Genetic Control of Cell Communication in *C. elegans* Development

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Summary

Cell communication is crucial for many aspects of growth and differentiation during the development of the nematode *Caenorhabditis elegans*. Two genes, *glp-1* and *lin-12*, mediate a number of known cell–cell interactions. Genetic and molecular analyses of these two genes lead to the conclusion that they are structurally and functionally related. We summarize these studies as well as those involving the identification of other genes that interact with *glp-1* and/or *lin-12*.

Introduction: Cell–Cell Interactions in Development

Over the past decade, it has become clear that cell communication plays a critical role in regulating the development of the nematode *Caenorhabditis elegans*. The development, in particular, of three major tissues in the animal depend on cell interactions: the germline tissue and pharynx of both sexes, and the vulva of the hermaphrodite (Fig. 1). In the developmental pathways that give rise to these structures, two types of cell–cell signalling have been delineated: (1) induction, in which cells of one tissue regulate the fate of cells in a second tissue, and (2) lateral signalling, in which equivalent cells within a single tissue interact with each other causing them to assume different fates. In this review, we discuss two examples of induction mediated by the *glp-1* (*g*erm*l*ine *p*roliferation defective-1) gene and two examples of lateral signalling mediated by the *lin-12* (abnormal cell *lin*eage-12) gene. These interactions are summarized in Table 1.

One case of induction mediated by *glp-1* is the regulation of germ line proliferation by a somatic gonadal cell, called the distal tip cell[1]. The distal tip cell is situated at the end of the germline tube. Normally, all germline nuclei near the distal tip cell remain in the mitotic cell cycle and serve as stem cells, or precursor cells, for the production of gametes in the adult. Germline nuclei located more proximally enter meiosis; meiotic cells mature as they move down the germ line tube and differentiate as sperm or oocytes in the proximal region of the gonad. All germline nuclei, both mitotic and meiotic, share a common cytoplasm; therefore, the germ line is a syncytium. However, each nucleus occupies its own partially membrane-bound region of cytoplasm at the periphery of the germ line tube. A more extensive description of *C. elegans* gonadal anatomy can be found elsewhere[1,2]. If, at any time during post-embryonic development, the distal tip cell is removed by laser microsurgery, germline nuclei that are normally in mitosis leave the mitotic cell cycle and enter meiosis[1]. Therefore, the distal tip cell induces germline growth and/or prevents premature entry into meiosis during larval development and adulthood.

The second inductive event mediated by *glp-1* occurs between cells in the early embryo, probably between the 4- and 28-cell stages[3]. Each blastomere of the early embryo has a distinct and invariant lineage: the AB blastomere generates much of the hypodermis and nervous system of the animal and the EMS blastomere produces, for the most part, gut and muscle[4]. The pharynx is a neuro-muscular organ at the front of the animal; it is composed of an anterior part derived from the AB blastomere and a posterior part coming from EMS[4]. The function of the pharynx is to pump food into the animal's intestine. Development of a complete pharynx depends on an inductive event between EMS (or one of its descendants) and descendants of the AB blastomere: if EMS is removed from the early embryo, then AB does not give rise to pharyngeal mesoderm[3].

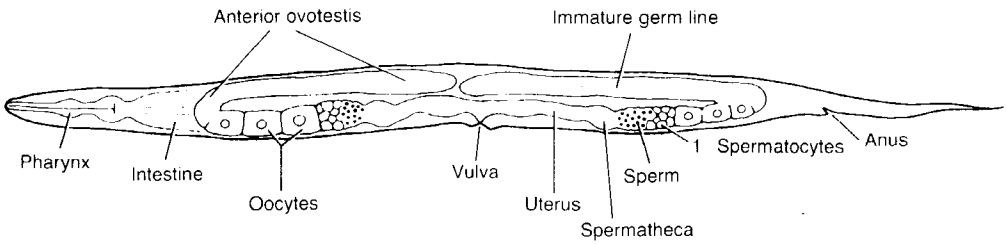

Fig. 1. Schematic diagram of a young adult hermaphrodite showing the position of the pharynx, the germ line, and the vulva. Development of each of these tissues depends on cell interactions. The gonad is composed of two U-shaped tubes, called anterior and posterior ovotestes. Each ovotestis is composed of germ line tissue which is immature distally and differentiating as sperm or oocytes in the proximal region. The somatic cells in the gonad include the distal tip cell, which is located at the distal end and the uterus and spermathecae proximally. In addition, maturing oocytes are completely ensheathed by somatic gonadal cells and the immature germ line of each ovotestis is partially encapsulated by two somatic cells. (Adapted from Fig. 1 of Kimble, J. E. (1981). Genetic control of sex determination in the germ line of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond. B* **322**, 11–18.)

Table 1. *Some regulatory cell interactions in* C. elegans *development*

Signalling cell	Receiving cell	Normal fate of receiving cell	Fate of receiving cell after removal of signalling cell	Deduced interaction	Time of interaction
Interactions between cells of non-equivalent developmental potential (*glp-1*-mediated):					
Distal tip cell (DTC)	Germ line	Continued mitosis	Germ cells enter meiosis	DTC induces germline mitosis	L2→adult
EMS-derived cell	AB-derived cell	AB forms anterior pharynx	AB does not form anterior pharynx	EMS-derived cell induces AB-derived cell to form anterior pharynx	Between 4- and 28-cell stages of embryo
Interactions between cells of equivalent developmental potential (*lin-12*-mediated):					
Anchor cell precursor	Ventral uterine precursor	VU (2° fate)	AC (1° fate)	AC precursor inhibits VU precursor from becoming AC	L2
Vulval precursor P6.p	Vulval precursors P5.p & P7.p	Follows 2° VPC lineage	Follows 1° VPC lineage	P6.p inhibits P5.p & P7.p from becoming a 1° VPC	L3

L2, second larval stage; L3, third larval stage; EMS and AB, embryonic blastomeres; AC, anchor cell; VU, ventral uterine precursor cell; VPC, vulval precursor cell.

The animal therefore has EMS-derived posterior pharyngeal mesoderm, but does not have a complete pharynx. In contrast, if AB is removed from the early embryo, MS gives rise to pharyngeal mesoderm as normal. Therefore, EMS (or one of its descendants) induces pharyngeal development in AB derived cells.

The *lin-12* gene is required for lateral signalling between numerous cells during late embryonic and postembryonic development[5]. In this review, we focus on two of these signals, both required for development of the vulva, the vulva being the opening in the hermaphrodite hypodermis through which embryos are laid. The first of the *lin-12* lateral signals is necessary for specification of the anchor cell (AC), which, in turn, induces the vulva[6]. [Since neither *lin-12* nor *glp-1* is known to affect vulval induction by the AC once the AC is formed, this example of cell signalling is not discussed further in this review.] In the wild-type, the AC is produced by one of two cells in the somatic gonad[7]: one of the two cells becomes an AC while the remaining cell becomes a ventral uterine precursor cell (VU). If either cell of the pair is ablated, however, then the remaining cell adopts the AC, or 1°, fate[6]. Therefore, the AC and VU precursors are equivalent in their ability to become AC. The cell that adopts the 1° fate is thought to inhibit its neighbor from following that same fate, the neighbor subsequently adopting a VU 2° fate[6]. The AC/VU pair is an example of an 'equivalence group'[6]: cells with equivalent developmental potential that interact with each other to adopt distinct fates.

A second example of lateral signalling within an equivalence group is found in six hypodermal cells that all have the potential to become vulval precursor cells (VPCs). These six cells interact during the third larval stage (L3) to adopt one of three different fates, 1°, 2°, or 3°[8,9]. The cells that follow 1° and 2° lineages produce cells in the vulva; cells that follow a 3° lineage generate hypodermis.

Genetics of *glp-1* and *lin-12*

The zygotic phenotype of *glp-1* mutants mimics the effect of ablating the distal tip cell early in larval development. Animals homozygous for a *glp-1* null mutation have very little germ line tissue: germ cells leave mitosis early in postembryonic development, enter meiosis, and differentiate, producing sperm (Table 2)[10]. Because *glp-1* hermaphrodites have such a minuscule germ line and produce only sperm, they are sterile.

Using temperature sensitive (*ts*) or leaky mutants, an effect of *glp-1* mutations on embryogenesis is also observed[10,11]. When *glp-1(ts)* hermaphrodites are raised at permissive temperature, they possess a wild-type germ line and are fertile. However, when such adults are shifted to restrictive temperature as adults, they produce functional gametes but their progeny die as embryos. These embryos have no anterior pharynx and their morphogenesis is abnormal[11]. Embryonic lethality depends strictly on the *maternal* genotype: heterozygous [*glp-1(+)/glp-1(ts)*] progeny of *glp-1(ts)* homozygous mothers and *glp-1(+)* fathers do not survive but homozygous *glp-1(0)/glp-1(0)* progeny of heterozygous mothers do survive[10,11]. Hence, both hypodermal development and induction of anterior

Table 2. *Mutant phenotypes of* glp-1 *and* lin-12[a,b]

Gene	Genotype	Phenotype	Defective interaction	Site of action	tsp
glp-1[6,7]	m(+/lf);z(lf/lf)	sterile: all germ cells enter meiosis	distal tip cell–germ line	germ line	L2→adult
	m(lf/lf);z(lf/lf) or m(lf/lf);z(lf/+)	embryonic lethal: anterior pharynx not formed	EMS-derived cell–AB-derived cell	nd	2- to 28-cell stage embryo
lin-12[5]	lf/lf	AC–VU: both cells follow 1° fate (AC)	AC–VU	VU	L2
		VPCs: no cells follow 2° VPC fate Non-functional vulva develops	among VPCs	nd	L3
	gf/gf or gf/+	AC–VU: both cells follow 2° fate (VU)	AC–VU		
		VPCs: all six cells follow 2° fate Multiple abnormal vulvae develop (Muv)	among VPCs		

[a] tsp, temperature sensitive period; m, maternal genotype; z, zygotic genotype; nd, experiment not done; lf, loss of function mutation; gf, gain of function mutation; EMS and AB, embryonic blastomeres; AC, anchor cell; VU, ventral uterine precursor; VPC, vulval precursor cell.
[b] The mutant phenotype of *lin-12* is only partially described in this table. For other cell fates affected by *lin-12* see reference 8.

pharynx depend on maternal *glp-1* product. Unlike development of the anterior pharynx, development of the embryonic hypodermis is not known to rely on cell interactions.

Although many different tissues are affected in *lin-12* mutants, the most obvious and best characterized phenotype is defective vulval development[5]. Two types of *lin-12* alleles have been isolated: recessive loss-of-function mutants [*lin-12(lf)*], in which both cells in a two-cell equivalence group adopt the 1° fate, and dominant gain-of-function mutants [*lin-12(gf)*] in which both cells adopt the 2° fate[5]. For example, in the AC/VU pair, *lin-12* activity is necessary for adoption of the VU (2°) fate: in *lin-12(lf)* mutants, both members of the AC/VU pair become ACs and in *lin-12(gf)* mutants, both members become VUs. In the hypodermal equivalence group that generates the vulva, *lin-12* has similar effects. No cells of the group assume the 2° fate in *lin-12(lf)* mutants and therefore a non-functional vulva forms. In contrast, in *lin-12(gf)* mutants, all cells of the equivalence group assume the 2° fate and therefore multiple non-functional vulvae form (a Multivulva or Muv phenotype).

Temperature shift experiments with *ts* mutations indicate that *glp-1* and *lin-12* products are required at those times when the relevant cell–cell interaction occurs. Thus, *glp-1* is needed during two periods of development: first during early embryogenesis when the anterior pharynx is induced and second postembryonically when germ line proliferation depends on the distal tip cell[10,11]. Similarly, *lin-12* is required in L2 for specification of the VU fate and in L3 for the correct choice of cell fates among the VPCs[5]. Therefore, *glp-1* and *lin-12* appear to act directly in these various cell communication events.

Are *glp-1* and *lin-12* part of the signalling mechanism or the receiving/transducing mechanism? Examination of genetic mosaic animals indicates that both act in the receiving cell. Thus, *glp-1(+)* is required in the germline and not in the distal tip cell for normal germ line proliferation[10]. Similarly, *lin-12(+)* is required in the VU precursor and not in the AC precursor for adoption of the VU fate[12]. Therefore, both genes are likely to play a role in receiving and/or transducing a signal[10,12]. Although not all cell interactions dependent on *glp-1* or *lin-12* have been analyzed in this manner, it seems likely that these genes play a similar role in the other cell signalling events as well.

Molecular Biology of *glp-1* and *lin-12*

The *glp-1* and *lin-12* genes lie in inverted (head to head) orientation approximately 20 kilobases (kb) apart on linkage group *III*[13,14]. Each gene appears to produce a single transcript[13]. Remarkably, the two genes are similar both in organization and in predicted amino acid sequence (50–60% identical)[14]. They are therefore likely to have evolved by gene duplication. Furthermore, both *glp-1* and *lin-12* are homologous to *Notch*[14–17], a *Drosophila* gene that is thought to function in an embryonic cell interaction controlling the choice between the epidermal and neuroblast fates[18,19]. *Notch* is also expressed in many proliferating tissues throughout development[20–22]. These three genes identify a new gene family whose members mediate regulatory cell interactions during development. Recently, this family has grown by the discovery of new members in *Xenopus* (C. Coffman, C. Kintner, and W. Harris, personal communication) and man (L. Ellisen and J. Sklar, personal communication).

The predicted proteins of *glp-1*, *lin-12* and *Notch* are similar both in organization and sequence. All have an

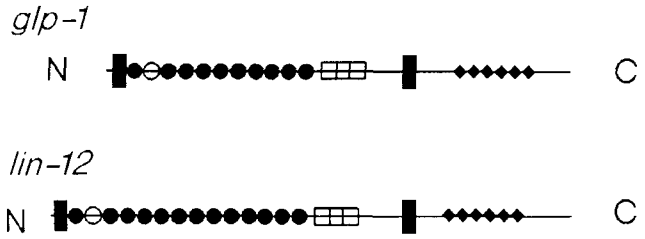

Fig. 2. The predicted *glp-1* and *lin-12* proteins have a similar organization[14,15]. N, amino terminus; C, carboxy terminus. The predicted proteins of *glp-1* and *lin-12* have two kinds of cysteine-rich sequence elements in the extracellular portion of the protein and a third repeated sequence motif in the cytoplasmic portion of the protein. The EGF-like motif (shaded circle) is found in a variety of molecules whereas the LNG motif (open square) is unique to *lin-12*, *Notch*, *glp-1*, and the two vertebrate homologues. The repeated sequence motif seen in the cytoplasmic domain (diamond) was originally identified in two yeast genes, *cdc10* and *SWI6*. A hydrophobic region of the appropriate size for a membrane-spanning domain is present as well as a signal sequence at the amino terminus (shaded bars). The open circle found between the first two EGF-like repeats designates a non-repeated, cysteine-rich region that is similar in the two genes.

N-terminal hydrophobic region that could function as a signal sequence and a second hydrophobic region followed by a stretch of positively charged amino acids that could be a transmembrane domain. Indeed, biochemical studies demonstrate that *Notch* is a membrane protein with extracellular and intracellular domains as predicted[20,21]. By analogy, we predict that both *glp-1* and *lin-12* are also membrane proteins with a similar transmembrane orientation.

The proteins encoded by these genes have three different repeated sequence motifs (Fig. 2)[14–17]. First, multiple copies of the cysteine-rich EGF-like motif make up the bulk of the extracellular region. This repeat is found in many membrane proteins, including the epidermal growth factor (EGF) precursor and the low density lipoprotein (LDL) receptor. A second repeated cysteine-rich motif that is unique to *lin-12*, *Notch*, and *glp-1*, the LNG motif, is found in three copies closer to the transmembrane domain. Finally, in the putative intracellular portion, there are six copies of a sequence motif originally found in two yeast genes, *cdc10* (required in *S. pombe* for initiation of the cell cycle)[23,24] and *SWI6* (required in *S. cerevisiae* for *HO* gene transcription during G_1)[25,26]. Recently, this repeat has been found in other genes, including *fem-1*, a *C. elegans* sex determination gene[27] and vertebrate erythrocyte ankyrin, a family of proteins thought to attach ctyoskeletal elements to the cell membrane[28].

Since the *glp-1* and *lin-12* proteins are almost certainly membrane proteins, it has been proposed that they are the receptors for ligands in cell–cell interactions (Fig. 2)[13–15,29].

Are *glp-1* and *lin-12* Functionally Related?

The molecular similarity between the predicted *glp-1* and *lin-12* products suggests that the two proteins may function by similar mechanisms. The phenotypes of *glp-1* and *lin-12* null mutants affect a non-overlapping set of cell interactions[5,10,11] and transcript analysis suggests that each gene is regulated uniquely in a tissue- and stage-specific manner[13]. However, several lines of evidence, relying on both genetic and molecular data, indicate that the function of these two genes may be more related than was previously thought.

First, *glp-1* may function in the adult soma, and *lin-12* may be able to substitute for this function. These conclusions are suggested by characterization of one unusual allele, *glp-1(q35)*. *glp-1(q35)* has a semi-dominant Muv (multivulval) phenotype[10], suggestive of the *lin-12(gf)* phenotype. This postembryonic somatic phenotype of *glp-1(q35)* was unexpected since the three processes affected by typical *glp-1* null alleles all depend on germ line expression. This *glp-1(q35)* somatic effect suggests that wild-type *glp-1* product may normally be present and functioning in the soma. *glp-1(q35)* DNA contains a premature stop codon and is predicted to generate a truncated product missing the carboxy terminal 122 amino acids (E. Maine, unpublished data). Since this mutation probably would not affect tissue-specific expression, it is likely that *glp-1* is usually expressed in the soma. Indeed, Northern analyses support this conclusion[13]. Why do typical *glp-1* mutations not have a postembryonic somatic phenotype? Perhaps *lin-12* is able to substitute for *glp-1* in the soma. Given the molecular similarity between *lin-12* and *glp-1*, it seems reasonable that they may have some functions that are redundant in the soma. The Muv phenotype of *glp-1(q35)* may arise if *glp-1(q35)* makes an aberrant product that interferes with the normal regulation of vulval development. To speculate further, since homology between the two genes is relatively low in the carboxy terminal region (~28%), perhaps these sequences are important for functionally distinguishing the two proteins. Since the *glp-1(q35)* protein lacks this region, perhaps it is able to interact with the *lin-12* protein to confer a partial *lin-12(gf)* Muv phenotype on the animal.

Second, *glp-1* may be able to receive a signal intended for *lin-12*. This possibility is suggested by studies of the *lin-12* germ line phenotype (G. Seydoux, T. Schedl, and I. Greenwald, *Cell*, in press). Germ line proliferation is abnormal in *lin-12(lf)* mutants: mitotic cells are present at the proximal end of the gonad in addition to the normal site of proliferation at the distal end. From a combination of genetic analyses and cell ablation experiments, Seydoux and her colleagues demonstrate that this unusual proliferation in the germ line of *lin-12* mutants depends on the AC and on *glp-1* activity. Furthermore, in wild-type animals, when cells neighboring the AC are ablated, proximal germline mitosis ensues. Seydoux speculates that the same mechanism may underlie proximal mitosis in *lin-12* mutants and in ablated wild-type animals and that a positive regulatory signal normally received by the *lin-12* product may also be able to activate the *glp-1* product.

Table 3. *Suppression of* glp-1(lf) *by mutations that alter body morphology*[1]

Suppressor	*glp-1* genotype	germline phenotype presence of mitotic germ cells in adult[2]	embryonic phenotype % survival[3]
–	*glp-1(+)*	+	>99
–	*glp-1(−)*	−	0
dpy-1(e1)	*glp-1(−)*	+	15
dpy-2(q292)	*glp-1(−)*	+	15
dpy-3(e27)	*glp-1(−)*	+	38
dpy-7(q288)	*glp-1(−)*	+	12
dpy-8(q287)	*glp-1(−)*	+	19
dpy-9(e12)	*glp-1(−)*	+	11
dpy-10(q291)	*glp-1(−)*	+	15
sqt-1(e1350)	*glp-1(−)*	+	2

[1] Results summarized here are from ref. 29. All animals grown at 20°.
[2] Presence of mitotically dividing germline nuclei was assayed two days after hermaphrodites became young adults.
[3] Data shown here include all embryos produced by at least ten animals. In general, the percentage of healthy progeny was much higher at the beginning of the egg laying period than at the end of it.

If the *lin-12* product does not bind this signal, either because it is mutant or because the cells in which it is expressed have been ablated, then the *glp-1* product that is present in the germ line may do so instead.

Third, *glp-1* and *lin-12* may be functionally redundant during embryogenesis. The *lin-12 glp-1* double mutant, unlike either single mutant, dies during early larval development (J. Kimble, unpublished). Therefore, in *glp-1* and *lin-12* single mutants, the presence of the wild-type product of the other gene is essential for survival. Hence, there appear to be certain developmental processes in which either *lin-12* or *glp-1* can function. Mutant hunts have identified two genes, designated *lag-1* and *lag-2*, with a phenotype similar to that of the *lin-12 glp-1* double mutant (E. Lambie, S. Christensen, and J. Kimble, unpublished data). The products of the *lag* genes may interact with the products of both *lin-12* and *glp-1* in the process of cell communication.

Suppressors of *glp-1*

One way to identify other genes involved in *glp-1*/*lin-12* mediated cell signalling is to isolate extragenic suppressors and/or enhancers of *glp-1* and *lin-12* mutations. Such mutations may identify genes that act specifically in signal production or transduction. Suppressors of *glp-1* (ref. 29; E. Maine, unpublished; J. Priess, personal communication) and of *lin-12* (M. Sundarem and I. Greenwald, personal communication; F. Tax and J. Thomas, personal communication) have been generated, and the bulk of these are under genetic analysis. Since most of this work is unpublished, we only discuss our suppressors of *glp-1*.

We have isolated numerous suppressors of *glp-1* to date including two distinct types of unlinked recessive suppressors and a set of dominant suppressors that are closely linked to *glp-1* (ref. 29; E. Maine, unpublished data) (Tables 3, 4). Each suppressor rescues all three

Table 4. glp-1(lf) *recessive suppressors without a visible phenotype*

Gene	Number of alleles	Chromosome
sog-1	5	*I*
sog-2	1	*II*
sog-3	1	*IV*
sog-4	2	nd
sog-5	1	*X*

mutant phenotypes of *glp-1(lf)* alleles: underproliferation in the germ line, absent anterior pharynx, and abnormal embryonic morphogenesis. However, the recessive suppressors do not bypass a need for *glp-1* function altogether because they do not suppress putative null alleles. One class of *glp-1* recessive suppressors has a striking visible phenotype: animals are shorter than normal or 'dumpy' (Dpy)[29]. The *dpy* mutations isolated as *glp-1* suppressors map to four previously identified genes, *dpy-2*, *dpy-7*, *dpy-8*, and *dpy-10*. Other independently isolated alleles of these genes (e.g., see ref. 30) also suppress the *glp-1(lf)* phenotype. Systematic testing of mutants with altered body shape revealed four other genes that interact with *glp-1* in a similar fashion, *dpy-1*, *dpy-3*, *dpy-9*, and *sqt-1* (Table 3). Clearly, a change in body morphology alone is insufficient for suppression, because mutations in many other *dpy* genes cannot suppress *glp-1(lf)*. Two suppressor genes, *sqt-1*, and *dpy-10*, encode collagens (ref. 31; J. Kramer, personal communication); the products of the other six genes are not known. Collagen is a major component of extracellular matrix, and therefore we have proposed that the putative extracellular domain of *glp-1* interacts with the extracellular matrix of the gonad (ref. 29; Fig. 2). If collagen mutations alter the structure of the gonadal basement membrane, this change may indirectly lead to an increase in the availability of *glp-1* gene product to receive the distal tip cell signal. This model requires that a partially functional *glp-1* be present and explains the lack of suppression of null mutations.

A second set of recessive suppressors results in no

obvious visible phenotype. These mutations identify at least five genes designated *sog*, for *s*uppressor *o*f *glp-1* (Table 4). The lack of a visible mutant phenotype suggests that the *sog* genes may be functionally redundant. This idea is supported by the fact that some suppressors interact even though they map to different genes. For example, the transheterozygote *sog-1(q298)/+*; *sog-5(q345)/+*; *glp-1(ts)* is fertile and has viable progeny (E. Maine, unpublished data).

Finally, nineteen semi-dominant suppressors of *glp-1* have been isolated; each maps genetically to the region of the *glp-1* gene. Molecular analysis is underway to determine whether they are intragenic revertants or mutations in a nearby gene such as *lin-12*. Since the predicted *lin-12* and *glp-1* gene products are very similar, perhaps the time and site of *lin-12* gene expression can be modified by mutation so that *lin-12* protein is available to substitute for *glp-1*.

Two Alternative Models for the Action of *glp-1* and *lin-12* in Development

Many models can be proposed for the regulation of *glp-1* activity. As examples, we present two extremely different models in Fig. 3. One invokes positive control by a diffusible signal and the other invokes negative control by a component of the extracellular matrix. Similar models can also be proposed for the regulation of *lin-12* in the AC/VU decision. For each model, we assume that *glp-1* encodes a transmembrane protein acting in the germ line to promote continued mitotic divisions. We do not extend these models to the early embryo, because we lack fundamental knowledge about the specific embryonic cells involved in induction of the anterior pharynx, the molecular identity of the maternal contribution of *glp-1*, and the state of the extracellular matrix around the mature oocyte and in the early embryo. However, one can imagine that either model might explain the regulation of maternal *glp-1*.

In the model for positive regulation, an activating signal is produced by the distal tip cell and transduction of that signal by *glp-1* leads to germ line mitosis. For the AC–VU interaction, a similar model would postulate production of an activating signal by the AC and signal transduction by *lin-12* to direct VU differentiation.

In the model for negative regulation, the *glp-1* protein is bound to a component of the extracellular matrix except at the distal end of the germ line tube. We select the extracellular matrix as the regulator, in part because of the identification of collagen suppressors (see above) and in part because there are no somatic cells other than the distal tip cell in the vicinity. In this model, *glp-1* protein would be active at the distal end of the germ line because the distal tip cell prevents contact between *glp-1* and the matrix. Therefore, no signal from the distal tip cell is required. For the AC–VU interaction, a similar model would postulate inactivation of the *lin-12* protein in the AC precursor, but not in the VU precursor. The mechanism of inactivation in the anchor cell could involve binding to a nearby component, either the extracellular matrix or another cell. The *lin-12* protein in the VU precursor would remain functional because it lacked these contacts.

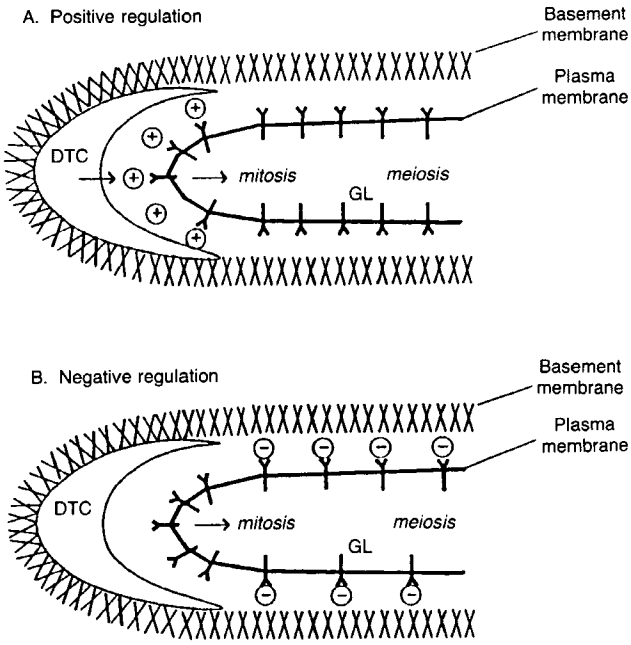

Fig. 3. Models for the regulation of *glp-1*. DTC, distal tip cell; GL, germ line. Diagrams (A) and (B) show the distal end of the gonad, including germ line, somatic distal tip cell, and surrounding basement membrane (extracellular matrix). Germ line nuclei at the distal end of the syncytium are mitotic; more proximal nuclei enter meiosis. Presence of the distal tip cell is required for continued germ line mitosis. A basement membrane surrounds both the distal tip cell and germ line, separating them from the surrounding pseudocoelom (see ref. 2). (In the animal, these structures are tightly apposed; we have left spaces between them to allow signalling and receiving molecules to be drawn.) We propose that *glp-1* encodes a protein (Y) located in the germline plasma membrane. A. Positive regulation: The *glp-1* protein must be activated in the distal region. A positive signal (+) is produced by the distal tip cell and binds the *glp-1* receptor. Activated *glp-1* protein induces mitosis. B. Negative regulation: The *glp-1* protein must be inactivated in the proximal region. A negative signal (−), perhaps a component of the extracellular matrix, binds the *glp-1* receptor outside the distal region. Inactivated *glp-1* protein permits entry into meiosis. The distal tip cell prevents the negative regulation of *glp-1* by blocking the interaction of *glp-1* with the extracellular matrix.

At the current time, there is no means of choosing between these two models. Although a signal from the distal tip cell or anchor cell fits better with current models of signal transduction, no gene or molecule has been identified that might be either signal.

Future Directions

The work on *glp-1* and *lin-12* summarized here provides some clues about how cells interact with each other and/or the extracellular matrix to influence cell fate during development. Current studies are directed towards understanding the function(s) of the *glp-1* and

lin-12 proteins themselves and towards identifying and analyzing other genes (*glp-1* and *lin-12* suppressors, *lag-1* and *lag-2*) that interact with *glp-1* and/or *lin-12*.

Acknowledgements

We thank many of our colleagues, especially J. Ahringer, J. Austin, V. Bardwell, S. Crittendon, T. Evans, C. Fox, V. Kokoyanni, P. Kuwabara, E. Lambie, T. Schedl, and M. Sheets for discussions during the course of this work and critical comments on the manuscript. We thank G. Seydoux, T. Schedl, and I. Greenwald, C. Coffman, C. Kintner, and W. Harris, L. Ellison and J. Sklar, and J. Kramer for communicating results prior to publication. Leanne Olds provided the technical illustrations. This research was supported by a U.S. Public Health Service Grant GM31816 and Research Career Development Award HD00630 to J. K. E.M.M. was supported by Public Health Service Grant GM11569. Many nematode strains used in this study were provided by the *Caenorhabditis elegans* Genetics Center, which is supported by contract N01-AG-9-2113 between the National Institutes of Health and the Curator of the University of Missouri.

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