Genetic control of cellular interactions in Caenorhabditis elegans development

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Abstract. During development of the nematode, Caenorhabditis elegans, cell interactions play a significant role in controlling cell fate. Regulatory cells in the somatic gonad control proliferation in the germline and induce formation of the vulva in the hypodermis. In the early embryo, mesodermal cells are induced in a process similar to embryonic induction. In addition, interactions between precursor cells of equivalent developmental potential direct one cell to adopt one fate and the other to adopt a second fate. Two genes have been identified in C. elegans that appear to mediate cell interactions. The glp-1 gene is required for embryonic induction and continued germline proliferation; lin-12 is required for cells of equal developmental potential to adopt different fates. Genetic mosaics indicate that glp-1 may be part of the receiving mechanism in controlling germline proliferation.

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Interactions between cells are critical to the control of growth and differentiation of multicellular organisms. Such interactions were first observed in classical experiments with sea urchin and frog embryos (e.g. Driesch 1891, Spemann & Mangold 1924). More recently, cellular interactions that regulate development 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, central to the control of these interactions. Remarkably, these two genes appear to encode similar proteins, indicating that they arose during evolution by duplication and that diverse regulatory interactions in development may rely on a similar underlying biochemical mechanism.

The three types of regulatory interactions that we discuss are summarized in Table 1. They include control of germline proliferation by the distal tip cell (Kimble & White 1981), induction of pharyngeal mesoderm in the embryo (Priess & Thomson 1987), and regulation among precursor cells of equivalent developmental potential to adopt different fates (Sulston & White 1980,
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**TABLE 1 Regulatory cell interactions in *Caenorhabditis 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>Interaction deduced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distal tip cell (dtc)</td>
<td>Germline</td>
<td>Germ cells in mitosis near dtc</td>
<td>Germ cells enter meiosis near dtc</td>
<td>Dtc signals germline to continue mitosis</td>
</tr>
<tr>
<td>P₁ blastomere</td>
<td>AB blastomere</td>
<td>AB generates cells of anterior pharynx</td>
<td>AB does not generate anterior pharynx</td>
<td>P₁ signals AB to produce anterior pharynx</td>
</tr>
<tr>
<td>₁° cell&lt;sup&gt;b&lt;/sup&gt;</td>
<td>₂° cell</td>
<td>₂° fate</td>
<td>₂°→₁°</td>
<td>₁° inhibits ₂° from becoming ₁°</td>
</tr>
<tr>
<td>e.g. anchor cell precursor, AC</td>
<td>e.g. ventral uterine precursor, VU</td>
<td>e.g. ventral uterus</td>
<td>e.g. VU becomes AC</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Only cell interactions discussed in this review are listed.
<sup>b</sup>Cells in equivalence groups are primary (₁°), secondary (₂°), or tertiary (₃°). Only one example is provided in this table: the anchor cell precursor is ₁° and the ventral uterine precursor is ₂°.

Kimble 1981, Sternberg & Horvitz 1986). The discovery of each of these interactions emerged from one basic type of experiment. In each case, when one cell (the signalling cell) was physically removed, either by ablation with a laser microbeam or by needle puncture, the fate of a neighbouring cell (the receiving cell) was dramatically altered. For example, in the gonad, a somatic cell is located at the distal end of a tube of germline cells. [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.] Normally, germ cells located close to the distal tip cell are in the mitotic cell cycle, whereas more proximal germ cells enter meiosis. When the distal tip cell is killed, in either sex and at any time during post-embryonic development, germ cells leave the mitotic cell cycle, enter meiosis and undergo gametogenesis. Thus, the distal tip cell must signal to cells of the germline to continue mitosis. The mitogenic influence of the distal tip cell is required for growth of the germline during post-embryonic development and for maintenance of a stem cell population of germ cells in the adult. Other experiments suggest that the distal tip cell signal acts over a distance and that its position, usually at the distal tip of the testis or ovotestis, establishes the polarity of the gonad.

Two genes have been identified that are involved in control of the interactions outlined in Table 1. The mutant phenotypes of these genes are summarized in Table 2 and are described briefly below. One of these genes, *glp-1*, was identified...
in a specific attempt to find genes that control cellular interactions (Austin & Kimble 1987). When the distal tip cell is ablated, germ cells that would normally be mitotic enter meiosis and differentiate. Therefore, the mutant phenotype we sought was entry into meiosis by germ cells that normally would be in mitosis. In a screen of approximately 20,000 mutant chromosomes, six mutations were isolated with this phenotype. All fell in a single complementation group on chromosome III. This locus was named glp-1 for germ line proliferation defective. The glp-1 locus was identified independently in a general screen for maternal effect mutants that result in defective embryogenesis (Priess et al 1987). The lin-12 locus was identified in a general screen for mutants that were defective in the cell lineages of the vulva (Ferguson & Horvitz 1985).

The glp-1 gene is necessary for control of continued mitoses in the post-embryonic germline (Austin & Kimble 1987, Priess et al 1987). Mutant animals lacking glp-1 (but born of a heterozygous glp-1/+ mother) produce only 4–8 germ cells (Table 2). These few germ cells enter meiosis and undergo spermatogenesis. The processes of meiosis and gametogenesis are normal in glp-1 mutant animals. Experiments using a temperature-sensitive allele of glp-1 indicate that the glp-1 product is required for germline growth from larval stage L2 throughout adulthood. It is thus central to proliferation of the germline during larval development and to maintenance of a stem cell population in the adult. Similarly, experiments in which the distal tip cell was removed at various times of development showed that this regulatory cell must signal to the germline throughout larval development and adulthood for continued germline mitoses.

**TABLE 2 Mutant phenotypes of glp-1 and lin-12**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genetic descriptor</th>
<th>Mutant phenotype</th>
<th>Temperature-sensitive period</th>
<th>Defective interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>glp-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>m(+/--); z(--/-)</td>
<td>Germ cells enter meiosis near dtc</td>
<td>From L2 through adult</td>
<td>Distal tip cell/germline</td>
</tr>
<tr>
<td>glp-1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>m(+/--); z(--/-)</td>
<td>AB does not generate anterior pharynx</td>
<td>Early embryo (4–28 cells)</td>
<td>P&lt;sub&gt;1&lt;/sub&gt;/AB</td>
</tr>
<tr>
<td>lin-12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>recessive (If)</td>
<td>2&lt;sup&gt;0&lt;/sup&gt; → 1&lt;sup&gt;0&lt;/sup&gt;</td>
<td>For VU→AC: L2/L3 moult</td>
<td>1&lt;sup&gt;0&lt;/sup&gt;/2&lt;sup&gt;0&lt;/sup&gt;</td>
</tr>
<tr>
<td>lin-12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>dominant (gf)</td>
<td>1&lt;sup&gt;0&lt;/sup&gt; → 2&lt;sup&gt;0&lt;/sup&gt;</td>
<td>—</td>
<td>1&lt;sup&gt;0&lt;/sup&gt;/2&lt;sup&gt;0&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

m, maternal genotype; z, zygotic genotype; If, loss-of-function; gf, gain-of-function; dtc, distal tip cell; VU, ventral uterine precursor; AC, anchor cell precursor. In all cases, the temperature-sensitive period of gene function corresponds to the time of development at which the relevant interaction is taking place.

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In addition to this germline phenotype, *glp-1* mutants have an embryonic phenotype that indicates the need for maternal *glp-1* product in embryogenesis (Austin & Kimble 1987, Priess et al 1987). This embryonic phenotype was observed in conditional mutants of *glp-1*. At permissive temperature, *glp-1(ts)* homozygotes produce a normal number of germ cells, but when shifted as adults to the restrictive temperature, progeny do not survive. *glp-1(ts)/glp-1(+) heterozygous cross-progeny of a *glp-1(ts)* mother do not survive; therefore the *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 pharynx development (Priess & Thomson 1987, Austin & Kimble 1987). The embryos have a near normal number of cells, but do not possess the anterior half of their pharynx (Table 2) and do not change from a ball of cells to an elongated worm. Since they do possess the posterior part of the pharynx, differentiation of pharyngeal cells per se is not affected. Using reciprocal shift experiments, the stage of embryogenesis at which the maternal *glp-1* product is required has been found to be from 4–28 cells of embryogenesis. This temperature-sensitive period corresponds extremely well with the time at which anterior pharynx development is induced (Priess & Thomson 1987). This induction event appears to be an interaction between the AB blastomere (or its descendants) and the P1 blastomere (or its descendants). Although no interactions are known to be required for hypodermal morphogenesis, such interactions may exist.

What role does *glp-1* play in regulating cellular interactions? Is it a component of the signalling mechanism or the receiving mechanism? To address this question, we examined genetic mosaic animals in which either the distal tip cell or the germline was defective for *glp-1* (Austin & Kimble 1987). Our results are summarized in Table 3. They indicate that the *glp-1* activity necessary for germline mitoses is produced by the germline. This implies that *glp-1* encodes a component of the receiving machinery for the distal tip cell-germline interaction. The mosaic analysis also shows that *glp-1* activity in the distal tip cell is neither necessary nor sufficient for germline development. Thus, *glp-1* does not encode the distal tip cell signal.

The effects of *lin-12* mutations on development have been extensively reviewed (Sternberg & Horvitz 1984, Greenwald 1987). Here, I emphasize one point: *lin-12* is involved in the regulation of interactions between cells in equivalence groups. Two types of *lin-12* allele have been isolated. Recessive alleles (loss-of-function, *lf*) transform cells of equivalent potential to one fate, and dominant alleles (gain-of-function, *gf*) transform the same cells to a second fate. Thus, in wild-type animals, where two equivalent cells can adopt either a primary or secondary fate, in *lin-12(lf)* mutants both cells adopt the primary fate, and in *lin-12(gf)* mutants both adopt the secondary fate. Opposite effects on development by *lf* and *gf* alleles have been observed in other genes that regulate cell fate (e.g. Sternberg & Horvitz 1984).
TABLE 3  Genetic mosaic experiments with glp-1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distal tip cell</td>
<td>Germline</td>
</tr>
<tr>
<td>Wild-type</td>
<td>glp-1(+)</td>
<td>glp-1(+)</td>
</tr>
<tr>
<td>Mutant</td>
<td>glp-1(-)</td>
<td>glp-1(-)</td>
</tr>
<tr>
<td>Mosaic</td>
<td>glp-1(+)</td>
<td>glp-1(-)</td>
</tr>
<tr>
<td>Mosaic</td>
<td>glp-1(-)</td>
<td>glp-1(+)</td>
</tr>
</tbody>
</table>

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

By genetic analysis, glp-1 was shown to map 0.02% to the right of lin-12 (Fig. 1) (Austin & Kimble 1987). This region of the C. elegans genome is represented by a large series of overlapping cosmids (Greenwald et al 1987). It was therefore possible to use the appropriate cosmids as hybridization probes in an attempt to identify the glp-1 gene. We found that the ZK506 cosmid, which neighbours lin-12 (Fig. 2), detects alterations in two different glp-1 mutations on Southern blots (Austin & Kimble, in preparation). One EMS-induced (ethyl methanesulphonate) mutation, q172, is associated with a small, 300 bp deletion in a 1.5 kb EcoRI fragment of this cosmid; a gamma-ray induced mutation, qDf2, is associated with a large deletion that breaks in the same 1.5 kb EcoRI fragment and removes the rest of the DNA in ZK506 to the right of that fragment (Fig. 3). The right end of qDf2 has not yet been determined. From this region, we have identified one major transcript of 4.8 kb that is likely to encode the glp-1 product (Fig. 3).

Fortuitously, in a search of the C. elegans genome for lin-12 homologues, a gene in the ZK506 cosmid was identified (J. Yochem & I. Greenwald, personal communication). When a phage bearing this lin-12 homologue was used as a

FIG. 1.  Genetic position of lin-12 and glp-1. glp-1 maps 0.02% to the right of lin-12 on chromosome III. Reproduced with permission (and minor modification) from Austin & Kimble (1987).
FIG. 2. The *lin-12* contig. Numerous overlapping cosmids have been identified in the region of *lin-12*. The *lin-12* gene, carried on C26F5, is indicated by an enclosed black rectangle. The *glp-1* gene maps to a neighbouring cosmid, ZK506 (Austin & Kimble, in preparation). This figure was prepared from a cosmid map provided by A. Coulson and J. Sulston (unpublished data).
hybridization probe to q172 DNA, the 300 bp deletion associated with this glp-I mutation was detected on a Southern blot (J. Austin & J. Kimble, in preparation). Therefore, glp-I is likely to be identical to the lin-12 homologue. Another gene homologous to lin-12 has been identified in Drosophila (Wharton et al. 1985). This gene, Notch, regulates the decision between differentiation as an epidermal precursor cell and a neuroblast. Thus, all three homologous genes, glp-I, lin-12 and Notch influence a developmental decision of cell fate. Presumably, all do so by mediating a cellular interaction that regulates this decision. Since the deduced amino acid sequences of lin-12 and Notch have the molecular characteristics predicted for membrane proteins (Yochem et al. 1988, Greenwald 1985, Wharton et al. 1985), glp-I may also encode a membrane protein. The functions of glp-I, lin-12 and Notch in mediating cellular interactions are not known. A simple possibility is that each encodes a receptor. For glp-I, the receptor would bind the signal emitted by the distal tip cell (or P1). Alternatively, it may encode a component of that receptor, or some other membrane protein that is essential to transduction of the distal tip cell (or P1) signal.

Fig. 4 presents one model for the molecular function of glp-I in the germline. We show the glp-I product as a receptor located in the membrane of the germline syncytium. Upon binding of the signalling molecule produced by the distal tip cell, this receptor transduces the signal to direct continued mitotic divisions in the germline. We suggest that the glp-I protein may be present throughout the germline tissue and that it is the position of the distal tip cell and its signal which determines where glp-I will actively direct germline mitoses. This model is supported by results from genetic mosaic analysis that place glp-I function in the germline, and by the homology of glp-I with lin-12, which suggests that glp-I may encode a membrane protein. Although this model is extremely speculative, it serves as a useful starting point for understanding how the glp-I gene product functions.
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![Diagram](image)

**FIG. 4.** Model for *glp-1* function. 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, we have drawn the distal tip cell at some distance from the germline to permit 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 & Ward 1988).

Based on the genetic mosaic experiments of Austin & Kimble (1987) and the predicted *glp-1* molecular structure of J. Yochem and I. Greenwald (personal communication), we propose that *glp-1* encodes a component of the membrane receptor for the distal tip cell and P₁ signals. In this figure we show the *glp-1* product as a receptor (Y) that is present throughout the germline. This distribution of *glp-1* product is suggested for simplicity; there is no evidence to date of its localization. We propose that the distal tip cell emits a signal (●) that binds the *glp-1* receptor locally. Since the distal tip cell signal appears to act over a distance (Kimble & White 1981), we propose that the ligand-activated *glp-1* generates a second messenger that diffuses in the germline syncytium to exert its mitogenic influence on germline nuclei more proximally.

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? Is the *glp-1* protein a component of the receptor itself as depicted in Fig. 4 or does it serve some other function that is critical to transduction of the signal? What is the distribution of the *glp-1* protein in the germline? And how is *glp-1* function limited to the AB cell in the embryo? Is the *glp-1* protein inherited on the membrane of the oocyte and distributed to all blastomeres? If so, a localized signal may activate *glp-1* to induce pharyngeal differentiation in AB. The answers to these questions are now accessible. Starting with the cloned *glp-1* gene, we can analyse the nature of the *glp-1* products and their distribution during development and we can investigate the regulation and function of *glp-1* after its reintroduction into the *C. elegans* germline and embryo.
Acknowledgements

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DISCUSSION

Garcia-Bellido: The genetic mosaic analysis you presented has been done with only one allele. Have you tried any other alleles?

Kimble: No, the alleles we have available in my lab are very similar to each other, and we have no reason to believe one would be different from another in a mosaic analysis. We prefer to concentrate on the molecular analysis of glp-1 for the moment and to generate appropriate nucleic acid and antibody probes for localization studies.

Weisblat: One of the glp-1 alleles reported by Priess et al (1987) does not affect the germline, but does alter embryos. Is it known where the mutation in this allele is located?

Kimble: No, that is not yet known.

Weisblat: The residual signal that you see on Northern blots of glp-1 RNA obtained from animals with no germline, could this be somatic?

Kimble: That is one possibility. The mutant we used with a reduced number of germ cells has about 20 instead of the normal 2000. The small amount of RNA seen in that mutant might be present in those few germ cells, or it might be present in a somatic tissue.

Meinhardt: How do you explain the mutant phenotype of lin-12, where regions of germline proliferation are found at both distal and proximal ends of a single germline tube? Do you think that some sort of inhibition doesn’t work any more?

Kimble: Greenwald and her colleagues showed some years ago that many different cells are transformed from one fate to another in lin-12 mutants. We postulate that one of the somatic gonadal cells in the proximal area has been transformed so that it now has distal tip cell activity. This newly transformed cell would then influence neighbouring germ cells to remain mitotic.

Nüsslein-Volhard: Is this the lack-of-function phenotype of lin-12?

Kimble: Yes.

Nüsslein-Volhard: According to your model, one expects that there is a group of genes which should have the same phenotype as glp-1.

Kimble: That is right, but we did not isolate other mutants with a phenotype like that of glp-1. There are several possible explanations for failure to detect other genes involved in this process. There may be redundant genes; if so, elimination of one would not cause a mutant phenotype. Such genes may be used for more than one cell interaction, in which case elimination of one might cause a different mutant phenotype such as embryonic lethality. The maternal rescue of glp-1 embryos permitted us to see the germline phenotype. If other genes are involved in the same processes as glp-1 but are not rescued, they might be zygotic lethal.
Wieschaus: The *glp-I* product is used for cell interactions in the early embryo. It is then not used until the animal grows up and has a gonad. Are there other cell interactions that occur in the worm outside those two time points?

Kimble: There are several other cell interactions that occur during *C. elegans* development. The best known is anchor cell induction where a single regulatory cell in the somatic gonad, the anchor cell, induces underlying hypodermal cells to generate a vulva. Although there are genes that are candidates for mediating this interaction, the evidence is not as compelling as it is for *glp-I* and *lin-12*. There are many genes known that are required for normal proliferation of the germ line and for normal development of the vulva. Any or all of these genes may mediate the cell interactions that influence these developmental events.

Garcia-Bellido: Are there other regions in the genome homologous to *lin-12* or *glp-I*?

Kimble: John Yochem is characterizing *lin-12* homologues of *C. elegans* in the laboratory of Iva Greenwald at Princeton University, but has only preliminary data at the moment.

Davidson: In that syncytial gonad, what are the real dimensions over which the second messenger is supposed to act?

Kimble: That is a difficult question to answer because we cannot detect cells as they make the decision to leave mitosis and enter meiosis. As an estimate of the order of magnitude, I would say about 100 μm.

Garcia-Bellido: Have you looked at where the *lin-12* dominant is expressed?

Kimble: Genetic mosaics of *lin-12* are currently being examined in Greenwald's lab. I would like to point out one important difference between cell interactions influenced by *glp-I* and those influenced by *lin-12*. Cell interactions that depend on *glp-I* activity occur between two very different types of cells (e.g. the somatic gonadal distal tip cell and germ cells). The decision regulated is between two fates (e.g. mitosis and meiosis) in only one of the interacting cells. In contrast, interactions that depend on *lin-12* occur between equivalent cells. In mutants lacking *lin-12*, both cells follow one fate; in mutants bearing gain-of-function dominant mutations in *lin-12*, both cells follow the alternate fate. We have not found a gain-of-function mutant of *glp-I*; in such mutants, we would predict that germ cells normally in meiosis might remain in mitosis instead.

Struhl: What is the phenotype of the *glp-1 lin-12* double mutant?

Kimble: We are constructing that double mutant now, so the result is not yet known.

Lawrence: Could you tell us more about *Notch*.

Kimble: *Notch* is one of the neurogenic loci in *Drosophila*. In wild-type development there is a sheet of cells from which neuroblasts delaminate. The remaining cells generate epidermal cells. Activity of the *Notch* gene is required in the epidermal cells to prevent them from delaminating and becoming neuroblasts; if *Notch* is absent, all cells delaminate and enter the nerve lineage.
Nüsslein-Volhard: Only in the ventral ectoderm, whereas Notch is expressed everywhere.

Struhl: There is one critical similarity: the laser ablation experiments of Doe and Goodman (1985) showed that if you remove the cell that would normally delaminate, then another cell from the epidermis would delaminate. This suggests that the cells somehow decide which is going to go out and which is going to stay. That's very similar to the lin-12 situation, where only one of the two cells makes the decision and the other makes the alternative decision.

Kimble: Yes; and in addition, the cell interactions influenced by Notch are more similar to those influenced by lin-12 than to those influenced by glp-1.

Cabrera: You presented evidence that glp-1 functions in the germline and is therefore part of the receiving mechanism in the distal tip cell–germline interaction. This contradicts the result obtained in Drosophila where Notch is clearly non-autonomous.

Wieschaus: One of the issues that complicates autonomy or non-autonomy of Notch is that the result depends on the size of the mosaic patch. If you produce very large patches of Notch, generally you get the Notch phenotype. If you produce a single Notch cell in the epidermis, that cell can remain epidermal, even though Notch would normally cause it to become a neuroblast. Anything bigger than one cell, even two adjacent cells, and you begin to see an autonomous effect of Notch.

McKeown: That just tells you the distance over which the signalling molecule works.

Wieschaus: If there are two adjacent cells, each of those cells is surrounded on most of its interfaces by wild-type cells, yet that is not enough for a Notch cell to be rescued by this type of non-autonomy. It must be totally surrounded by wild-type cells. I can't think whether that's going to influence how you want to interpret the cellular basis for the non-autonomy but it seems that it should.

McKeown: Gerhard Technau and Jose Campos-Ortegas (1987) have done experiments in which they have taken Notch- blastomeres and transferred them into wild-type embryos. Rather than developing as only neural cells, they are capable of developing as both ectodermal and neural cells. The implication is that Notch- embryos are not missing a receptor: they are missing the signal. That's exactly the opposite of what you are saying here.

Kimble: The difficulty interpreting the Notch mosaic results suggests to me that we may not understand some important facts about these loci. One possible complication is that the genes may produce more than one product. Perhaps, the same gene can produce both signal and receptor. For example, the entire glp-1 gene might encode a membrane protein that acts as a receptor, whereas a protein made from the 5' half of glp-1 would consist of EGF-like peptides and might generate the signal.
How do I accommodate our genetic mosaic results with such a suggestion? I must postulate that the mutation used for mosaic analysis is located at the 3' end and does not affect production of the 5' transcript. We have preliminary results that most of our glp-1 mutations map to the 3' region of the gene. This includes the mutation used for mosaic analysis.

Heasman: We have done some preliminary experiments on isolated mouse germ cells. Can we draw some parallels here? We take germ cells at the time that they are just beginning to migrate; they are mitotic cells and in culture those cells divide for a while and then slow down and appear to become meiotic cells and have a different phenotype. However, when we use a medium conditioned by the germ ridges from embryos, we can maintain the mitotic state in culture. This activity is blocked by antibodies against the EGF receptor. Furthermore, in the W mutants where there are no germ cells, conditioning of the medium by germ ridges does not stimulate mitosis. I wonder whether a similar EGF-type growth factor is involved in both embryonic systems.

Kimble: It was recently reported that the W locus encodes a protein similar to the c-kit proto-oncogene, a tyrosine kinase receptor (Geissler et al 1988). To answer your question, I believe that the maintenance of stem cell populations of mitotically dividing cells may rely on similar mechanisms in both C. elegans and in higher vertebrates and in both germ cells and other tissues. For example, there is evidence that haemopoietic stem cells depend on the 'microenvironment,' so they too may require interaction with other cells to continue proliferation.

Kornberg: You said initially that the allele you did the mosaic analysis with was a null allele. How did you define it as a null?

Kimble: Our proposal that this allele is a null allele is based on our characterization of multiple glp-1 alleles. These include one small internal deficiency and one large deficiency that breaks in the middle of the gene. All strong alleles, including the deficiencies and the allele used for mosaic analysis, are fully penetrant and have identical phenotypes. However, it is certainly possible that these mutants knock out only one function of the glp-1 gene.

Cabrera: Does this putative intracellular domain of glp-1, lin-12 or Notch have homology to a tyrosine kinase domain?

Kimble: No. These genes are homologous in their putative extracellular domains to EGF; they are not homologous to the EGF receptor, which is a tyrosine kinase.

Wolpert: I find it somewhat ironic that you are using Caenorhabditis to talk about cellular interactions and receptors and so forth, and very little about lineage. Is there really no autonomous generation of diversity? I am not talking about the very early stages where there are obviously cytoplasmic differences.

Kimble: A mechanism of cell determination relying strictly on cell lineage is much more difficult to prove experimentally than a mechanism relying on
cellular interactions. Alterations in cell fate after laser ablation of individual cells can be used convincingly to delineate cell interactions, but the lack of an effect in the same experiment does not provide equally compelling evidence that cellular interactions have no influence.

**Davidson:** Nevertheless, is it not fair to say that the experiments done by Priess & Thomson (1987), which demonstrate the requirement for cellular interaction in the anterior pharynx, do not give the same kind of result for the development of the posterior pharynx? There are other analogous situations where one part of the given structure is made one way and the other part is made in another way.

**Kimble:** That is possible, but again relies on interpretation of a negative result.

**Davidson:** It's a little stronger than just a negative result. The same experimental protocol results in one thing in one region and another in another place.

**Wieschaus:** You showed this terribly abnormal embryo which lacks glp-1 activity. Then you showed Jim Priess' embryo which had only an abnormal pharynx. That suggests that the glp-1 product must control more than just pharynx development and therefore that cellular interactions must be playing roles in other events in embryogenesis. Can you use your temperature-sensitive mutant to investigate this under precisely controlled conditions?

**Kimble:** We have shown that both embryonic morphogenesis and anterior pharynx development require maternal glp-1 product between the stages of four and 28 cells. If we shift these glp-1 mutants from the permissive to the restrictive temperature at the 8-cell stage, larvae are formed, but the anterior pharynx does not develop. This result suggests that embryonic morphogenesis depends on some event occurring between four and eight cells, while pharynx induction depends on an event taking place between eight and 28 cells.

**Wieschaus:** Have you done pulses of permissive or restrictive temperature?

**Kimble:** No.

**Nüsslein-Volhard:** Have you done in situ hybridization or antibody staining?

**Kimble:** We have begun in situ experiments, but have not yet developed antibodies. We have no results to report yet.

**Morata:** A few years ago you said that the equivalence group could be defined as units of interactions in the ventral nerve cord in the nematode. You implied that they could be equivalent to compartments in *Drosophila*. Have you identified genes which specifically recognize these equivalence groups?

**Cabrera:** I think there is a substantial difference between equivalence groups and compartments. In the former, cellular interactions take place to specify different cell lineages. In the latter, boundaries are created between cells. Within these boundaries, different building strategies are implemented, such as anteroposterior or dorsoventral polarity and proximodistal differential cell growth.
Kimble: The analogy I drew between equivalence groups in \textit{C. elegans} and compartments in \textit{Drosophila} was based on the idea that, in each case, distinct groups of cells might be set aside during embryogenesis to establish specific regions of the animal's anatomy.

References

Doe CQ, Goodman CS 1985 The role of cell interactions and cell lineage in the determination of neuronal precursor cells. Dev Biol 111:206–219