Development 1989 Supplement, 53–57
Printed in Great Britain © The Company of Biologists Limited 1989

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 mitoic 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-

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

Signalling cell	Receiving cell	Normal fate of receiving cell	Fate of receiving cell after removal of signal	Deduced interaction
Distal tip cell (dtc)	Germline	Continued mitotic proliferation	Germ cells enter meiosis	Dtc induces germline to continue mitosis
P_1 blastomere	AB blastomere	AB gives rise to anterior pharynx	AB does not produce anterior pharynx	P_1 induces AB to produce anterior pharynx
1° cell	2° cell	2° fate	Cell that would be 2° becomes 1°	1° cell inhibits 2° cell from becoming 1°

* Only cell interactions discussed in this review are listed.

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

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

gene	genotype	phenotype	defective interaction
*glp-1**	m(+/−);z(−/−) m(−/−);z(+/−) or m(−/−);z(−/−)	all germ cells enter meiosis anterior pharynx not formed	distal tip cell–germline P_1/AB
lin-12†	lf/lf gf/gf or gf/+	both cells follow 1° fate both cells follow 2° fate	signalling between cells in equivalence groups

m, maternal genotype; z, zygotic genotype; lf, loss-of-function, gf, gain-of-function.
* Austin and Kimble (1987); Priess *et al.* (1987). † Greenwald *et al* (1983).

Table 3. *Genetic mosaic experiments with* glp-1*

	Genotype		Phenotype
Animal	Distal tip cell	Germline	No. germline nuclei
Wild-type	*glp-1(+)*	*glp-1(+)*	~2000
Mutant	*glp-1(−)*	*glp-1(−)*	4–8
Mosaic	*glp-1(+)*	*glp-1(−)*	~2000
Mosaic	*glp-1(−)*	*glp-1(+)*	4–8

*The results summarized here are from Austin and Kimble (1987).

is required for induction of the pharynx during embryogenesis, but we think it likely 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

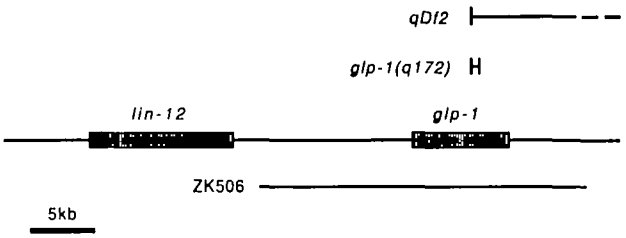

Fig. 1. Map of the *lin-12* – *glp-1* region showing the relative positions of the two genes. The extent of the genomic region contained in the cosmid ZK506 is shown below the map. The extent of the deletions in *glp-1(q172)* and *qDf2* is shown above the map. The right endpoint of *qDf2* has not been determined but it is to the right of the region contained in ZK506.

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.

Fortuitously, a search of the *C. elegans* genome for *lin-12* homologues identified one such homologue in the cosmid ZK506 (Yochem and Greenwald, 1989). The region of *lin-12* homology was centered on the transcription unit that we had identified as *glp-1*. Subsequent sequence analysis has shown that *glp-1* is similar both in sequence and organization to *lin-12* (Yochem and Greenwald, 1989). *lin-12* is also homologous to a *Drosophila* gene, *Notch* (Greenwald, 1985; Yochem *et al.* 1988). *Notch* has been shown to be required for the decision between differentiation as an epidermal precursor or a neuroblast which takes place during *Drosophila* embryogenesis (Poulson, 1937). Thus all three homologous genes, *glp-1*, *lin-12* and *Notch*, influence a developmental decision of cell fate. In each case, it is thought that this developmental decision occurs as the result of cell–cell interactions. The deduced amino acid sequences of all three genes have the molecular characteristics predicted for membrane proteins (Yochem and Greenwald, 1989; Yochem *et al.* 1988; Wharton *et al.* 1985). Although the functions of *glp-1*, *lin-12* and *Notch* in mediating cell interactions are not known, a simple possibility is that each encodes a receptor. In the case of *glp-1*, its product might be the receptor that binds the signal emitted by the distal tip cell.

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

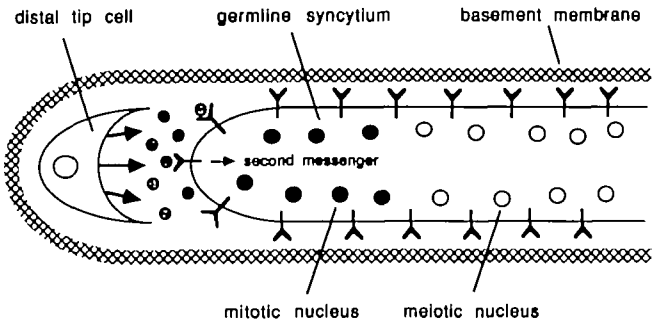

Fig. 2. Model for *glp-1* function in the germline. Diagram shows the distal portion of the *C. elegans* germline with its associated distal tip cell and basement membrane. The distal tip cell is a somatic cell that signals to the germline syncytium. Germline nuclei at the distal end of the syncytium remain in mitosis; germline nuclei further proximal enter meiosis. In this figure, the distal tip cell is shown at some distance from the germline in order to allow the drawing of signal molecules in the intercellular space. In the animal, the membrane of the distal tip cell is closely juxtaposed to the membrane of the germline. No basement membrane separates the distal tip cell from the germline; instead a basement membrane encapsulates both and separates the gonad from the surrounding pseudocoelom (Kimble and Ward, 1988).

Based on the genetic mosaic experiments of Austin and Kimble (1987) and the predicted *glp-1* molecular structure (Yochem and Greenwald, 1989), we propose that *glp-1* encodes a component of the membrane receptor for the signal produced by the distal tip cell. In this figure we show the *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 (O) that binds the *glp-1* receptor locally. Since the distal tip cell signal appears to act over a distance (Kimble and White, 1981), we propose that the ligand-activated *glp-1* generates a second messenger that diffuses in the germline syncytium. Experiments that show that mutations in collagen genes can suppress the phenotypes of mutations in *glp-1* suggest a role for the basement membrane in the distal tip cell–germline interaction; whether *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 *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 *glp-1* will actively direct germline proliferation.

One approach to the identification of genes that interact with *glp-1* and *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 *glp-1* germline and embryonic phenotypes has been identified (Table 4) (Maine and Kimble, 1989). These mutations suppress hypomorphic and conditional alleles of *glp-1* but not putative null alleles, indicating that they do not simply bypass the requirement for *glp-1* activity. In addition to suppressing the *glp-1* phenotype, these mutations cause an alteration in body morphology: suppressor homozygotes (*sup/sup*) are

Table 4. *Suppression of the* glp-1 *phenotype by mutations that affect body morphology**

Suppressor	*glp-1* genotype	germline phenotype: presence of mitotic germ cells in adult†	embryonic phenotype % hatching‡
–	*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

* Results summarized here are from Maine and Kimble (1989). All animals grown at 20°C.

† Presence of mitotically dividing germline nuclei in *sup/sup; glp-1(−)/glp-1(−)* hermaphrodites was assayed two days after they became young adults.

‡ Percentage of hatching was measured for eggs laid by *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.

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 *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 *dpy* genes do not have this effect. Two of the suppressor genes, *sqt-1* and *dpy-10*, have been shown to encode collagens (Kramer *et al.* 1988; J. Kramer, personal communication). Suppression of the *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 *glp-1* phenotype is caused by effects on the basement membrane surrounding both the germline and the distal tip cell (Fig. 2).

Conclusions

The identification of *glp-1* and *lin-12* is an important first step towards understanding the mechanism of cell–cell interactions in *C. elegans*. Several major questions remain unanswered. Does the same *glp-1* product mediate both its germline and embryonic functions? If so, what are the signals and are they the same? Are the *glp-1* and *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 *glp-1* and *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

We are grateful to Geraldine Sedoux, John Yochem, Iva Greenwald and Jim Kramer for sharing unpublished results. This research was supported by a U.S. Public Health Service grant GM31816 and a Research Career Development Award HD00630 to J.K. J.A. is a trainee of the NIH Predoctoral Training Program in Genetics GM07133. E.M. is supported by Public Health Service Grant GM11569.

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