glp-1 can substitute for lin-12 in specifying cell fate decisions in Caenorhabditis elegans

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SUMMARY

Members of the lin-12/Notch gene family encode receptors for intercellular signals and are found throughout the animal kingdom. In many animals, the presence of at least two lin-12/Notch genes raises the issue of the significance of this duplication and divergence. In Caenorhabditis elegans, two lin-12/Notch genes, lin-12 and glp-1, encode proteins that are 50% identical, with different numbers of epidermal growth factor-like motifs in their extracellular domains. Many of the cell fate decisions mediated by lin-12 and glp-1 are distinct. Here, we express glp-1 protein under the control of lin-12 regulatory sequences in animals lacking endogenous lin-12 activity and find that glp-1 can substitute for lin-12 in mediating cell fate decisions. These results imply that the lin-12 and glp-1 proteins are biochemically interchangeable, sharing common ligand and effector proteins, and that the discrete lin-12 and glp-1 mutant phenotypes result from differential gene expression. In addition, these results suggest that the duplicate lin-12/Notch genes found in vertebrates may also be biochemically interchangeable.

Key words: lin-12, glp-1, lin-12/Notch gene family, cell-cell interactions, receptors

INTRODUCTION

Receptor-mediated intercellular signalling events control many cell fate choices during animal development. The lin-12 and glp-1 genes encode related transmembrane proteins, which appear to function as receptors for intercellular signals that specify cell fates (Greenwald et al., 1983; Austin and Kimble, 1987, 1989; Priess et al., 1987; Seydoux and Greenwald, 1989; Yochem and Greenwald, 1989). lin-12 and glp-1 are members of the lin-12/Notch family of proteins, which all possess several structural features [diagrammed in Fig. 1 of the Results section], including tandem epidermal growth factor (EGF)-like motifs and lin-12/Notch repeat (LNR) motifs in their extracellular domains, and tandem cdc10/SWI6 repeats (sometimes called ankyrin repeats) in their intracellular domains (reviewed in Greenwald and Rubin, 1992).

lin-12 and glp-1 are located close to one another in the genome and are thought to have arisen by a gene duplication event, since corresponding exons average about 50% nucleotide identity and several splice sites are precisely conserved (Yochem and Greenwald, 1989). The two genes mediate distinct cell fate decisions, since the phenotypes of null mutations in each gene are distinct (Greenwald et al., 1983; Austin and Kimble, 1987; Priess et al., 1987). In addition, they are genetically redundant for other cell fate decisions, since the lin-12(0) glp-1(0) double mutant displays a highly penetrant early larval lethality associated with cell fate transformations (Lambie and Kimble, 1991).

Like Caenorhabditis elegans, vertebrates have at least two lin-12/Notch genes, which are expressed in distinct but overlapping patterns during development (Gallahan and Callahan, 1987; Ellis et al., 1991; Weinmaster et al., 1991, 1992; Franco del Amo et al., 1992; Lardelli and Lendahl, 1993). These observations raise the issue of the functional significance of the duplication and divergence. This issue can be addressed in C. elegans, where genetic analysis has revealed the functions of the two lin-12/Notch genes, lin-12 and glp-1. There have been several considerations arguing against the biochemical interchangeability of the lin-12 and glp-1 proteins but, until this study, there was no direct test of this issue.

Several lines of indirect evidence suggested interchangeability. (1) Studies of laser-operated wild-type hermaphrodites and lin-12(0) mutants demonstrated that a signal from the anchor cell, which is a known source of ligand for lin-12, can inappropriately activate glp-1. These observations suggest that the normal ligand for lin-12 activates glp-1, although there are other possible explanations (Seydoux et al., 1990). (2) lin-12 and glp-1 are genetically redundant for certain cell fate decisions during embryogenesis, suggesting that the lin-12 and glp-1 proteins may be biochemically interchangeable receptors in the affected cells (Lambie and Kimble, 1991). However, genetic redundancy might also
result if lin-12 and glp-1 are components of parallel signalling pathways in the affected cells, or act in different cells, which function redundantly in controlling the fates of the affected cells through cell-cell interactions. (3) Certain gain-of-function mutations in glp-1 behave like lin-12 gain-of-function mutations in one lin-12-mediated cell fate decision (Mango et al., 1991; Roehl and Kimble, 1993), suggesting that glp-1 may be able to mediate this decision. However, the glp-1 mutant proteins have neomorphic properties and may not necessarily reflect the properties of the glp-1(+ ) protein (see Discussion). (4) Genetic screens have defined genes that appear to be involved in both lin-12- and glp-1-mediated cell fate decisions, suggesting that the lin-12 and glp-1 proteins interact with common proteins (Lambie and Kimble, 1991; Sundaram and Greenwald, 1993b; F. Tax, J. Thomas and H. R. Horvitz, personal communication).

Other observations suggested that the lin-12 and glp-1 proteins may not be interchangeable. (1) Although lin-12 and glp-1 display about 50% amino acid sequence identity, the two proteins have different numbers of epidermal growth factor-like motifs (a possible ligand-binding region; Rebay et al., 1991) and several stretches of amino acids in both the extracellular and intracellular domains that show little conservation (Yochem and Greenwald, 1989). (2) Apparent gene-specific suppressor mutations suggest that some proteins might interact specifically with either lin-12 or glp-1, and not necessarily both (Maine and Kimble, 1989, 1993; F. Tax, J. Thomas and H. R. Horvitz, personal communication; A. M. Powell and J. Press, personal communication). It is therefore possible that glp-1 is unable to interact with certain proteins that can interact with lin-12, and vice versa.

We have directly addressed the issue of biochemical interchangability by assaying the ability of lin-12/glp-1 gene chimeras that express glp-1(+ ) or lin-12/glp-1 protein chimeras under the control of lin-12 regulatory sequences to mediate lin-12-specific cell fate decisions. Our results imply that the lin-12 and glp-1 proteins are biochemically interchangable and share common ligand and effector proteins, and suggest that the proteins encoded by the duplicate vertebrate lin-12/Notch genes may also be biochemically interchangable.

MATERIALS AND METHODS

General methods and strains

Methods for handling and culturing C. elegans have been described by Brenner (1974). The wild-type parent for all strains used was C. elegans var. Bristol strain N2 (Brenner, 1974). The LG III mutations used were as follows: unc-36(e251) and unc-32(e189) (Brenner, 1974); lin-12(n941) and lin-12(n137 n720), which are lin-12(0) alleles (Greenwald et al., 1983); glp-1(q46) (Austin and Kimble, 1988); and qC1, a crossover suppressor for LGIII that is marked with dpy-19(e1259) and glp-1(q355) (J. Austin and J. Kimble, personal communication). The lin-11::lacZ reporter gene used was nls2 IV, an integrated array composed of plasmids containing lin-11(+ ) and lin-11::lacZ transgenes (Freyd, 1991; G. Freyd and H. R. Horvitz, personal communication). The mutation him-5(e1467) V was used to increase the frequency of self progeny males (Hodgkin et al., 1979). arEx29-34 are some of the extrachromosomal arrays generated in this study. arEx29, arEx31 and arEx33 express lin-12(+) protein from lin-12(+) transgenes on the array, and are composed of the plasmids pRF4 and p101i (plasmids are described below). arEx30, arEx32 and arEx34 express glp-1(+) protein under lin-12 regulation from lin-12/glp-1 chimeric genes on the array, and are composed of the plasmids pRF4 and p3.4ggi.

Plasmids

p101i expresses lin-12(+) protein under lin-12 regulation

p101i contains a 15.1 kb insert of lin-12 genomic DNA in Bluescript (Stratagene), from the BamH1 site at −3.4 kb to the BstEII site at +11.7 kb, numbered from +1 at the ATG initiation codon (Yochem et al., 1988).

lin-12/glp-1 gene chimeras

The starting plasmids for the construction of all lin-12/glp-1 chimeric genes were p101i and p66dAB, which contains a 9.9 kb glp-1(+ ) genomic fragment extending from the EcoRI site at −2.5 kb to the BamH1 site at +7.4 kb, numbered from +1 at the ATG initiation codon (Yochem and Greenwald, 1989) in Bluescript (Stratagene). Essentially, lin-12/glp-1 chimeric genes contain the 5′ flanking region of lin-12, beginning at the BamH1 site at −3.4 kb; coding region containing different amounts of 5′ lin-12 genomic DNA from p101i and 3′ glp-1 genomic DNA from p66dAB; and the 3′ flanking region of glp-1, extending to the BamH1 site at +7.4 kb.

p3.4ggi carries a lin-12/glp-1 gene chimera that expresses glp-1(+ ) protein under lin-12 regulation

The chimeric gene diagrammed schematically in Fig. 1 contains lin-12 5′ genomic DNA through the BamH1 site at +0.005 kb joined to glp-1 genomic DNA at position +0.005 kb (via a PCR-engineered BamH1 site). In addition, this construct contains a 1.5 kb PCR fragment from the first intron of lin-12 that was introduced into the third intron of the glp-1 genomic portion of the chimera at a StuI site at +5.6 kb. A chimeric gene that was otherwise identical but lacking the lin-12 first intron failed to give appropriate expression (see below and Results).

Plasmids containing lin-12/glp-1 chimeric genes that express chimeric proteins under lin-12 regulation

The protein chimeras encoded by these genes are shown schematically in Fig. 1. (1) The gene chimera that encodes protein chimera 1 contains lin-12 genomic DNA through the Psrl site at +9 kb joined to glp-1 genomic DNA at the Psrl site at +5.7 kb. (2) The gene chimera that encodes protein chimera 2 contains lin-12 genomic DNA through the StuI site at +2.2 kb joined to glp-1 genomic DNA at the SmaI site at +1.5 kb. (3) The gene chimera that encodes protein chimera 3 contains lin-12 genomic DNA through the SpeI site at +0.4 kb joined to a glp-1 fragment at the HindIII site at +0.2 kb (the HindIII site at +0.2 kb was adapted to a SpeI site).

Transgenic lines and evidence for regulatory sequences needed for lin-12-specific expression

Transgenic lines were established by microinjection into the hermaphroditic germ line (Fire, 1986; Mello et al., 1991). By accepted convention, ‘Ex’ is used to represent extrachromosomal arrays, and ‘Is’ to represent attached arrays. pRF4, a plasmid containing a cloned dominant rol-6(su1006) marker gene (Mello et al., 1991), was microinjected at a concentration of 100 μg/ml along with plasmids containing chimeric genes at a concentration of 6 μg/ml to create extrachromosomal arrays formed from the plasmid mixture. F1 Roller progeny were picked, and F2 Roller progeny used to establish lines. To assess rescue of lin-12(0) hermaphrodite phenotypes, plasmid mixtures were injected into a recipient strain of genotype unc-36 lin-12[941]/wC1, which enabled lin-12(0); arEx segregrants from established lines to be recognized by their Unc-36 and Rol-6 phenotypes. For the data shown in Fig. 3,
A. Constructs that encode lin-12(+) or glp-1(+) proteins under lin-12 regulation

B. Proteins that have lin-12 activity

Fig. 1. (A) Schematic diagram of transgenes that provide lin-12(+) or glp-1(+) protein under the control of lin-12 regulatory sequences. The first line depicts the genomic region encompassing the lin-12(+) coding sequence that can complement a lin-12(0) mutant. The second line depicts the lin-12/glp-1 protein. Essentially, this chimera contains the 3.4 kb 5' flanking region and ATG from the lin-12(+) gene attached in frame to the glp-1(+) coding region, with 0.9 kb of glp-1 3' flanking region (see below and Materials and Methods). In addition, the first intron of the lin-12 gene, which appears to contain a necessary enhancer, was added to the third intron of the glp-1 genomic region. See Materials and Methods for a detailed description of the constructs.

(B) Schematic diagram of wild-type and chimERIC proteins expressed by various gene chimeras studied. All members of the lin-12/Notch family have the same general organization of epidermal growth factor (EGF)-like motifs, three lin-12/Notch repeat (LNR) motifs and six cdc10/SWI6 motifs.

lin-12(+) or glp-1(+) proteins under lin-12 regulation.

the recipient strain had the genotype unc-36 lin-12(n941)/unc-32: nls2, and for the data shown in Fig. 4, the recipient strain had the genotype unc-36 lin-12(n941)/qC1: him-5. Plasmids containing lin-12/glp-1 chimeric genes that express chimeric proteins 1 or 2 were first injected into wild-type strain N2 (Brenner, 1974) and the resulting extrachromosomal arrays were then crossed into an unc-36 lin-12(n941) background.

Five independent extrachromosomal arrays containing the lin-12/glp-1 chimeric gene that expresses glp-1(+) protein shown in Fig. 1 were obtained; one of these was tested and found to rescue the lethality of lin-12(n941) glp-1(q46). When a chimeric gene similar to that shown in Fig. 1 but lacking the first intron of lin-12 was tried (i.e., the chimeric gene contained 3.4 kb of 5’ flanking sequence from lin-12 fused to the glp-1 genomic coding region), no extrachromosomal arrays were found to rescue either the sterility or vulval phenotype of lin-12(n941) or the lethality of lin-12(n941) glp-1(q46). These results strongly suggest that an enhancer element exists in the first intron of lin-12 which is required for lin-12-specific expression.

lin-12/glp-1 chimeric genes expressing chimeric proteins 1-3 were tested for the ability to rescue the sterility and vulval defects of lin-12(n941) and the lethality of the lin-12(n941) glp-1(q46) double mutant (Lambie and Kimble, 1991). The unc-36 marker was also present. At least 40 animals of each genotype were examined. Two independent extrachromosomal arrays expressing chimera 1 and three independent arrays expressing chimera 2 rescued the sterility and vulval defects of lin-12(n941); for each chimera, one of the arrays was tested and found to rescue the lethality of lin-12(n941) glp-1(q46) [but not the germline prolifer-
unclear (Sundaram and Greenwald, 1993a). Since analysis of sequences required for \textit{lin-12} expression has revealed that one or more of the last three introns is required for rescue of the egg-laying defect of \textit{lin-12} hypomorphs (H. A. W., unpublished observations), we think that the lack of complete restoration of egg-laying ability by chimeric genes reflects the absence of an essential sequence element(s).

Reciprocal experiments with \textit{lin-12} under the control of \textit{glp-1} promoter elements were not feasible due to the inability of our group and others (A. Fire and J. Priess, personal communication) to complement \textit{glp-1} mutants efficiently with \textit{glp-1} genomic DNA. We speculate that this difficulty is due to a general lack of expression of extrachromosomal arrays in the germline, where \textit{glp-1} function is needed (Austin and Kimble, 1987).

**RESULTS**

**Genes encoding \textit{lin-12}(+), \textit{glp-1}(+) or \textit{lin-12}/\textit{glp-1} chimeric proteins under \textit{lin-12} regulation**

We first identified a \textit{lin-12} genomic clone that can rescue the sterility and vulval defects of hermaphrodites lacking endogenous \textit{lin-12} activity [\textit{lin-12}(0) mutants] (Figs 1 and 2; see also Materials and Methods). We then constructed different \textit{lin-12}/\textit{glp-1} gene chimeras that replace different amounts of the 3′ genomic region of \textit{lin-12}(+) with the corresponding genomic region of the \textit{glp-1}(+) gene (Fig. 1 and Materials and Methods), and assessed the ability of these chimeric genes to complement the sterility and gross vulval defects of \textit{lin-12}(0) hermaphrodites, and the lethality of the \textit{lin-12} \textit{glp-1} double mutant. As described below and summarized in Table 1, various chimeric genes, which encode the intact \textit{glp-1}(+) protein or a \textit{lin-12}/\textit{glp-1} chimeric protein under the control of \textit{lin-12} regulatory sequences, have \textit{lin-12}(+) activity by these assays. We also describe in detail the ability of \textit{glp-1}(+) protein to mediate \textit{lin-12}-specific cell fate decisions (summarized in Table 2).

Although most experimental details are given in Materials and Methods, we note here two relevant points. First, all experimental plasmids were injected at the same concentration, so that the resulting extrachromosomal arrays are expected to contain similar numbers of copies of the transgenes being tested (Fire, 1986; Mello et al., 1991). Second, \textit{lin-12}/\textit{glp-1} chimeric genes involved the replacement of \textit{lin-12} intron and 3′ flanking sequences, and therefore of potential sequence elements required for \textit{lin-12}-specific activity.

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**Table 1. \textit{glp-1}(+) and \textit{lin-12}/\textit{glp-1} chimeric proteins have \textit{lin-12} activity**

<table>
<thead>
<tr>
<th>Protein encoded by transgene</th>
<th>\textit{lin-12}(n941) Fertility</th>
<th>\textit{lin-12}(n941) Vulva</th>
<th>\textit{lin-12}(n941) glp-1(q46) Viability</th>
<th>Glp</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>\textit{lin-12}(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>\textit{glp-1}(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Chimera 1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chimera 2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chimera 3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

All animals examined were also homozygous for the chromosomal marker \textit{unc-36(e251)} (Brenner, 1974). Details about the phenotypes of transgenic lines, including a discussion of the egg-laying phenotype, are presented in Materials and Methods.

\textit{lin-12}(n941) hermaphrodites are sterile but have sperm and oocytes (Greenwald et al., 1983; Seydoux et al., 1990).

\textit{lin-12}(n941) hermaphrodites have a large vulval protrusion with characteristic morphology (Greenwald et al., 1983; Seydoux et al., 1993).

\textit{lin-12}(n941) glp-1(q46) animals arrest as larvae (Lambie and Kimble, 1991).

\textit{Glp}, germline proliferation defective; a few sperm are present (Austin and Kimble, 1987; Priess et al., 1987).

\textit{5% of hermaphrodites lacked the characteristic large vulval protrusion (see Materials and Methods).}

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Fig. 2. General morphology of hermaphrodites of various genotypes. The \textit{lin-12}(+) hermaphrodite is from the wild-type strain N2. The \textit{lin-12}(0); \textit{Ex}[\textit{lin-12}(+)] hermaphrodite [complete genotype: \textit{unc-36} \textit{lin-12}(n941); \textit{arEx29}] is provided with \textit{lin-12}(+) protein from \textit{lin-12}(+) transgenes on the extrachromosomal array. The \textit{lin-12}(0); \textit{Ex}[\textit{glp-1}(+)] hermaphrodite [complete genotype: \textit{unc-36} \textit{lin-12}(n941); \textit{arEx30}] expresses \textit{glp-1}(+) protein from \textit{lin-12}/\textit{glp-1} chimeric genes on the extrachromosomal array. For the preceding three genotypes, an arrowhead marks the vulva; fertilized eggs can also be seen inside the uterus. The \textit{lin-12}(0) hermaphrodite [complete genotype: \textit{unc-36} \textit{lin-12}(n941)] is sterile and an arrow marks the characteristic large vulval protrusion.
expression, with sequences from the \textit{glp-1} gene. We found at least one such \textit{lin-12}-specific regulatory element in the first intron of \textit{lin-12} and inferred the existence of another elsewhere in the gene (see Materials and Methods). Thus, minor differences in behavior of \textit{lin-12}/\textit{glp-1} chimeric genes may result from differences in gene expression rather than differences in protein function.

\textbf{The ability of \textit{glp-1}(+) protein to mediate cell fate choice during vulval development}

We examined the vulval anatomy of \textit{lin-12(0)} hermaphrodites carrying \textit{lin-12(+)} transgenes, which express \textit{lin-12(+)} protein, or \textit{lin-12/glp-1} chimeric genes expressing \textit{glp-1(+) protein}. Most \textit{lin-12(0)} hermaphrodites carrying \textit{lin-12(+)} transgenes or \textit{lin-12/glp-1} chimeric genes expressing \textit{glp-1 (+)}

\textbf{Fig. 3.} Vulval development in hermaphrodites of various genotypes. For each genotype, the left panel shows an L3 hermaphrodite, with an AC indicated by an arrowhead. The right panel shows the vulval invagination in a mid-L4 hermaphrodite, with arrows marking cells that have the morphology and position of “N” descendants of a vulval precursor cell that adopted the 2° fate (Sulston and Horvitz, 1977; Sternberg and Horvitz, 1986). Abbreviated and complete genotypes are as shown in Fig. 2.
1(+) protein had a single vulval invagination with generally normal morphology (Table 2, Fig. 3). None of the hermaphrodites displayed the Multivulva phenotype associated with inappropriate activation of lin-12, which is characterized by up to six pseudovulvae (Greenwald et al., 1983; Greenwald and Seydoux, 1990; Struhl et al., 1993).

In wild-type hermaphrodites, there are six VPCs, consecutively numbered P3.p-P8.p. Each has the potential to choose one of three fates, termed ‘1°’, ‘2°’ and ‘3°’; normally only P5.p and P7.p choose the 2° fate (Sulston and White, 1980; Sternberg and Horvitz, 1986). This decision has been shown to require intercellular signalling and to be mediated by lin-12: in lin-12(0) hermaphrodites, none of the VPCs express the 2° fate, while in certain lin-12(d) hermaphrodites, all VPCs express the 2° fate. Although vulval development appeared to be normal in lin-12(0) hermaphrodites carrying either lin-12(+)-transgenes or lin-12/gpl-1 chimeric genes expressing glp-1(+) protein, we wanted to examine directly the cell fate choices of the VPCs. We therefore examined the expression pattern of a lin-11::lacZ gene, which is a reporter for the choice of the 2° fate (Freyd, 1991; G. Freyd and H. R. Horvitz, personal communication). We saw wild-type expression of the lin-11::lacZ reporter gene in appropriate VPC descendants of lin-12(0) hermaphrodites carrying lin-12(+)-transgenes or lin-12/gpl-1 chimeric genes expressing glp-1(+) protein (Fig. 4), implying that glp-1 can mediate choice of the 2° fate by a VPC.

**Fig. 4.** Expression of a reporter gene for the 2° fate by VPC descendants in hermaphrodites of various genotypes. The lin-11::lacZ reporter gene is a marker for the choice of the 2° fate by a VPC; in wild type, two VPC descendants adopt the 2° fate, and two descendants of each of those cells stain (Freyd, 1991; G. Freyd and H. R. Horvitz, personal communication). The lin-12(0); Ex[lin-12(+)] hermaphroditic has the complete genotype unc-36 lin-12(n941); nls2; arEx31. The lin-12(0); Ex[glp-1(+)] hermaphroditic has the complete genotype unc-36 lin-12(n941); nls2; arEx32.

### Table 2. glp-1(+) can substitute for lin-12(+) in specific cell fate decisions

<table>
<thead>
<tr>
<th>Relevant Genotype</th>
<th>Hermaphrodites</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z1.ppp/Z4.aaa</td>
<td>lin-12(d)</td>
</tr>
<tr>
<td>lin-12(0)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>lin-12(+)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>lin-12(0)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>lin-12(0); Ex[lin-12(+)]</td>
<td>1 (40/46)</td>
<td>1 (40/46)</td>
</tr>
<tr>
<td>lin-12(0); Ex[glp-1(+)]</td>
<td>1 (39/43)</td>
<td>0 (0/0)</td>
</tr>
</tbody>
</table>

The slight difference between the transgene expressing lin-12(+) protein, which results in a low penetrance gain-of-function phenotype, and the transgene expressing glp-1(+) protein, which does not, probably reflects differences in regulatory elements present in the two constructs (see text and Materials and Methods).

a. abn, abnormal vulva (see Figs 2 and 3).

b. The other six hermaphrodites lacked an anchor cell, which we attribute to the fact that the extrachromosomal array is likely to contain many copies of the lin-12(+) gene (see Mello et al., 1991). These hermaphrodites did not display any vulval induction, as would be expected for essentially wild-type lin-12(+).

c. The other four hermaphrodites had 2 ACs, which we attribute to inadequate expression from the extrachromosomal array or mosaicism.

d. The one remaining hermaphrodite had an abnormal vulval invagination, which we attribute to inadequate expression from the extrachromosomal array or mosaicism.

e. The other four hermaphrodites had a second small invagination adjacent to the normal vulval invagination.

f. The other two males had one ectopic hook, which we attribute to the likely presence of multiple copies of the lin-12(+) gene.

g. The other male had no hook, which we attribute to inadequate expression from the extrachromosomal array or mosaicism.

The slight difference between the transgene expressing lin-12(+) protein, which results in a low penetrance gain-of-function phenotype, and the transgene expressing glp-1(+) protein, which does not, probably reflects differences in regulatory elements present in the two constructs (see text and Materials and Methods).
We have shown that several lin-12-mediated cell fate decisions can occur normally if the glp-1(+) or several lin-12/glp-1 chimeric proteins are substituted for the lin-12 protein. Since we did not observe the phenotypes associated with inappropriate lin-12 activation, the activity of the glp-1(+), protein appears to be regulated appropriately by a ligand that normally interacts in these cells only with lin-12(+). Moreover, since normal cell fates are specified, glp-1(+) appears to be able to interact appropriately with downstream effector molecules that normally interact with lin-12(+). The ability of glp-1(+) to substitute for lin-12(+) implies that the two proteins, despite considerable sequence divergence and a difference in the number of
epidermal growth factor-like motifs in the extracellular domains, are biochemically interchangeable. The interchangeability of corresponding domains inferred from the ability of lin-12/glp-1 chimeric proteins to substitute for lin-12(+) underscores this point. 

One of the cell fate decisions normally mediated only by lin-12 that we found could be mediated by glp-1(+) protein is the decision of vulval precursor cells (VPCs) to express the 2° fate. Certain gain-of-function mutations in glp-1, which resemble lin-12 gain-of-function mutations in causing ectopic expression of the 2° fate (Mango et al., 1991; Roehl and Kimble, 1993), suggested indirectly that glp-1 might be able to mediate this decision. However, the glp-1 mutant proteins examined have neomorphic (novel) activity, so their properties do not necessarily reflect those of the glp-1(+) proteins. These glp-1 gain-of-function mutations are truncations and appear to cause ligand-independent activation of the glp-1 protein, so the ability of glp-1 to interact with the normal ligand for lin-12 cannot be assessed. Moreover, these glp-1 gain-of-function mutations alter the intracellular domain of glp-1 and hence might confer an ability to interact with a downstream effector protein that normally interacts only with lin-12. Indeed, our finding that glp-1(+) protein can mediate VPC fate choice strengthens the conclusions about the function of domains of lin-12/Notch proteins based on the behavior of glp-1 mutant proteins in VPC development (Mango et al., 1991; Roehl and Kimble, 1993).

Although lin-12 and glp-1 are required for distinct cell fate decisions (Greenwald et al., 1983; Austin and Kimble, 1987; Priess et al., 1987), the two genes appear to be genetically redundant for other cell fate decisions during embryogenesis, since the lin-12 glp-1 double mutant displays a highly penetrant early larval lethality associated with cell fate transformations (Lambie and Kimble, 1991). There are various plausible explanations for the genetic redundancy of lin-12 and glp-1 with respect to embryonic cell fate decisions. For example, lin-12 and glp-1 may be biochemically interchangeable receptors in the affected cells, components of parallel signalling pathways in the affected cells, or act in different cells, which function redundantly in controlling the fates of the affected cells through cell-cell interactions. Our finding that lin-12 and glp-1 are biochemically interchangeable for a number of postembryonic decisions does not distinguish among these explanations, but is consistent with the hypothesis that they function interchangeably in the affected embryonic cells (Lambie and Kimble, 1991). Thus, a simple explanation for all of the available data is that lin-12 and glp-1 use the same ligand and downstream effector molecules, with certain cells expressing lin-12 or glp-1 only, while certain other cells express both.

Our results with lin-12/glp-1 gene chimeras strongly suggest that there is an enhancer in the first intron of lin-12 that is necessary for lin-12-specific expression: a lin-12/glp-1 chimeric gene that contains the 5′ flanking sequence from lin-12 and the remainder of the coding region from glp-1 does not rescue a lin-12(00) mutant or a lin-12 glp-1 double mutant, but the addition of the first intron of lin-12 to this gene chimera leads to efficient rescue (Fig. 2 legend; see also Materials and Methods). This observation is interesting in light of the proposal that the ancestral gene more closely resembled glp-1, and that a duplicate of the ancestral gene was placed under the control of different regulatory elements to give rise to a new gene, lin-12 (Yochem and Greenwald, 1989). Indeed, it is possible that this kind of duplication event involving lin-12/Notch genes in other taxa has been used to increase the developmental potential of certain lineages or groups of cells.

Studies of the duplicate C. elegans genes may be relevant to a consideration of the duplicate (or multiple) mammalian genes. The known mammalian lin-12/Notch proteins show the same degree of relatedness as lin-12 and glp-1. Rat-Notch1 and rat-Notch2 proteins are 56% identical (Weinmaster et al., 1992). In mice, the MotchA and MotchB proteins are 60% identical, and the protein encoded by int-3, a proto-oncogene that is the third known member of the lin-12/Notch family, has fewer EGF-like motifs than the two Motch proteins and is 60% identical to MotchA in its intracellular domain (Franco del Amo et al., 1992; Reaume et al., 1992; Robbins et al., 1992; Lardelli and Lendahl, 1993). Moreover, a comparison of the expression pattern of the two rat-Notch genes as well as two of the three mouse homologs has revealed that they are expressed in some common embryonic tissues as well as in different ones (Weinmaster et al., 1992; Lardelli and Lendahl, 1993), which, assuming the expression pattern reflects the requirement for gene activity, is reminiscent of the situation with lin-12 and glp-1. Our finding that the glp-1 protein can substitute for lin-12 in cell fate decisions suggests that that the duplicate mammalian genes may also be biochemically interchangeable. Thus, the phenotypes of ‘knock-out’ mutations in the individual mammalian lin-12/Notch genes may only partially reveal the true requirement for the activity of individual lin-12/Notch genes.

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REFERENCES


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