Regulation of Induction by GLP1, a Localized Cell Surface Receptor in *Caenorhabditis elegans*

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During the development of the nematode *Caenorhabditis elegans*, cell interactions influence fundamental decisions of growth, differentiation, and pattern formation from early embryogenesis through adulthood (for review, see Lambie and Kimble 1991a). The powerful genetics and cellular simplicity of *C. elegans* make it an ideal organism to analyze the mechanisms by which cell interactions regulate cell fate. Specific cell interactions can be defined at the level of individual cells, and genes controlling those interactions can be identified and manipulated both genetically and molecularly. In this paper, we focus on the role of a single gene, *glp-1* (for *g*erm*l*ine *p*roliferation defective), in the control of cell fate by inductive signaling.

The activity of *glp-1* is required for at least two inductive events during *C. elegans* development. First, maternal *glp-1* is essential in the early embryo for induction of pharyngeal tissue (Austin and Kimble 1987; Priess et al. 1987). During normal development, two cells of the 4-cell embryo, called ABa and EMS, interact to generate a complete pharynx. Specifically, EMS (or its descendants) signals ABa (or its descendants) to make the anterior portion of the pharynx; this cell interaction occurs during the 4- to 28-cell stage of embryogenesis. In the absence of EMS, ABa does not generate pharyngeal tissue (Priess and Thomson 1987; Bowerman et al. 1992). Similarly, in the absence of *glp-1* activity, ABa makes no pharyngeal tissue (Austin and Kimble 1987; Priess et al. 1987). Therefore, a simple hypothesis is that *glp-1* directly mediates induction of ABa to form pharyngeal tissue. In addition to its postulated role in pharyngeal induction, maternal *glp-1* is required for hypodermal development and morphogenesis of the embryo. Second, zygotic *glp-1* is required for induction of germ-line proliferation during larval development and adulthood (Austin and Kimble 1987; Priess et al. 1987). During normal development, a single somatic cell, called the distal tip cell, signals the adjacent germ-line tissue to divide mitotically. In the absence of the distal tip cell, the germ line fails to proliferate: the few germ-line nuclei present leave mitosis, enter meiosis, and differentiate (Kimble and White 1981). Similarly, in the absence of *glp-1* gene activity, germ-line nuclei leave mitosis, enter meiosis, and differentiate (Austin and Kimble 1987; Priess et al. 1987). The simplest explanation of these results is that *glp-1* directly mediates the induction of germ-line proliferation by the distal tip cell.

The *glp-1* gene functions in the germ-line tissue rather than the distal tip cell and has been postulated to be part of the receiving mechanism in inductive signaling (Austin and Kimble 1987). Specifically, the germ line fails to proliferate in genetic mosaic animals whose distal tip cell has wild-type *glp-1*, but whose germ line lacks *glp-1*. In contrast, the germ line proliferates in mosaic animals whose germ line has wild-type *glp-1* and whose distal tip cell lacks *glp-1*. Therefore, *glp-1* functions in the germ line rather than the distal tip cell. A similar mosaic analysis cannot be done for maternal *glp-1* to clarify its role in embryogenesis; however, by analogy with the result obtained in the distal tip cell/germ-line mosaics, we predict that *glp-1* is required in ABa (or its descendants), which is the receiving cell rather than the signaling cell in pharyngeal induction.

The *glp-1* gene is a member of the LNG family, which is named for its founding members, *lin-12*, *Notch*, and *glp-1* (Austin and Kimble 1989; Yochem and Greenwald 1989). The *lin-12* and *Notch* genes, like *glp-1*, mediate cell interactions that regulate cell fate (Greenwald et al. 1983; Lehmann et al. 1983). Other members of the family include *Xenopus Xotch* (Coffman et al. 1990), rat *Notch* (Weinmaster et al. 1991), mouse *int-3* (Robbins et al. 1992), and human *TAN-1* (Ellisen et al. 1991). The developmental roles of the vertebrate LNG proteins are not understood, although lesions in TAN-1 and *int-3* protein are associated with lymphoid and mammary tumors, respectively (Ellisen et al. 1991; Robbins et al. 1992).

LNG proteins appear to be transmembrane proteins (Greenwald 1985; Wharton et al. 1985; Yochem et al. 1988; Austin and Kimble 1989; Yochem and Greenwald 1989; Coffman et al. 1990; Ellisen et al. 1991; Weinmaster et al. 1991). All possess one hydrophobic sequence at the amino terminus, which is thought to be a signal sequence, and a second hydrophobic sequence in the middle of the protein, which is thought to be a membrane-spanning domain. The *Notch* protein has been shown definitively to be a membrane protein with one transmembrane domain (Johansen et al. 1989; Kidd et al. 1989); the *glp-1* and *lin-12* proteins have been shown to be membrane associated (S. Crittenden and J. Kimble, unpubl.; see below), and by analogy with *Notch*, they, and all LNG proteins, are likely to be transmembrane proteins.

LNG proteins are composed of three types of repeated motifs (Greenwald 1985; Wharton et al. 1985;

Yochem et al. 1988; Austin and Kimble 1989; Yochem and Greenwald 1989; Coffman et al. 1990; Ellisen et al. 1991; Weinmaster et al. 1991). Here we focus on the *glp-1* protein, which we call GLP1. In the putative extracellular domain of GLP1 are ten EGF-like repeats and three cysteine-rich LNG repeats (other names for the LNG repeat include the *Notch* repeat [Wharton et al. 1985], the B-type cysteine-rich repeat [Kidd et al. 1989], or the LNR [*lin-12/Notch* repeat] motif [Yochem and Greenwald 1989]). The LNG repeats, which are unique to LNG proteins, are located just outside the single membrane-spanning domain; the tandem array of EGF-like repeats is found at the amino terminus. In the putative intracellular domain of GLP1 are six copies of the *cdc10*/SWI6 motif, which was originally discovered in two yeast regulatory genes (Aves et al. 1985; Breeden and Nasmyth 1987a,b). More recently, repeats of the *cdc10*/SWI6 motif have been found in the cytoskeletal protein ankyrin (Lux et al. 1990). All members of the LNG family have three LNG repeats extracellularly and six *cdc10*/SWI6 repeats intracellularly. The number of EGF-like repeats, however, is variable: GLP1 has ten EGF-like repeats, *lin-12* protein has 13 repeats, and *Notch* and the vertebrate homologs have 36 repeats. Therefore, there appears to be a *Notch* subfamily of proteins with 36 EGF-like repeats within the LNG superfamily. Nonetheless, the conservation of protein architecture among members of the LNG family, in addition to the similarity in their developmental roles, suggests that these proteins all function by a similar molecular mechanism.

In addition to being similar in structure and function, the *glp-1* and *lin-12* proteins are functionally interchangeable. Three lines of evidence support this idea. First is the phenotype of the *lin-12 glp-1* double mutant (Lambie and Kimble 1991b). Whereas both *lin-12* and *glp-1* single mutants usually reach adulthood (when derived from a *glp-1*/ + mother), the *lin-12 glp-1* double mutant invariably arrests in the first larval stage. Therefore, in *glp-1* and *lin-12* single mutants, the presence of the wild-type product of the other gene is essential for survival. This means that certain developmental processes can rely on either *glp-1* or *lin-12* function. Second is the similarity in the effects of certain dominant (d) mutations of *lin-12* and *glp-1* on vulval development (Greenwald et al. 1983; Mango et al. 1991). Normally, a cell in the somatic gonad, the anchor cell, signals to the underlying vulval precursor cells to form a vulva (Kimble 1981); in addition, the vulval precursor cells interact among themselves to ensure that only one vulva is made (Sulston and White 1980). In *lin-12(d)* and *glp-1(q35)* mutations, many pseudovulvae are made (Muv phenotype) in an anchor-cell-independent manner (Greenwald et al. 1983; Mango et al. 1991). Third is the effect of *lin-12* loss-of-function mutations on germ-line development (Seydoux et al. 1990). Normally, the germ line is organized such that mitotic cells are distal and meiotic cells are proximal (see Fig. 1). In *lin-12(lf)* mutants,

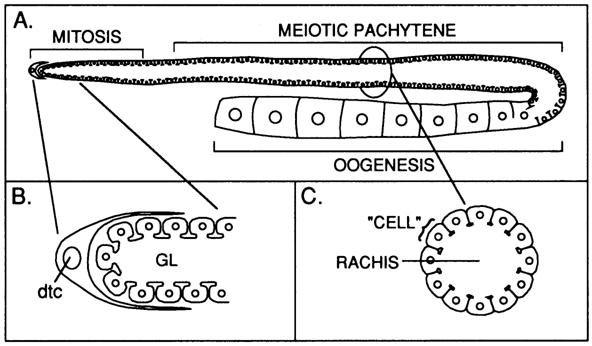

Figure 1. Anatomy of the adult hermaphrodite germ line. (*A*) Each ovotestis is a U-shaped structure with mitotic germ-line nuclei found distally, nuclei in meiotic pachytene found more proximally, and differentiating gametes found most proximally (Hirsh et al. 1976). (*B*) The distal tip cell sends out cytoplasmic processes that embrace the last few germ "cell" diameters in the germ-line tube (D. Hall, pers. comm.). (*C*) The germ-line tissue is a syncytium with most nuclei distributed around the periphery of the germ-line tube. Each germ-line nucleus is partially partitioned from a central core of cytoplasm, or rachis, by membranes; a germ "cell" refers to a unit composed of one nucleus plus its surrounding cytoplasm and membranes. For a review of gonadal anatomy, see Kimble and Ward (1988).

mitotic germ-line nuclei are present in an aberrant position at the proximal end of the gonad in addition to being found at the normal distal site. Seydoux and colleagues speculate that a signal from the anchor cell normally meant for LIN12, instead activates GLP1.

The functional overlap between *lin-12* and *glp-1* proteins implies that the major differences in the developmental roles of the *glp-1* and *lin-12* genes rely on differences in regulation of the two genes. The *glp-1* gene is primarily responsible for germ-line dependent interactions (embryonic induction of the pharynx depends on maternal *glp-1*, which is thought to be produced by the germ line). The *lin-12* gene, in contrast to *glp-1*, is primarily responsible for somatic interactions and specifically for signaling between cells of equivalent developmental potential (lateral signaling) (Greenwald et al. 1983). One simple mechanism to achieve this disparity in the roles of functionally similar proteins is to confine the expression of each to distinct tissues. By Northern blot analysis, it has been shown that *glp-1* RNA accumulates primarily in the germ line, whereas *lin-12* RNA is preferentially somatic (Austin and Kimble 1989). In addition, analysis of the *glp-1(q35)* mutation indicates that the somatic activity of *glp-1* may be inhibited posttranslationally (Mango et al. 1991). Therefore, two regulatory mechanisms have already been discovered that could explain the restriction of *glp-1* activity to the germ line.

In this paper, we address two fundamental questions about the function and regulation of GLP1. First, where is the GLP1 protein found during development? The current models for GLP1 function postulate that it acts as a receptor for an inductive signal. Is the location of GLP1 consistent with this model? Second, what are the critical functional domains in the GLP1 protein?

The conservation of sequence elements among the LNG proteins suggests that the repeated motifs may be critical. What are the molecular defects associated with *glp-1* mutants? Do the sites of the molecular lesions provide insight into the functional domains of GLP1 and LNG proteins generally?

MATERIALS AND METHODS

Methods used in the course of this work are described in detail elsewhere (Kodoyianni et al. 1992; S. Crittenden et al., in prep.). Briefly, for GLP1 protein localization, we used affinity-purified rat polyclonal antibodies raised against bacterially produced pGEX fusion proteins (Smith and Johnson 1988). Immunocytochemistry was done using germ lines extruded from the animal and with early embryos. Photographs were obtained on a BioRad MRC-600 laser scanning confocal microscope. For mapping mutations, lesions were localized roughly within the *GLP-1* gene, either by DNA-RNA mismatch (Myers et al. 1985) or by chemical cleavage of DNA-DNA hybrids (Cotton et al. 1988), and then the specific nucleotide changes were ascertained by DNA sequencing.

RESULTS

GLP1 Is Localized in Both Germ Line and Embryo

To learn the location of the GLP1 protein during development, we raised polyclonal antibodies against two fragments: One contained four EGF-like repeats (EGFL-7 to EGFL-10) and the other consisted of the three LNG repeats (S. Crittenden et al., in prep.). These antibodies recognize GLP1 in fixed tissues by immunocytochemistry and also in protein extracts by Western blot. We find the distribution of GLP1 to be restricted in both germ line and embryo.

Germ line. The germ line is a syncytial tissue: In the distal portion of the gonad, germ-line nuclei are arranged around a central core of cytoplasm and each nucleus is partially enclosed by membrane (Fig. 1). Within the germ-line syncytium, the most distal germ-line "cells" are mitotic, whereas more proximal "cells" have left the mitotic cell cycle and entered meiosis (Fig. 1) (Hirsh et al. 1976). We use the term germ "cell" to refer to a unit composed of one nucleus plus its surrounding cytoplasm and membranes (see Fig. 1C). GLP1 is associated with the plasma membranes of the distal-most germ-line "cells," which are in the mitotic cell cycle (Fig. 2). GLP1 is not detected in the distal tip cell. The germ-line staining of GLP1 is most intense at the distal end and becomes weaker more proximally. In the region where germ cells leave the mitotic cell cycle and enter meiosis, GLP1 becomes located within the cytoplasm of the syncytium (Fig. 2). No GLP1 is found in oocytes (data not shown). From these results, we make two conclusions. First, the localization of GLP1 to the mitotic region is consistent with the idea that GLP1 is a membrane receptor involved in induction of germ-line proliferation by the distal tip cell. Second,

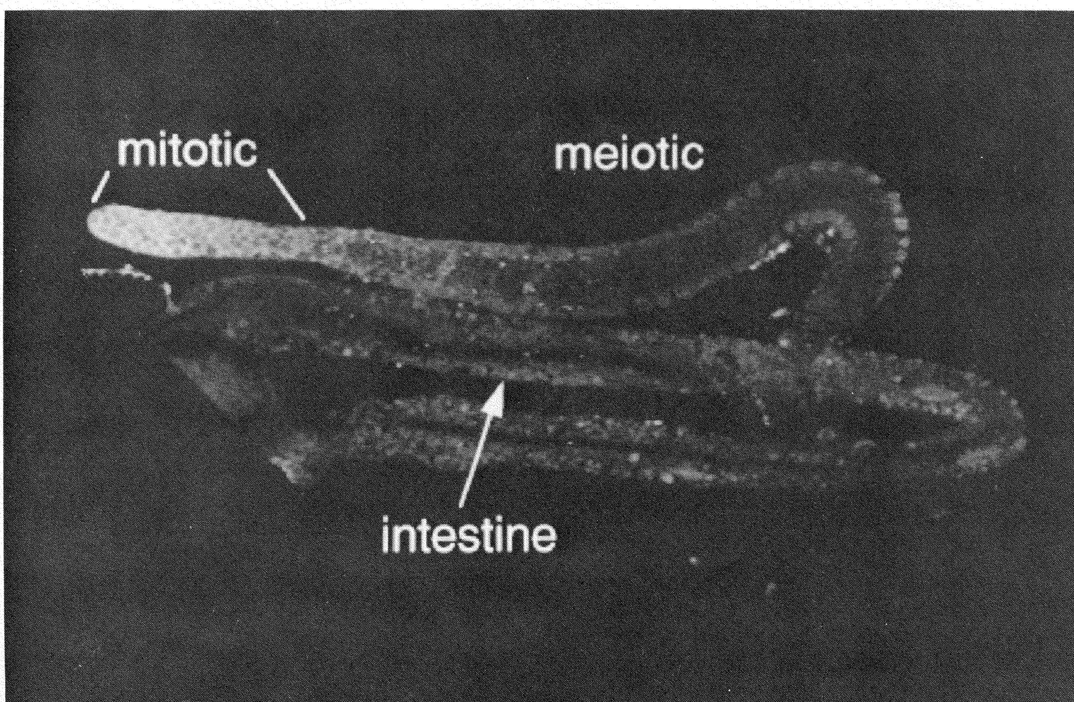

Figure 2. GLP1 is localized to the distal mitotic region of the germ line. An ovotestis and intestine were extruded from a wild-type hermaphrodite adult, stained with affinity-purified antibodies to the LNG region of GLP1, and examined using a BioRad MRC-600 confocal microscope. In the extruded gonad, GLP1 is associated with membranes of germ-line "cells" in the distal mitotic region. The level of GLP1 staining decreases as germ-line "cells" leave mitosis and enter meiosis. GLP1 is not detected in the intestine. For more detail, see S. Crittenden et al. (in prep.).

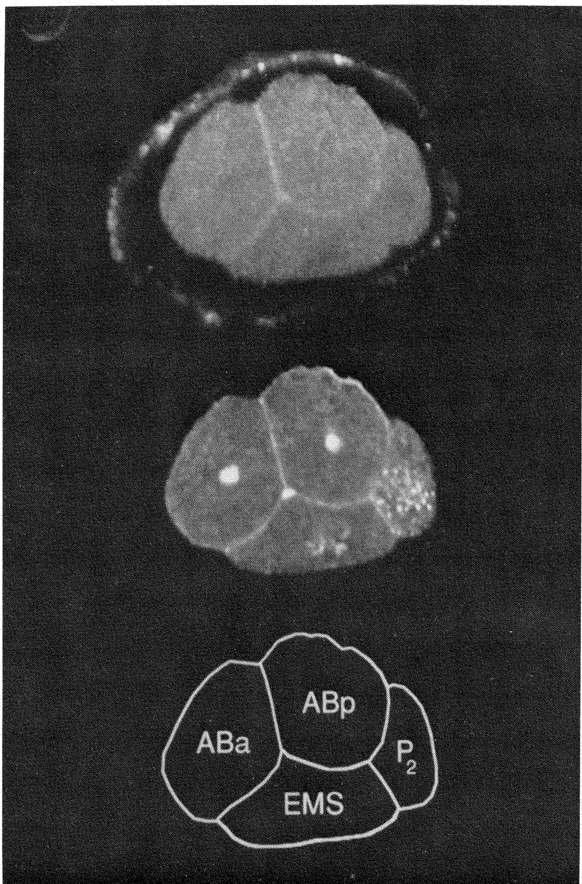

Figure 3. GLP1 is localized to ABa and ABp in the 4-cell embryo. Wild-type embryos were double stained with affinity-purified rat antibodies to the LNG region of GLP1 (fluorescein) and with mouse antibodies to actin, DNA, and P granules (rhodamine). These embryos were then examined using a BioRad MRC-600 confocal microscope. Anterior is left; dorsal is up. (*Top*) GLP1 staining is associated with membranes of the two daughters of AB, which are called ABa and ABp. GLP1 is found at regions of cell-cell contact including membranes between the two AB daughters and those between the AB daughters and the other two blastomeres at the 4-cell stage, EMS and P_2. (*Middle*) Actin staining reveals the boundaries of the blastomeres. DNA staining shows nuclei, and in this focal plane only the nuclei of ABa and ABp are seen. P granule staining is localized to P_2 and hence to the posterior end of the embryo. (*Bottom*) Line drawing of the embryo shown above with blastomeres labeled. For more detail, see S. Crittenden et al. (in prep.).

the failure to detect GLP1 in oocytes suggests that *glp-1* RNA rather than protein is contributed maternally to the embryo.

Early embryo. The embryo divides by an invariant pattern of cell divisions to generate a first larval stage worm (Sulston et al. 1983). The first division of the zygote is asymmetric, generating a larger blastomere, AB, and a smaller blastomere, P_1. AB gives rise primarily to hypodermis, nerves, and the anterior part of the pharynx; in contrast, P_1 gives rise primarily to muscle, intestine, germ line, and the posterior part of the pharynx. The daughters of AB, designated ABa and ABp, are equivalent in developmental potential (Priess and Thomson 1987). We find that GLP1 is associated with the plasma membranes of ABa and ABp at the 4-cell stage (Fig. 3); GLP1 is also found in all AB descendants through the 28-cell stage of embryogenesis (S. Crittenden et al., in prep.). These results bear on three aspects of GLP1 function in the embryo. First, the presence of GLP1 in ABa fulfills the prediction that GLP1 acts in the receiving cell of the inductive interaction between EMS and ABa (or their descendants). Second, the presence of GLP1 from the 4- to 28-cell stage corresponds remarkably well with the period of *glp-1(ts)* temperature sensitivity, supporting the idea that *glp-1* acts during this early phase of embryogenesis. Third, the presence of GLP1 in both ABa and ABp supports the proposal by Priess and Thomson (1987) that these two blastomeres are equivalent in developmental potential.

The Repeated Motifs in GLP1 Are Essential

To begin to delineate the functional domains of GLP1, we have characterized 17 *glp-1* mutations with varying phenotypes. Figure 4 summarizes the locations of the *glp-1* mutations within the GLP1 protein, their phenotypes, and their molecular defects. Our results are described below by phenotypic class.

In one phenotypic class are the putative null mutations (NULL, Fig. 4) (Austin and Kimble 1987; Kodoyianni et al. 1992; S. Crittenden et al., in prep.). All are nonconditional and have the same severe loss-of-function phenotype: a total failure of germ-line proliferation. The putative null alleles include two non-

←——— DOMAINS OF GLP1 ———→

TYPE OF MUTATION	EGF-like 1	2	3	4	5	6	7	8	9	10	LNG 1	2	3	*cdc10*/ SWI6 1	2	3	4	5	6	C-terminus
NONSENSE			↑ NULL								↑ NULL									↑ MX (cs)
MISSENSE	↑ E	↑(2) E		↑ E			↑ NULL					↑ NULL		↑(2) TS			↑(4) TS			
DELETION												[---------] NULL								

Figure 4. The molecular basis of *glp-1* mutations. *Domains of the* glp-1 *protein* (GLP1): Regions of GLP1 are represented from left to right as they are organized within GLP1, with the tandem array of EGF-like repeats found most amino-terminal and the unique carboxyl terminus found most carboxy-terminal. The transmembrane domain, located between the LNG repeats and *cdc10*/SWI6 (or ankyrin) repeats, has been omitted because no mutations mapped to this region. Individual repeats are numbered beginning from the most amino-terminal repeat. *Phenotypes of* glp-1 *mutations:* The various *glp-1* mutations have a variety of phenotypes: (NULL) phenotype similar to that of a null allele; (MX) mixed phenotype has both gain- and loss-of-function defects; (E) phenotype is more severe in embryo than germ line. *Number of* glp-1 *mutations:* An arrow with no parentheses signifies a single allele; when (*n*) is found next to an arrow, the number in parentheses indicates how many independently isolated alleles map to the specified repeat. See text for further explanation. (TS) Heat sensitive; (cs) cold sensitive. For more detail, see Kodoyianni et al. (1992).

sense mutations, two missense mutations, and one in-frame deletion (Fig. 1). No GLP1 is detected in mutants homozygous for the most amino-terminal nonsense mutation, *glp-1(q175)*; therefore, *glp-1(q175)* is likely to be a protein null. In contrast, GLP1 protein is detected in mutants homozygous for the in-frame deletion that removes most of the LNG repeats. Because GLP1 protein is present but activity is eliminated, the LNG repeats must be essential for GLP1 function. The presence or absence of GLP1 in the other null mutants has not yet been determined; yet the finding of a missense mutation in the seventh EGF-like repeat that leads to a null phenotype suggests that this EGF-like repeat is essential to GLP1 function.

A second phenotypic class includes six temperature-sensitive mutations (TS, Fig. 4) (Austin and Kimble 1987; Priess et al. 1987; Kodoyianni et al. 1992; S. Crittenden et al., in prep.). The effect of the *glp-1(ts)* mutations on germ-line proliferation is either as severe as that of the null mutations or nearly as severe. All six *glp-1(ts)* mutations are missense mutations located in the *cdc10*/SWI6 repeats, and GLP1 protein can be detected in *glp-1(ts)* homozygous mutants at restrictive temperature. Therefore, the *cdc10*/SWI6 repeats must be essential for GLP1 activity.

In a third phenotypic class are mutations with a more severe phenotype in the embryo than in the germ line (E, Fig. 4) (Austin and Kimble 1987; Priess et al. 1987; Kodoyianni et al. 1992). All four *glp-1(E)* mutations are missense mutations located in the amino-terminal EGF-like repeats. Three of the four are partial loss-of-function mutations and one (that in EGFL-1) may interfere with an embryo-specific function. The presence or absence of GLP1 in the *glp-1(E)* mutants has not yet been determined.

Finally, in a class by itself, is the allele *glp-1(q35)*, which has mixed gain- and loss-of-function character (MX, Fig. 1) (Austin and Kimble 1987; Mango et al. 1991; S. Crittenden et al., in prep.). This unique allele has a dominant multivulva (Muv) phenotype, which is typical of *lin-12* gain-of-function alleles, and a recessive germ-line proliferation defect, which is typical of *glp-1* loss-of-function alleles. *glp-1(q35)* is a nonsense mutation that is predicted to remove a unique stretch of amino acids from the carboxyl-terminus, leaving all of the repeated motifs intact. Examination of GLP1(q35) protein both by immunocytochemistry and on Western blots supports the notion that this protein is indeed present in truncated form. From our analysis of *glp-1(q35)*, we have proposed that the carboxy-terminal region deleted in GLP1(q35) protein may normally regulate the somatic expression of GLP1. Furthermore, we have provided evidence that the GLP1(q35) protein, when expressed at normal abundance, is functional and that the unique carboxy-terminal domain is not essential for its activity.

Positive Regulation of *glp-1*

The two partial loss-of-function *glp-1* mutations mapping to the second EGF-like repeat have a unique effect on germ-line development. The ovotestis of a wild-type hermaphrodite has about 1000 germ-line descendants, and that of a *glp-1(0)* hermaphrodite has only a few germ-line descendants (4–8). Intriguingly, the EGFL-2 missense mutants can possess either ovotestes with a virtually normal mitotic germ line or ovotestes with no mitotic germ line at all (Austin and Kimble 1987; Kodoyianni et al. 1992). Indeed, even within a single animal, one ovotestis can appear wild-type and the other Glp. This "all-or-none effect" on germ-line proliferation shows that *glp-1* is regulated independently in the two ovotestes and suggests that a positive feedback control can maintain *glp-1* in an active state.

DISCUSSION

GLP1 and the Germ Line

In the germ line, the distribution of GLP1 is restricted to the distal mitotic region of the syncytium (this paper; S. Crittenden et al., in prep.). GLP1 is found in membranes adjacent to the distal tip cell and also in membranes of mitotic germ cells that are not adjacent to the distal tip cell. Since the distal tip cell signals the germ line to remain in mitosis, this localization is consistent with a role of GLP1 in receiving that signal.

The mechanism by which GLP1 is localized in the germ-line syncytium is not understood. GLP1 germ-line localization might rely on transcriptional, post-transcriptional, or posttranslational controls. One simple model is that the distal tip cell could exert its control over germ-line proliferation by producing a ligand that binds and stabilizes GLP1. According to this model, the extent of GLP1 would be determined by the turnover of GLP1 plus its diffusion rate through the germ-line membrane. A gradient of GLP1 would thus be formed once GLP1 was released from the distal tip cell ligand. However, many alternatives exist, and there is no compelling reason to favor any one model at present.

The significance of GLP1 localization is not understood. It seems likely that the restriction of GLP1 to the mitotic region of the germ line reflects not only a requirement for its presence in that region, but also a requirement for its absence in more proximal cells. If this is true, the presence of Glp in more proximal germ-line nuclei might affect the differentiation of those germ-line nuclei or, as discussed in the next section, it might interfere with early embryogenesis.

GLP1 and the Early Embryo

In the early embryo, the distribution of GLP1 is restricted to the two anterior blastomeres, ABa and ABp, at the 4-cell stage, and to their descendants through the 28-cell stage (this paper; S. Crittenden et al., in prep.). Of particular importance to its role in pharyngeal induction, GLP1 is present in ABa membrane immediately adjacent to EMS membrane. Because ABa (or its descendants) is the receiving cell in pharyngeal induction and EMS (or its descendants) is the signaling cell, this location is consistent with the proposed role for GLP1 as a membrane receptor for the inducing signal.

In addition to its location in ABa, GLP1 is found in its developmentally equivalent sister ABp. This observation is intriguing in light of the similarities between *lin-12* and *glp-1*. As mentioned earlier, the *lin-12* and *glp-1* proteins overlap with each other functionally (Lambie and Kimble 1991b; Mango et al. 1991). The primary role of *lin-12* in development is to mediate lateral signaling—signaling that allows equivalent cells to adopt distinct fates (Greenwald et al. 1983). Therefore, the finding that GLP1 is present in two equivalent blastomeres that subsequently adopt individual fates suggests the possibility that GLP1 mediates lateral signaling between ABa and ABp (or their descendants), much as *lin-12* mediates lateral signaling between somatic precursor cells during postembryonic development. If this is true, the role of GLP1 in early embryogenesis may be much broader than previously considered.

The mechanism by which GLP1 is localized in the early embryo is likely to be posttranscriptional. This idea is based on our inability to detect GLP1 protein in oocytes, and on the embryonic requirement for maternal *glp-1*. Therefore, *glp-1* is likely to be contributed to embryos as maternal mRNA. Expression of that RNA might be regulated either by RNA localization or by localized activation of the RNA. One intriguing possibility is that the *glp-1* 3′UTR may control its temporal and spatial expression within the early embryo. In *Drosophila* and *Xenopus*, the regulated localization of maternal RNA is mediated by elements with the 3′UTRs of localized RNAs (Mowry and Melton 1992; MacDonald 1993). Alternatively, the localized appearance of GLP1 may result from posttranslational regulation, perhaps through the spatial control of protein stability.

The significance of the GLP1 restriction to AB descendants during early embryogenesis is not understood. As in the germ line, it seems likely that embryonic GLP1 restriction reflects not only a requirement for its presence in AB descendants, but also a requirement for its absence in the descendants of EMS and P_2.

GLP1 and the Control of Cell Fate

How does GLP1 mediate inductive interactions? By analogy with other membrane receptors, we postulate that the extracellular domain of GLP1 fields extracellular cues and that the intracellular domain acts in some manner to influence cell fate.The extracellular domain consists primarily of an amino-terminal array of EGF-like repeats and three LNG repeats, whereas the intracellular domain possesses six *cdc10*/SWI6 repeats and a unique carboxy-terminal tail. We find that each of twelve missense mutations with a loss-of-function phenotype maps to one or another of the tandemly repeated motifs, suggesting that the repeated motifs are essential to GLP1 function.

The specific functions of the various repeated motifs of GLP1 remain a matter of speculation. It seems likely that the intracellular domain is of particular importance for regulation of cell fate and that the *cdc10*/SWI6 repeats of GLP1 are the key structural elements mediating this function. A series of missense mutations within these repeats drastically reduce and perhaps eliminate GLP1 activity at restrictive temperature (Kodoyianni et al. 1992; S. Crittenden et al., in prep.). Therefore, the *cdc10*/SWI6 repeats are clearly critical to GLP1 function. Furthermore, a GLP1 protein with much of the unique carboxyl terminus removed, but with the *cdc10*/SWI6 repeats intact, remains functional. What might the *cdc10*/SWI6 repeats be doing? In several other proteins, *cdc10*/SWI6 repeats have been implicated in mediating protein-protein interactions (Lux et al. 1990; Haskill et al. 1991; LaMarco et al. 1991; Nolan et al. 1991; Thompson et al. 1991). Therefore, it is likely that the six *cdc10*/SWI6 repeats of LNG proteins are also important for protein-protein interactions. Furthermore, many *cdc10*/SWI6 repeat-containing proteins either bind or regulate a putative transcription factor: SWI4 and SWI6 of *Saccharomyces cerevisiae* (Andrews and Herskowitz 1989 a,b), *fem-1* of *C. elegans* (Spence et al. 1990), cactus of *Drosophila* (C. Nüsslein-Volhard, pers. comm.), IκB (Haskill et al. 1991), and the $\beta1$ subunit of GABP from mammals (Thompson et al. 1991).

We speculate that the *cdc10*/SWI6 repeats of GLP1, and indeed these repeats of all LNG proteins, may function by binding regulatory proteins that are critical for the specification of cell fate. The *cdc10*/SWI6 repeats of GLP1 might, for example, bind a protein and thereby prevent its entry into the nucleus. In the germ line of *C. elegans*, the bound factor might be a positive regulator of meiosis or a negative regulator of mitosis. According to this model, the LNG proteins could be thought of as the membrane receptor equivalents of cytoplasmic regulatory proteins that bear *cdc10*/SWI6 repeats (e.g., IκB). This model is invoked, in part, by analogy with tyrosine kinases, which can be found either as cytoplasmic proteins (e.g., c-*src*) or as membrane receptors (e.g., the EGF receptor).

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