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18. *lin-12* and *glp-1*: Homologous Genes With Overlapping Functions in *Caenorhabditis elegans*

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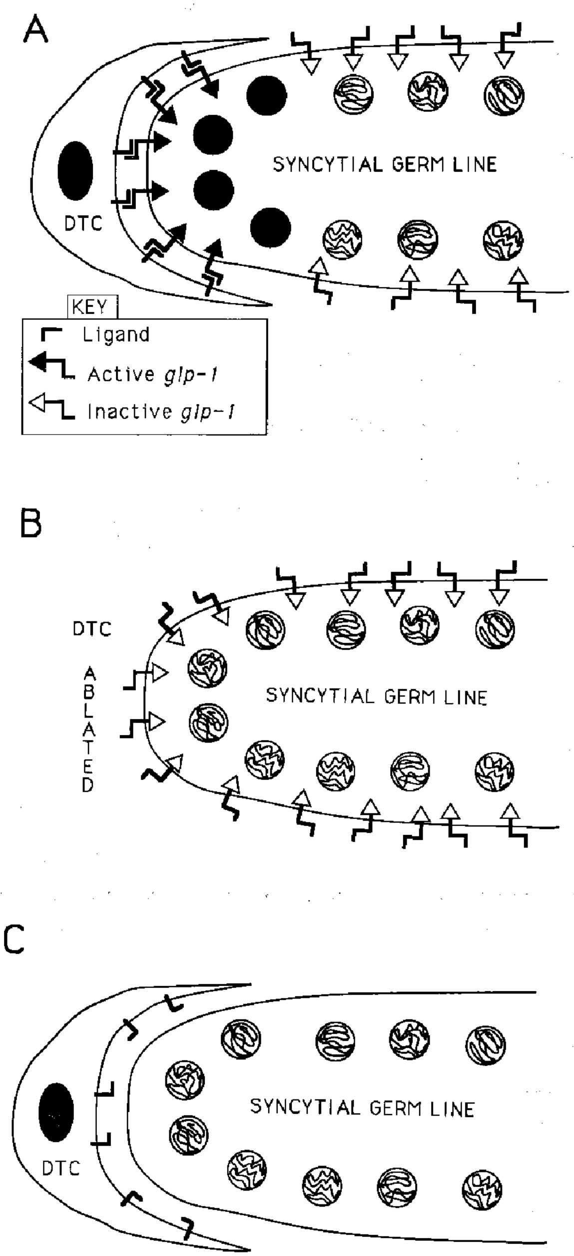
INTRODUCTION

During the development of the nematode, *Caenorhabditis elegans*, cell fates are determined via a combination of cell-autonomous and cell-nonautonomous mechanisms. The latter, regulative phenomena, require the existence of one or more intercellular signaling pathways. In this review, we consider the functions of two genes, *lin-12* (*lin*eage abnormal) and *glp-1* (*g*erm *l*ine *p*roliferation defective), that are required for intercellular signaling during nematode development.

glp-1 FUNCTIONS IN INDUCTIVE SIGNALING

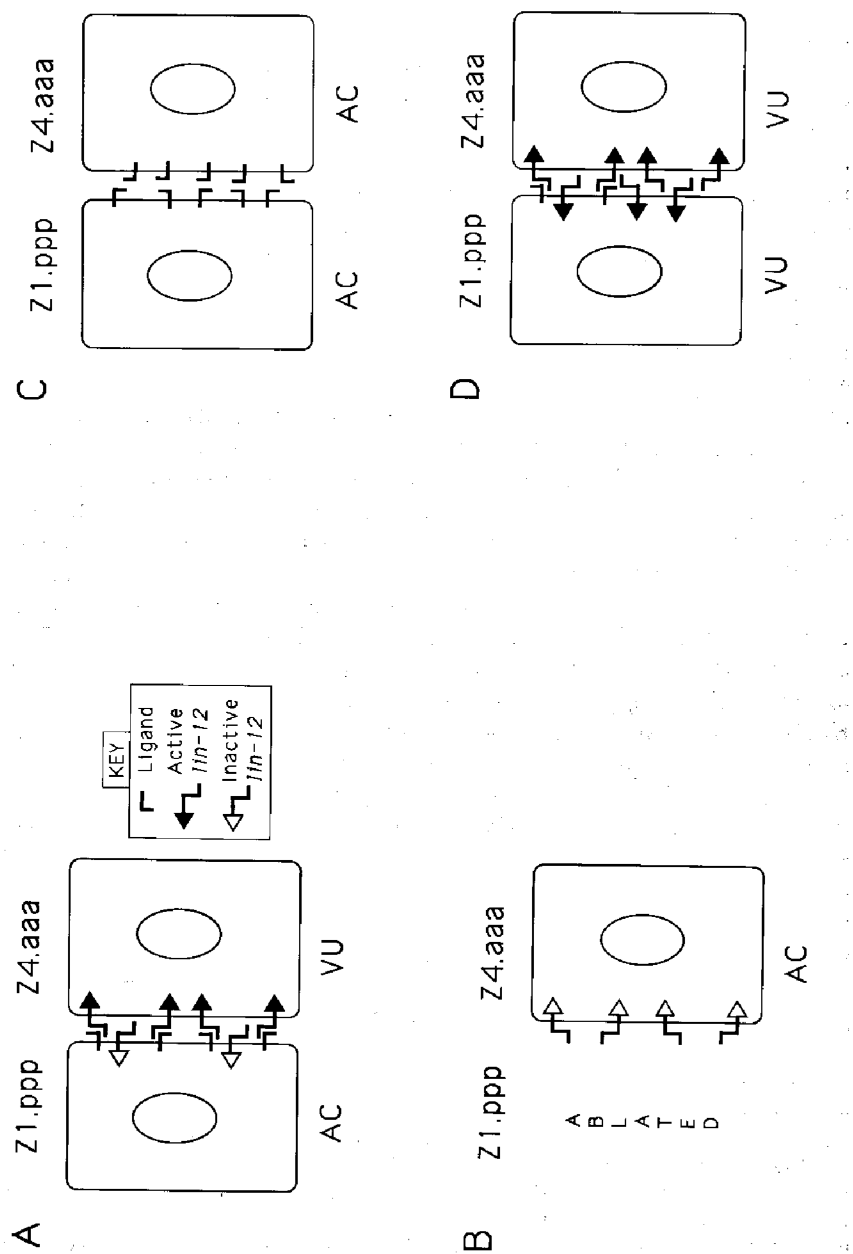
glp-1 is important for inductive signaling, i.e., communication between cells of dissimilar developmental potential. Two well-defined examples of *glp-1*-mediated induction have been identified (Austin and Kimble, 1987; Priess et al., 1987). The first involves the regulation of germline proliferation by the distal tip cell of the somatic gonad (Fig. 1A). The germline nuclei near the distal tip cell normally remain in the mitotic cell cycle, functioning as stem cells for the production of gametes in the adult (Nigon and Brun, 1955; Hirsh et al., 1976; Kimble and Hirsh, 1979). As germline nuclei move proximally, away from the distal tip region, they enter meiosis. The meiotic nuclei follow a progressive program of maturation as they move proximally, eventually differentiating into sperm or oocytes. All germline nuclei share a common cytoplasm (Hirsh et al., 1976). However, even in this syncytium, individual nuclei are compartmentalized into partially membrane-bound regions at the periphery of the gonad. The striking polarity of germline maturation has been shown to depend primarily on the distal tip cell. Removal of the distal tip cell (by laer microsurgery) at any time during development causes the distal germline nuclei to stop dividing mitotically and enter meiosis (Fig. 1B; Kimble and



White, 1981). Thus, the distal tip cell maintains the mitotic stem cell population, either by inducing mitosis or inhibiting entry into meiosis. The zygotic phenotype of *glp-1* mutants strongly resembles that of animals in which the distal tip cell has been ablated (Fig. 1C). The germline nuclei in homozygous *glp-1* null (0) mutants undergo only one or two mitotic divisions before entering meiosis, and eventually differentiate into sperm (Austin and Kimble, 1987; Priess et al., 1987). Consequently, *glp-1* hermaphrodites are sterile; no germline nuclei are available for the production of oocytes. The results of genetic mosaic experiments indicate that *glp-1* is required in the germ line but not the distal tip cell for normal germline proliferation (Austin and Kimble, 1987). Thus, *glp-1* functions in the receiving mechanism in this case of inductive signaling.

The second inductive event that is dependent on *glp-1* occurs early in embryogenesis, between the 4- and 28-cell stages. Each blastomere of the early *C. elegans* embryo follows a unique and invariant lineage: Most of the hypodermis and nervous system are generated by the blastomere AB, while the blastomere EMS primarily produces muscles, gut, and the somatic gonad (Sulston et al., 1983). The pharynx is a neuromuscular feeding organ located at the anterior of the animal; the anterior section of the pharynx is derived from AB and the posterior from EMS. Micromanipulation experiments have revealed that the production of anterior pharyngeal mesoderm by the descendants of AB is dependent upon an inductive signal from EMS (or one of its descendants). When EMS is removed, AB produces only ectodermal tissues (Priess and Thomson, 1987). The role of *glp-1* in this inductive event has been studied by utilizing temperature-sensitive mutants (Priess et al., 1987; Austin and Kimble, 1987). At permissive temperature, homozygous *glp-1(ts)* hermaphrodites are self-fertile and produce normal offspring. However, the embryos produced by such animals do not develop normally if they are shifted to restrictive temperature during early development (prior to the 28-cell stage). These embryos fail to hatch and they exhibit various defects in morphogenesis. Most notably, they lack anterior pharyngeal mesoderm. These lethal developmental defects are strictly dependent upon maternal genotype: *glp-1(+)/glp-1(ts)* heterozygotes produced by crossing a *glp-1(ts/glp-1(ts)* hermaphrodite with a wild-type male do not survive, but *glp-1(0)/glp-1(0)* progeny of a heterozygous hermaphrodite proceed through embryogenesis normally.

Fig. 1. Induction of germline mitosis by the distal tip cell is regulated by *glp-1*. Mitotically proliferating nuclei are represented by black circles, and meiotic nuclei by circles containing wavy lines. The distal tip cell (DTC) nucleus is shown as a shaded oval. The production of a ligand by the distal tip cell, its localization at the cell membrane, and the germline-specific transmembrane location of *glp-1* are hypothetical. The association of *glp-1* with ligand is shown as activating *glp-1* to produce a local mitogenic signal. **A:** Wild type. **B:** Wild type, distal tip cell ablated. **C:** *glp-1(0)*.

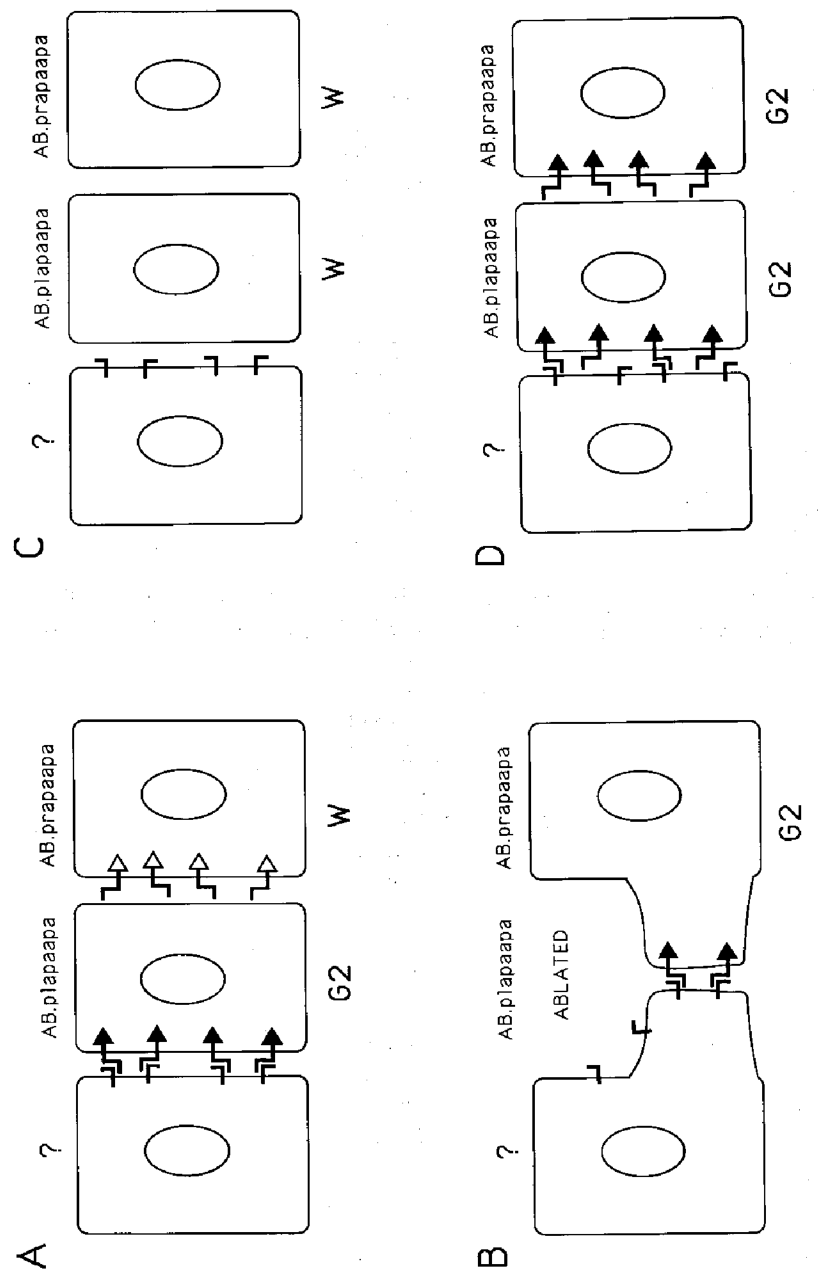


lin-12 FUNCTIONS IN LATERAL SIGNALING

Zygotic *lin-12* activity is important during the development of a number of different somatic tissues (Greenwald et al., 1983), but there is no apparent maternal requirement for *lin-12* (Lambie and Kimble, submitted). In most cases, *lin-12* has been implicated in lateral signaling, i.e., communication between cells of similar developmental potential. The best-characterized examples of *lin-12* function involve equivalence groups. An equivalence group, in its simplest form, consists of two cells with equivalent developmental potential, which cooperate such that one assumes a primary fate, while the other assumes a secondary fate (Sulston and White, 1980; Kimble, 1981). The primary fate is operationally defined as that adopted by a member of an equivalence group when its counterpart has been ablated by laser microsurgery. The restriction of fates within an equivalence group can be thought of as involving two separate decisions. First is the establishment of asymmetry, wherein one cell adopts the primary fate. Second is the propagation of asymmetry, in which a cell that has assumed the primary fate inhibits its neighbor from also adopting the primary fate. The role played by *lin-12* in each of these decisions appears to depend upon context. We consider three different equivalence groups here and how mutations in *lin-12* affect each.

The first two examples both involve the development of the vulva, the ventral opening in the hypodermis through which eggs are laid. The anchor cell (AC), which induces the underlying hypodermal cells to divide and produce the vulva, is derived from an equivalence group consisting of two cells of the somatic gonad, Z1.ppp and Z4.aaa (Fig. 2A; Kimble and Hirsh, 1979; Kimble, 1981). The primary fate is to become an AC, while the secondary fate is to become a ventral uterine precursor cell (VU) (Fig. 2B). The initial asymmetry in this equivalence group is apparently established stochastically, with either Z1.ppp or Z4.aaa adopting the primary fate. In recessive *lin-12* loss-of-function (lf) mutants, both Z1.ppp and Z4.aaa adopt the primary fate (Fig. 2C; Greenwald et al., 1983). Therefore, *lin-12* is required for the primary cell to signal its counterpart to assume the secondary fate. In dominant *lin-12* gain-of-function (gf) mutants, Z1.ppp and Z4.aaa both adopt the secondary fate (Fig. 2D; Greenwald et al., 1983). Moreover, if either Z1.aaa or Z4.ppp is ablated in a *lin12(gf)* mutant, the remaining cell adopts the secondary fate (Seydoux and Greenwald, 1989, 1990). This indicates that *lin-12* activity is sufficient to induce a cell to adopt the secondary fate. Genetic mosaic experiments have shown that *lin-12* activity is required only within the cell that adopts the

Fig. 2. Lateral signaling in the AC/VU equivalence group is regulated by *lin-12*. The production of a ligand by the AC cell, its localization at the cell membrane, and the transmembrane location of *lin-12* are hypothetical. **A:** Wild type. **B:** Wild type, Z1.ppp ablated. **C:** *lin-12(0)*. **D:** *lin-12(gf)*.



secondary fate, thus indicating that *lin-12* functions as part of the receiving mechanism in this case of lateral signaling (Seydoux and Greenwald, 1989).

The vulval precursor cells (VPCs) of the hypodermis are also members of an equivalence group. In this case, the primary fate is to execute a particular sublineage, while the secondary fate is to undergo a different sublineage (Sulston and White, 1980; Sternberg and Horvitz, 1986). The initial asymmetry is established by the AC, which induces adjacent VPCs to divide (Kimble, 1981). This induction is not dependent upon *lin-12* (Greenwald et al., 1983). The most proximal VPC assumes the primary fate and laterally signals its neighbors to assume the secondary fate (Sternberg, 1988). In *lin-12(lf)* mutants, this lateral signaling is defective and none of the VPCs adopt the secondary fate (Greenwald et al., 1983). In *lin-12(gf)* mutants, all members of the equivalence group adopt the secondary fate (Greenwald et al., 1983). Thus, in both of the cases considered so far, *lin-12* is essential for the assumption of the secondary fate; *lin-12(lf)* mutations apparently sever the communication between a primary cell and its neighbors, whereas *lin-12(gf)* mutations cause all cells to behave as if they are receiving a signal from an adjacent primary cell.

The situation is different in the third case considered here (Fig. 3). The embryonic blast cells, AB.plapaapa and AB.prapaapa, define an equivalence group (Fig. 3A,B; Sulston et al., 1983). The mechanism by which asymmetry is established is not known, but in wild-type animals, AB.plapaapa invariably assumes the primary fate (designated G2) and AB.prapaapa, the secondary fate (designated W). In *lin-12(lf)* mutants, both cells adopt the secondary fate, and in *lin-12(gf)* mutants both assume the primary fate (Fig. 3C,D; Greenwald et al., 1983). Thus, in this case *lin-12* does not appear to mediate the reception of a signal through which a primary cell induces its neighbor to assume the secondary fate. A simple explanation for the data would be that AB.plapaapa and AB.prapaapa compete for an extrinsically generated signal whose reception is mediated by *lin-12* and induces the primary fate. AB.plapaapa could be predisposed to adopting the primary fate if it intervened between AB.prapaapa and the source of the signal. This situation would be analogous to that observed in the development of the lateral hypodermis, where one member of an equivalence group can apparently intercept an extrinsic signal and thus prevent it from influencing other members of the same equivalence group (Waring and Kenyon, 1990).

In addition to its role in equivalence groups, *lin-12* regulates the fates of several pairs of nonequivalent, but homologous, cells (Greenwald et al., 1983).

Fig. 3. Lateral signaling in the G2/W equivalence group may be only indirectly regulated by *lin-12*. **A:** Wild type. **B:** Wild type, AB.plapaapa ablated. **C:** *lin-12(0)*. **D:** *lin-12(gf)*. Symbols are as in Figure 2. The production of a membrane bound ligand by an unidentified cell (labeled "?") that is ordinarily juxtaposed to AB.plapaapa, and the transmembrane location of *lin-12* are hypothetical.

For example, in male *C. elegans*, AB.plpppaaaa normally differentiates into a neuron (designated DA9), and its homologue, AB.prpppaaaa, executes a sublineage (designated Y). The fate of AB.plpppaaaa is unaffected by the ablation of AB.prpppaaaa, and vice versa; however, in a *lin-12(0)* mutant, both cells assume the DA9 fate, and, in a *lin-12(gf)* mutant, both execute the Y sublineage. *lin-12* may be required for intercellular signaling in such cases, but this signaling is not known to occur, either directly or indirectly, between the two cells whose fates are affected. Alternatively, *lin-12* may function in these contexts as part of an autocrine regulatory mechanism.

lin-12 AND *glp-1* ENCODE PUTATIVE TRANSMEMBRANE RECEPTORS

The *lin-12* and *glp-1* genes are located adjacently on chromosome III, separated by about 20 Kbp (Austin and Kimble, 1987; Yochem and Greenwald, 1989). They are transcribed convergently, and each gene produces a single major transcript (Austin and Kimble, 1989). *lin-12* and *glp-1* are remarkably similar in amino acid sequence (50–60% identical) and in overall molecular organization (Greenwald, 1985; Yochem et al., 1988; Yochem and Greenwald, 1989). It appears likely that they arose through the duplication of an ancestral gene. Moreover, both genes are homologous to the *Notch* gene of *Drosophila*, which is thought to function in a number of intercellular signaling events during fly development (Wharton et al., 1985; Kidd et al., 1986). *lin-12*, *glp-1*, and *Notch* are members of a gene family whose members mediate regulatory cell interactions during development. Additional members of this gene family have recently been isolated from several vertebrates, including *Xenopus* and *Homo sapiens* (Coffman et al., 1990; L. Ellisen and J. Sklar, personal communication).

A comparison of the predicted *lin-12* and *glp-1* proteins is diagramed in Figure 4. Each gene has an amino terminal hydrophobic region that could act as a signal sequence, and an internal hydrophobic region that could be a transmembrane domain. *Notch* has been shown to exist as a membrane-spanning protein, supporting the view that the region between the two hydrophobic segments exists as an extracellular domain, and the carboxy terminal portion as an intracellular domain (Kidd et al., 1989; Johansen et al., 1989; Fehon et al., 1990). The putative extracellular domains contain two main types of cysteine-rich repeated motifs: There are multiple tandem copies of a sequence homologous to epidermal growth factor (EGF), and three tandem copies of an "LNG" sequence, unique to the members of the *lin-12/Notch/glp-1* family (Greenwald, 1985; Yochem and Greenwald, 1989). The putative intracellular domain contains six tandem copies of a sequence first described in two yeast genes, the *cdc10* gene of *S. pombe*, and the *SWI6* gene of *S. cerevisiae* (Breeden and Nasmyth, 1987). This motif has also been found in other genes, e.g., the *fem-1* gene of *C. elegans* and the vertebrate erythrocyte ankyrin gene (Spence et al., 1990; Lux et al., 1990).

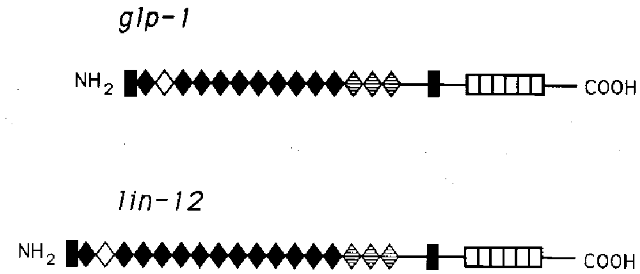


Fig. 4. The predicted protein products of *lin-12* and *glp-1* have extensive similarity. Sequence motifs shared by *lin-12* and *glp-1* are represented by the following symbols: filled rectangle, hydrophobic region; filled diamond, EGF-like repeat; open diamond, nonrepeated cysteine-rich element; striped diamond, LNG repeat; open rectangle, *cdc10/SWI6*-like repeat. Horizontal line indicates coding regions that are not strongly conserved.

The functions of these different domains are not known. However, the extracellular EGF-like motif has been found in a number of proteins that are thought to interact with transmembrane proteins and with components of the extracellular matrix (for review, see Davis, 1990). The *cdc10/SWI6* domain may mediate an interaction with the cytoskeleton, since there is evidence that erythrocyte ankyrin can anchor a membrane protein to the cytoskeleton (Bennett and Stenbuck, 1979).

The combined molecular and genetic data for *lin-12* and *glp-1* argue strongly that they function as transmembrane receptor proteins. Seydoux and Greenwald (1990) have proposed that *lin-12* and *glp-1* transduce intercellular signals by changes in their association state: Binding of an extracellular ligand would induce dimerization, which would activate the intracellular domain for signal transmission. As support for this model, they note that all eight of the *lin-12(gf)* mutations that have been sequenced are present within a restricted region of the putative extracellular domain, and could conceivably result in an increased, or ligand-independent, tendency toward dimerization. At present, there is no direct evidence that the *lin-12* protein forms dimers. In theory, *lin-12(gf)* mutations could affect any of a number of properties of the *lin-12* protein, including local conformation, posttranslational modification, turnover rate, and subcellular targeting.

The nature of the proposed extracellular ligand, whether diffusible, membrane bound, or affixed to an extracellular matrix, is unknown. It is possible that several ligands exist, and that they have different properties, or even opposing effects. Moreover, the effect of ligand binding could vary depending upon the identity of the cell expressing *lin-12* or *glp-1*. Hedgecock et al. (1990) have observed that lateral signaling between Z1.ppp and Z4.aaa is disrupted in mutants where the two cells are not closely apposed. This suggests that the AC to VU signal might not be readily diffusible.

Indeed, recent studies with transfected *Drosophila* tissue culture cells have revealed that the extracellular region of *Notch* protein binds in vivo to the extracellular region of another transmembrane protein, *Delta* (Fehon et al., 1990). *Delta* is one of five other zygotic neurogenic loci that have been identified in *Drosophila* (Lehman et al., 1983). Thus, *Delta* could act as an extracellular signal received by *Notch*. Other zygotic neurogenic loci have been found to encode a transmembrane channel protein (*big brain*: Rao et al., 1990), a G-protein (*groucho*: Preiss et al., 1988), and nuclear proteins (*Enhancer of split*: Klambt et al., 1989; *mastermind*: Smoller et al., 1990). These proteins are thought to act in conjunction with the products of the maternal neurogenic loci, *almondex* and *pecanex* (Perrimon et al., 1986; LaBonne et al., 1989), mediating one or more signal transduction pathways required for the specification of the nonneural ectodermal fate.

lin-12 AND *glp-1* ARE FUNCTIONALLY INTERCHANGEABLE

It is clear from the results of genetic studies that *lin-12* and *glp-1* play different roles during development. The presumed evolution of *lin-12* and *glp-1* from a common ancestor raises the question of how these homologous genes have assumed such disparate functions. In the early stages of their divergence, they must have interacted with the same proteins (and other factors) both extracellularly and intracellularly. Have these other proteins also evolved so that they interact specifically with either *lin-12* or *glp-1*, or is some other form of regulation responsible for dividing the functions of these two genes? One clue to this riddle lies in the observation that the bulk of *glp-1* RNA is expressed in the germline, whereas *lin-12* RNA is expressed primarily in the soma (Austin and Kimble, 1989). Thus, the separate roles of *lin-12* and *glp-1* could be instituted by limiting their expression to specific tissues. If so, *lin-12* and *glp-1* might actually be capable of functioning interchangeably, given the opportunity. Several lines of genetic and molecular evidence suggest that this is correct.

First, an unusual allele of *glp-1*, designated *q35*, has a semidominant multivulva phenotype resembling that seen in *lin-12(gf)* mutants (Austin and Kimble, 1987; S. Mango and J. Kimble, unpublished observations). This suggests that *glp-1(q35)* is expressed in a somatic tissue (the hypodermis), where it mimics the effect of excessive *lin-12* activity. The somatic effect of *glp-1(q35)* is surprising, but not irreconcilable with previous observations, since low levels of *glp-1* RNA have been detected in somatic tissues (Austin and Kimble, 1989). The *glp-1(q35)* mutation introduces a stop codon 122 amino acids from the normal carboxy terminus, and thus may affect some aspect of posttranscriptional regulation (E. Maine, personal communication). Indeed, since the homology between *lin-12* and *glp-1* is relatively low in the carboxy terminal region (about 28%), it may be that sequences contained within this region are involved in differentially regulating the activity or turnover rate of the two proteins.

A second indication that *glp-1* and *lin-12* may share functional interactions comes from analyses of the germline phenotype of *lin-12(lf)* mutants. In *lin-12(lf)* mutants, the germline nuclei in both the proximal and distal arms of the gonad undergo mitotic proliferation (Seydoux et al., 1990). The aberrant proximal germline proliferation is dependent upon the presence of the anchor cell and requires *glp-1* activity (presumably in the germline). This effect can be phenocopied by ablating the somatic gonadal cells that normally separate the AC from the germline. Seydoux et al. (1990) speculate that the lateral signal from the AC to the VU is capable of inducing mitosis in the germline by binding to and activating *glp-1*. In wild-type animals this signal would be intercepted by *lin-12* molecules expressed on the surface of the intervening somatic cells. This model is similar to that depicted in Figure 3, regarding the effect of *lin-12* mutations on the G2/W equivalence group.

Double mutant analysis has provided the most direct evidence that *lin-12* and *glp-1* are capable of functioning interchangeably. Unlike either single mutant, the *lin-12(lf) glp-1(lf)* double mutant invariably arrests and perishes in the first larval stage (Lambie and Kimble, submitted). These lethally arrested larvae exhibit a distinctive combination of abnormalities, collectively termed the Lag phenotype (*lin-12* and *glp-1*). Most or all of the defects observed appear to result from embryonic cell fate transformations; some structures are duplicated (e.g., the excretory pore) and others are absent (e.g., the rectum). The duplication of the excretory pore is thought to result from a failure in communication between the members of an equivalence group. Overall, these results are interpreted to mean that there are certain embryonic cell fate decisions where the zygotic expression of either *lin-12* or *glp-1* is sufficient for normal development. Given the molecular similarity between *lin-12* and *glp-1*, it seems reasonable to suppose that in these cases, *lin-12* and *glp-1* are capable of interacting with common intercellular and extracellular components of the signaling pathway.

IDENTIFICATION OF GENES THAT INTERACT WITH *lin-12* AND/OR *glp-1*

Several genetic screens have been performed in attempts to identify genes that interact with *lin-12* and/or *glp-1*. Suppressor screens have been successfully used to identify genes that modify the phenotypes produced by *lin-12(lf)* and *lin-12(gf)* mutations (M. Sundaram and I. Greenwald, personal communication; F. Tax, J. Thomas, and R. Horvitz, personal communication). Similar strategies have been used to isolate suppressors of *glp-1(ts)* mutants (Maine and Kimble, 1989; E. Maine, personal communication; J. Priess, personal communication). Two distinct types of unlinked recessive suppressors and a set of dominant suppressors that are linked to *glp-1* have been identified (for review, see Maine and Kimble, 1990). Several of the recessive suppressor loci have been found to encode collagens (Kramer et al., 1988; J. Kramer, personal communication). To explain these observations, Maine and Kimble (1989) have suggested that the extracellular matrix of the gonad regulates the activity state of *glp-1* via an interaction with the extracellular domain of *glp-1*, and that mutations in collagen genes could alter the structure of the gonadal basement membrane in such a way as to perturb this regulation.

A different approach for identifying genes that interact with *lin-12* and *glp-1* has been to search for genes that are required for the activities of both *lin-12* and *glp-1* (Lambie and Kimble, submitted). Two genes, *lag-1* and *lag-2*, have been identified in this way as being required for both *lin-12* and *glp-1* to function. Loss-of-function mutations in either gene are recessive and result in a Lag phenotype that is identical with that of *lin-12 glp-1* double mutants. Leaky or temperature-sensitive alleles of *lag-1* and *lag-2* produce postembryonic phenotypes resembling those seen in both *lin-12(lf)* (two anchor cells) and *glp-1(lf)* (failure of germline proliferation) single mutants. The functions of *lag-1* and *lag-2* are not known; however, in view of the similarity between *lin-12*, *glp-1*, and *Notch*, it seems possible that *lag-1* and/or *lag-2* are homologous to the zygotic neurogenic loci of *Drosophila*.

FUTURE DIRECTIONS

As our understanding of the molecular mechanisms that underlie the functioning of *lin-12* and *glp-1* has expanded, the apparent differences between inductive signaling and lateral signaling have correspondingly diminished. Detailed comparisons of the temporal and spatial distribution of the *lin-12* and *glp-1* gene products should soon be possible through the use of gene-specific antibodies. Transformation-mediated reverse genetics will permit the dissection of the various coding and noncoding sequences at the *lin-12* and *glp-1* loci. The molecular isolation and characterization of the various genes that are required for *lin-12* and/or *glp-1* to function will provide important insights regarding the functioning of *lin-12* and *glp-1*, and will also allow comparisons to be made between the molecular hardware of cell-cell communication as it exists in *C. elegans* and in other organisms.

ACKNOWLEDGMENTS

We wish to thank L. Ellisen, J. Sklar, M. Sundaram, I. Greenwald, R. Horvitz, F. Tax, J. Thomas, E. Maine, and S. Mango for communicating unpublished results, and Diane Church for comments on the manuscript. Research done in the authors' laboratory was funded by NIH grant GM31816 to J.K. E.J.L. was supported by postdoctoral fellowship DRG-989 from the Damon Runyon-Walter Winchell Cancer Fund.

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