Genetics of intercellular signalling in *C. elegans*

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Summary

Cell–cell interactions play a significant role in controlling cell fate during development of the nematode *Caenorhabditis elegans*. It has been found that two genes, *glp-1* and *lin-12*, are required for many of these decisions. *glp-1* is required for induction of mitotic proliferation in the germline by the somatic distal tip cell and for induction of the anterior pharynx early in embryogenesis. *lin-12* is required for the interactions between cells of equivalent developmental potential, which allow them to take on different fates. Comparison of these two genes on a molecular level indicates that they are similar in sequence and organization, suggesting that the mechanisms of these two different sets of cell–cell interactions are similar.

Key words: *Caenorhabditis elegans*, cell–cell interaction, cell fate, *glp-1, lin-12*.

Introduction

Interactions between cells have been shown to be crucial to the determination of cell fate in a variety of organisms. These interactions were first observed in classical experiments with sea urchin and frog embryos (e.g. Driesch, 1891; Spemann and Mangold, 1924). More recently, cell interactions that influence the determination of cell fate have been described in the nematode *Caenorhabditis elegans*. In this brief review, we describe several cellular interactions that influence development in *C. elegans* and two genes, *glp-1* and *lin-12*, that are required for these interactions. Remarkably, these two genes, which are required in different sets of cell–cell interactions, appear to encode similar proteins, indicating that diverse regulatory interactions during development may rely on a similar underlying biochemical mechanism.

Cell Interactions in *C. elegans*

The evidence for cell–cell interactions in *C. elegans* has come primarily from experiments in which particular cells were physically removed, by laser ablation or puncture with a needle, and the effect on development of other cells was monitored (Sulston and White, 1980; Kimble and White, 1981; Kimble, 1981; Sulston et al. 1983; Priess and Thomson, 1987). Three of the regulatory interactions that have been identified by these experiments are summarized in Table 1. They include control of germline proliferation by the distal tip cell (Kimble and White, 1981), induction of pharyngeal mesoderm in the embryo (Priess and Thomson, 1987), and regulation among precursor cells of equivalent developmental potential so that they adopt different fates (Sulston and White, 1980; Kimble, 1981; Sulston et al. 1983). We have focused on the interaction that takes place between the distal tip cell and the germline. In *C. elegans*, proliferation of germline cells occurs throughout the lifetime of the animal; germ cells located close to the distal tip cell are in the mitotic cell cycle while more proximal germ cells enter meiosis. [The germline tissue is actually a syncytium. However, each germline nucleus occupies its own membrane-bound alcove of cytoplasm located at the edge of a common anuclear cytoplasm (Hirsh et al. 1976). Each germline nucleus and its cytoplasm is called a germ cell for simplicity.] If the distal tip cell is destroyed at any time during postembryonic development, germ cells leave the mitotic cell cycle, enter meiosis and undergo gametogenesis (Kimble and White, 1981). Thus the distal tip cell must signal cells of the germline to continue dividing mitotically. By isolating loss-of-function mutations whose phenotypes mimic the effect of disrupting the interaction between distal tip cell and germline, we hope to identify the gene products that mediate this interaction. So far, we have identified one gene, *glp-1*, required for this interaction.

*glp-1* affects cell interactions needed for germline and pharynx development

The *glp-1* locus was identified in a screen for mutations affecting germline development (Austin and Kimble, 1987). Six recessive alleles of *glp-1* were isolated in a screen of 20000 mutagenized chromosomes; this fre-
quency suggests that these mutations result in a loss of glp-1 activity. Other alleles of glp-1 were independently identified in a screen for mutations that result in defective embryogenesis (Priess et al. 1987). In wild-type animals, two germline precursor cells give rise to approximately 2000 germ cells in the adult hermaphrodite. In glp-1(−) animals only 4–8 germ cells are produced in all; they undergo meiosis and form a small number of gametes (Table 2). This switch of the germ cells from mitosis to meiosis is similar to the effect of ablating the distal tip cell early in larval development (Kimble and White, 1981). Experiments using temperature-sensitive alleles have shown that there is a continuous requirement for glp-1 activity. This result parallels the observation seen for the distal tip cell–germline interaction: the presence of the distal tip cell is required throughout germline development for continued germ cell proliferation (Kimble and White, 1981).

In addition to their effect on germline development, mutations in glp-1 result in an embryonic phenotype that indicates a requirement for maternal glp-1 product during embryogenesis (Priess et al. 1987; Austin and Kimble, 1987). This embryonic phenotype can be observed using conditional mutations in glp-1. At permissive temperature, glp-1(ts) homozygotes produce a normal number of germ cells, but when shifted as adults to restrictive temperature, their progeny do not survive. Moreover, the glp-1(ts)/glp-1(+) heterozygous cross-progeny of a glp-1(ts) mother do not survive. Therefore, glp-1 product must be contributed by the mother for survival of her progeny. The lethal phenotype of glp-1(−) embryos includes defects in hypodermal morphogenesis and pharyngeal development (Priess et al. 1987). The embryos have a near normal number of cells, but they are missing the anterior half of their pharynx and they do not undergo the morphogenesis that normally changes a ball of cells into an elongated worm during embryogenesis (Table 2). It has been shown that, while development of the posterior pharynx occurs in a cell-autonomous manner, formation of the anterior pharynx requires an inductive interaction between the AB blastomere (or its descendants) and the P1 blastomere (or its descendants) (Table 1). Temperature-shift experiments have shown that maternal glp-1 product is required between the 4-cell and 28-cell stages of embryogenesis. Cell destruction experiments have shown that induction of the anterior pharynx occurs during this same early period of embryogenesis (Priess et al. 1987). It is not presently known whether inductive interactions are also required for proper formation of the hypodermis.

What role does glp-1 play in the cell–cell interactions controlling development of the germline and the pharynx; is it, for example, a component of the signalling mechanism or the receiving mechanism? To address this question, we examined genetic mosaic animals where either the distal tip cell or the germline was glp-1(−) (Austin and Kimble, 1987). Our results are summarized in Table 3. They indicate that the glp-1 activity necessary for continued proliferation of the germline is produced by the germline and not by the distal tip cell. This implies that glp-1 encodes a component of the receiving machinery for the distal tip cell–germline interaction rather than the distal tip cell signal. It is not possible to use these genetic mosaics to determine where maternally derived glp-1 gene product

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### Table 1. Regulatory cell interactions in C. elegans development*

<table>
<thead>
<tr>
<th>Signalling cell</th>
<th>Receiving cell</th>
<th>Normal fate of receiving cell</th>
<th>Fate of receiving cell after removal of signal</th>
<th>Deduced interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal tip cell (dtc)</td>
<td>Germline</td>
<td>Continued mitotic proliferation</td>
<td>Germ cells enter meiosis</td>
<td>Dtc induces germline to continue mitosis</td>
</tr>
<tr>
<td>P1 blastomere</td>
<td>AB blastomere</td>
<td>AB gives rise to anterior pharynx</td>
<td>AB does not produce anterior pharynx</td>
<td>P1 induces AB to produce anterior pharynx</td>
</tr>
<tr>
<td>1° cell</td>
<td>2° cell</td>
<td>2° fate</td>
<td>Cell that would be 2° becomes 1°</td>
<td>1° cell inhibits 2° cell from becoming 1°</td>
</tr>
</tbody>
</table>

*Only cell interactions discussed in this review are listed.

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### Table 2. Mutant phenotypes of glp-1 and lin-12

<table>
<thead>
<tr>
<th>gene</th>
<th>genotype</th>
<th>phenotype</th>
<th>defective interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>glp-1†</td>
<td>m(+/−):z(+/−)</td>
<td>all germ cells enter meiosis</td>
<td>distal tip cell–germline</td>
</tr>
<tr>
<td></td>
<td>m(−/−):z(+/−)</td>
<td>anterior pharynx not formed</td>
<td>P1/AB</td>
</tr>
<tr>
<td></td>
<td>or m(−/−):z(−/−)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lin-12†</td>
<td>If/If</td>
<td>both cells follow 1° fate</td>
<td>signalling between cells in equivalence groups</td>
</tr>
<tr>
<td></td>
<td>gI/gI or gI/+</td>
<td>both cells follow 2° fate</td>
<td></td>
</tr>
</tbody>
</table>

m, maternal genotype; z, zygotic genotype; If, loss-of-function, gI, gain-of-function.

The lin-12 locus was identified in a general screen for while in lin-12(gf) (gain-of-function) mutants both takes place between these cells in which the cell destroyed by laser ablation, the remaining cell will ent cells adopts a primary fate while the other cell different cell fates. Normally, one of a pair of equival-

cells is also adopting this fate (Kimble, 1981). Two types of glp-1, lin-12 alleles have been isolated. In lin-12(lf) (loss-of-

function) mutants, both cells adopt the primary fate, suggesting that an interaction adopts a secondary fate. If either of the two cells is

adopting this fate, implying that glp-1 acts on the receiving end of this interaction as well.

### lin-12 and equivalence groups

The lin-12 locus was identified in a general screen for mutations that produced defects in the cell lineages of the vulva (Greenwald et al. 1983). Mutations in this gene cause changes in cell fate similar to the results of laser ablation experiments, suggesting that this gene product is necessary for cell–cell signalling (Table 1 and Table 2) (Greenwald et al. 1983; Sternberg, 1988). In particular, lin-12 appears to be involved in the interactions that occur between cells in equivalence groups. Such sets of cells have equivalent developmental potential, but, as a result of cell–cell interactions, take on different cell fates. Normally, one of a pair of equivalent cells adopts a primary fate while the other cell adopts a secondary fate. If either of the two cells is destroyed by laser ablation, the remaining cell will adopt the primary fate, suggesting that an interaction takes place between these cells in which the cell adopting the primary fate prevents the other cell from also adopting this fate (Kimble, 1981). Two types of lin-12 alleles have been isolated. In lin-12(lf) (loss-of-function) mutants, both cells adopt the primary fate, while in lin-12(gf) (gain-of-function) mutants both adopt the secondary fate (Greenwald et al. 1983). Analysis of animals that are genetic mosaics for lin-12(+) indicates that lin-12 acts on the receiving end of these cell interactions (Seydoux and Greenwald, 1989).

### Molecular analyses of glp-1 and lin-12

We were able to identify the glp-1 gene on a molecular level by making use of its proximity to lin-12 (Austin and Kimble, 1989). glp-1 is located 0-02 map units to the right of lin-12 on LGIII (Austin and Kimble, 1987). Comparisons of genetic and molecular distances in the region surrounding lin-12 (Greenwald et al. 1987) indicated that this genetic distance would correspond to 20–40 kb. lin-12 has been cloned (Greenwald, 1985) and the region of the C. elegans genome surrounding lin-12 has been placed in a series of overlapping cosmids (Greenwald et al. 1987). Using these cosmids as hybridization probes, we examined the pattern of restriction fragments produced by DNA isolated from wild-type animals and animals carrying mutations in glp-1. We found that the cosmid ZK506 detected DNA alterations associated with three different glp-1 mutations (Fig. 1) (Austin and Kimble, 1989). Two of these mutations, glp-1(q172) and qDf2, contain deletions while glp-1(q339) contains a complex rearrangement. We have identified a single transcript produced from the region identified by these three glp-1 mutations. This transcript is altered in size by glp-1(q172), confirming that it is the glp-1 transcript.

**Mode of action of glp-1**

Fig. 2 presents one model for the molecular function of glp-1 in the germline. We show the glp-1 product as a receptor located in the membrane of the germline syncytium. Upon binding of the signalling molecule...
Based on the genetic mosaic experiments of Austin and Kimble (1987) and the predicted \textit{glp-1} molecular structure (Yochem and Greenwald, 1989), we propose that \textit{glp-1} encodes a component of the membrane receptor for the signal produced by the distal tip cell. In this figure we show the \textit{glp-1} product as a receptor (Y) that is present throughout the germline; this is one possibility but there is no evidence to date of its localization. We propose that the distal tip cell emits a signal (Q) that binds the \textit{glp-1} receptor locally. Since the distal tip cell signal appears to act over a distance (Kimble and White, 1981), we propose that \textit{glp-1} interacts directly with the basement membrane in the distal tip cell–germline interaction: whether \textit{glp-1} interacts directly with the basement membrane is not presently known.

produced by the distal tip cell, this receptor transduces the signal to direct continued mitotic divisions in the germline. The \textit{glp-1} protein may be present throughout the germline. In this case, it might be the position of the distal tip cell and its signal that determines where \textit{glp-1} will actively direct germline proliferation.

One approach to the identification of genes that interact with \textit{glp-1} and \textit{lin-12} is the isolation of mutations that act as phenotypic suppressors of mutations in these genes. A set of recessive suppressors of both the \textit{glp-1} germline and embryonic phenotypes has been identified (Table 4) (Maine and Kimble, 1989). These mutations suppress hypomorph and conditional alleles of \textit{glp-1} but not putative null alleles, indicating that they do not simply bypass the requirement for \textit{glp-1} activity. In addition to suppressing the \textit{glp-1} phenotype, these mutations cause an alteration in body morphology: suppressor homozygotes (\textit{sup/sup}) are shorter than normal [a Dumpy (Dpy) phenotype]. The suppressor mutations have all turned out to be located in previously identified genes. Alleles of these genes, isolated in screens for mutations that alter body morphology, also suppress mutations in \textit{glp-1}, indicating that the suppression is not due to unusual mutations in these genes. It has been shown that the change in body morphology is not sufficient for suppression, as mutations in other \textit{dpy} genes do not have this effect. Two of the suppressor genes, \textit{sqt-1} and \textit{dpy-10}, have been shown to encode collagens (Kramer et al. 1988; J. Kramer, personal communication). Suppression of the \textit{glp-1(−)} phenotype by mutations in genes encoding collagen suggests that there is a role for extracellular matrix in the interaction between distal tip cell and germline. One possibility is that suppression of the \textit{glp-1} phenotype is caused by effects on the basement membrane surrounding both the germline and the distal tip cell (Fig. 2).

### Table 4. Suppression of the \textit{glp-1} phenotype by mutations that affect body morphology*

<table>
<thead>
<tr>
<th>Suppressor</th>
<th>\textit{glp-1} genotype</th>
<th>germline phenotype: percentage of mitotic germ cells in adult†</th>
<th>embryonic phenotype: percentage hatching‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>\textit{glp-1}(+)</td>
<td>+</td>
<td>&gt;99</td>
</tr>
<tr>
<td>−</td>
<td>\textit{glp-1}(−)</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>dpy-1(e1)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>dpy-2(q292)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>dpy-3(s27)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>38</td>
</tr>
<tr>
<td>dpy-7(q288)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>12</td>
</tr>
<tr>
<td>dpy-8(q287)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>19</td>
</tr>
<tr>
<td>dpy-9(e12)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>11</td>
</tr>
<tr>
<td>dpy-10(q291)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>15</td>
</tr>
<tr>
<td>sqt-1(e1350)</td>
<td>\textit{glp-1}(−)</td>
<td>+</td>
<td>2</td>
</tr>
</tbody>
</table>

* Results summarized here are from Maine and Kimble (1989). All animals grown at 20°C.
† Presence of mitotically dividing germine nuclei in \textit{sup/sup}: \textit{glp-1(−)/glp-1(−)} hermaphrodites was assayed two days after they became young adults.
‡ Percentage of hatching was measured for eggs laid by \textit{sup/sup:glp-1(−)/glp-1(−)} hermaphrodites. Data shown here include all embryos produced by each animal. In general, the percentage of eggs that hatched was much higher at the beginning of the egg-laying period than at the end of it.

### Conclusions

The identification of \textit{glp-1} and \textit{lin-12} is an important first step towards understanding the mechanism of cell–cell interactions in \textit{C. elegans}. Several major questions remain unanswered. Does the same \textit{glp-1} product mediate both its germline and embryonic functions? If so, what are the signals and are they the same? Are the \textit{glp-1} and \textit{lin-12} proteins actually receptors for intercellular signals or do they serve some other function that is critical to transduction of the signal? Although the \textit{glp-1} and \textit{lin-12} genes code for similar proteins, genetic data suggest that these two proteins are required for different sets of cell–cell interactions. Is this difference in function due to differences in biochemical specificity or in the pattern of expression for
these two genes? Finally, the suppression of mutations in glp-1 by mutations in at least two collagen genes suggests a possible role for extracellular matrix in cell–cell interactions. Does glp-1 interact directly with the basement membrane surrounding the germline and, if so, how is this interaction changed by mutations in collagen genes? The answers to these questions are now accessible. Starting with the cloned glp-1 and lin-12 genes, it will be possible to analyze the regulation and function of their products.

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