unpublished; J. Yochem & I. Greenwald, personal communication). The deduced amino acid sequence of *glp-1* is extremely similar to that of *lin-12* (J. Yochem & I. Greenwald, personal communication); the sequence of *lin-12* predicts a membrane protein with an extracellular portion composed primarily of EGF-like repeats (Yochem *et al.* 1988). Analysis of genetic mosaic animals indicates that *glp-1* functions in the germline and may be part of the receiving mechanism in the regulatory interaction that controls its growth (Austin & Kimble, 1987). These data together place *glp-1* in the germline as a membrane protein that mediates induction of germline growth by the somatic distal tip cell.

Among the *glp-1* suppressors, only *sqt-1* has been molecularly isolated; the deduced amino acid sequence of *sqt-1* shows that it is a collagen gene (Kramer *et al.* 1988). The molecular identity of *sqt-1*, its mutant phenotypes, and its genetic interactions with *glp-1* together suggest that this gene encodes a collagen in the extracellular matrix of *C. elegans*. This suggestion does not preclude the possibility that the *sqt-1* gene product is also found in the cuticle, which is essentially a specialized form of the extracellular matrix. Indirect evidence suggests that the *dpy* suppressor genes may also act in formation of the extracellular matrix (Cox *et al.* 1980, 1984; Kramer *et al.* 1982; Kusch & Edgar, 1986). The distal arm of the gonad in *C. elegans* is composed primarily of germ cells plus the somatic distal tip cell (Klass *et al.* 1976; Kimble & Hirsh, 1979). A basement membrane encases both the germline and somatic distal cells; no extracellular matrix is visible between the distal tip cell and the germ cells (Kimble & Ward, 1988). We suggest that the *dpy* and *sqt-1* gene products participate in formation of the gonadal basement membrane.

The genetic interactions observed between *glp-1* and the *sqt-1* and *dpy* mutations may reflect molecular interactions between the *glp-1* protein and elements of the extracellular matrix. An influence of the extracellular matrix on inductive interactions has also been proposed from a variety of studies that correlate changes in the composition of the extracellular matrix with changes in induction of developmental fate (reviewed in McClay & Ettensohn, 1987; Ekblom & Thesleff, 1985; Ekblom *et al.* 1986; Hay, 1983, 1984).

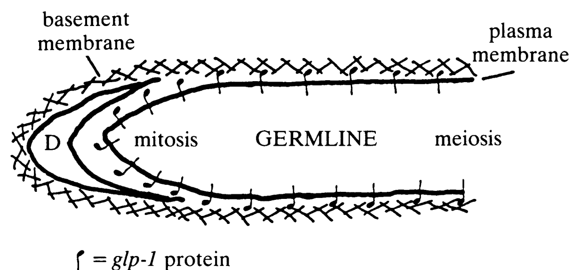

Fig. 4. Model for interaction between *glp-1* protein and the extracellular matrix. The distal gonad is schematized with *glp-1* protein as an integral membrane protein in the germline plasma membrane. The space between the basement membrane and the germline plasma membrane is greatly exaggerated; in actuality, the plasma membranes of the distal tip cell and the germline are closely apposed. D = distal tip cell. See text for further explanation.

Furthermore, certain growth factors bind components of the extracellular matrix (Gordon *et al.* 1987; Schweigerer *et al.* 1987); the EGF-like domains found in the putative extracellular domain of *glp-1* (J. Yochem & I. Greenwald, personal communication) may bind components of the extracellular matrix in an analogous fashion.

Fig. 4 shows one mechanism by which interactions between *glp-1* and the extracellular matrix may influence the germline decision between mitosis and meiosis. In this model, the *glp-1* protein stimulates mitosis directly and is present in both proximal and distal regions of the germline plasma membrane. Contacts between the extracellular domain of *glp-1* and the extracellular matrix would block or inactivate *glp-1* function so that these proximal germ cells enter meiosis. Distally, where no (visible) extracellular matrix is found between the distal tip cell and the germline plasma membrane, *glp-1* would not be inhibited and therefore would be free to stimulate mitosis. Disruption or alteration of the extracellular matrix in suppressor mutants may release *glp-1* protein located proximally and rescue the *glp-1* phenotype by allowing this additional *glp-1* protein to function. Alternative models, of course, remain possible. For instance, *glp-1* may not act directly to stimulate mitosis. One mechanism by which it might indirectly stimulate mitosis is to anchor a positive regulator of meiosis to the extracellular matrix and hence to restrict that regulator from the distal region. By this second model, germline mitoses might continue in the distal region simply because the distal tip cell prevents contact between the extracellular matrix and the germline plasma membrane.

In summary, suppression of the *glp-1* mutant phenotypes by *dpy* and *sqt-1* mutations has led us to propose an interaction between the extracellular domain of *glp-1* and components of the extracellular matrix. In the germline, we speculate that this interaction imposes spatial contraints on the decision between mitosis and meiosis. Although a similar process may also occur during embryogenesis, it is complicated by the fact that the embryonic function and suppression of *glp-1* depends on maternal products. We therefore focus on

the control of germline proliferation by the distal tip cell as an experimental model system for elucidating the function of *glp-1* in mediating inductive cellular interactions.

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