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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; Ellisen 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 either for or 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 activity 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. Priess, 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 interchangeability 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 interchangeable 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 interchangeable.

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, 1987); and *qC1*, a crossover suppressor for LG*III* that is marked with *dpy-19(e1259)* and *glp-1(q339)* (J. Austin and J. Kimble, personal communication). The *lin-11::lacZ* reporter gene used was *nIs2 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 *Bam*HI site at −3.4 kb to the *Bst*EII 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 *Eco*RI site at −2.5 kb to the *Bam*HI 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 *Bam*HI 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 *Bam*HI 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 *Bam*HI site at +0.005 kb joined to *glp-1* genomic DNA at position +0.005 kb (via a PCR-engineered *Bam*HI 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 *Stu*I 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 *Pst*I site at +9 kb joined to *glp-1* genomic DNA at the *Pst*I site at +5.7 kb. (2) The gene chimera that encodes protein chimera 2 contains *lin-12* genomic DNA through the *Stu*I site at +2.2 kb joined to *glp-1* genomic DNA at the *Sma*I site at +1.5 kb. (3) The gene chimera that encodes protein chimera 3 contains *lin-12* genomic DNA through the *Spe*I site at +0.4 kb joined to a *glp-1* fragment at the *Hin*dIII site at +0.2 kb (the *Hin*dIII site at +0.2 kb was adapted to a *Spe*I site).

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

Transgenic lines were established by microinjection into the hermaphrodite germline (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. F_1 Roller progeny were picked, and F_2 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(n941)/qC1*, which enabled *lin-12(0); arEx* segregants from established lines to be recognized by their Unc-36 and Rol-6 phenotypes. For the data shown in Fig. 3,

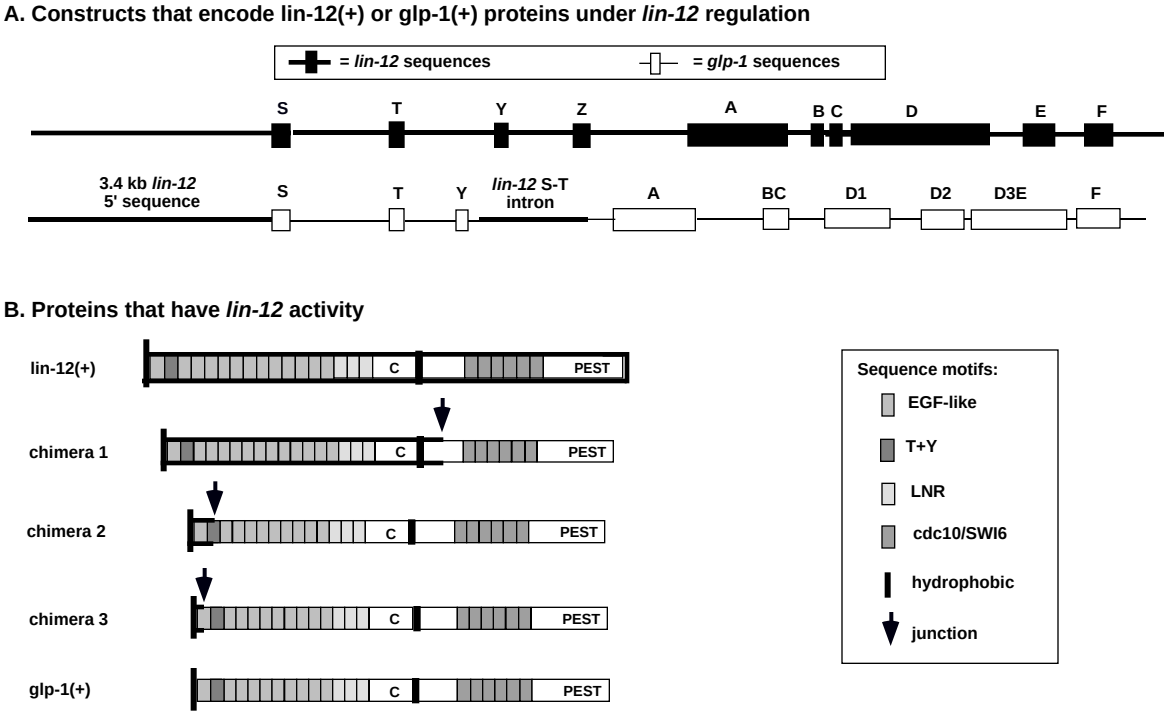

Fig. 1. (A) Schematic diagram of transgenes that provide lin-12(+) or glp-1(+) protein under the control of *lin-12* regulatory sequences. The first line depicts the genomic region encompassing the *lin-12(+)* coding sequence that can complement a *lin-12(0)* mutant. The second line depicts the *lin-12/glp-1* gene chimera that encodes 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 (Breeden and Nasmyth, 1987). They also have predicted signal and transmembrane hydrophobic sequences, represented by solid black; a pair of cysteine residues that is conserved in corresponding locations in an otherwise cysteine-poor region of the extracellular domain, represented by 'C'; and a 'PEST' sequence, apparently important for regulating protein stability (Rogers et al., 1986; Mango et al., 1991). Between the first and second EGF-like motif, lin-12 and glp-1 have a cysteine-based motif ('T+Y') that lacks some of the conserved amino acids of EGF-like motifs (Yochem et al., 1988). The *lin-12/glp-1* gene chimeras that encode lin-12/glp-1 protein chimeras 1-3 were created using conserved sites (detailed in Materials and Methods). The junctions are as follows: chimera 1, exon D; chimera 2, the T -Y (second) intron; chimera 3, the S-T (first) intron.

the recipient strain had the genotype *unc-36 lin-12(n941)/unc-32; nIs2*, 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 proliferation defect, a *glp-1*-specific phenotype]. Six independent extrachromosomal arrays expressing chimera 3, which has a hybrid first EGF-like motif, rescued the sterility defect well but only weakly rescued the vulval defects. The rescue by one array was quantified: 95% of the hermaphrodites were fertile, but only 5% of hermaphrodites lacked the abnormal vulval protrusion; however, this array rescues the lethality of *lin-12(n941) glp-1(q46)*.

Although the overt vulval defects of *lin-12(n941)* were rescued by most *lin-12/glp-1* chimeric genes, *lin-12(n941)* hermaphrodites carrying trangenes expressing either glp-1(+) or lin-12/glp-1 chimeric proteins were egg-laying defective. Hermaphrodites containing certain chimeric genes laid some eggs before turning into 'bags of worms'; others did not. This egg-laying defective phenotype resembles that of hypomorphic *lin-12* mutants; the cellular basis for the egg-laying defect of *lin-12* hypomorphs is

unclear (Sundaram and Greenwald, 1993a). Since analysis of sequences required for *lin-12* expression has revealed that one or more of the last three introns is required for rescue of the egg-laying defect of *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 *lin-12* under the control of *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 *glp-1* mutants efficiently with *glp-1* genomic DNA. We speculate that this difficulty is due to a general lack of expression of extrachromosomal arrays in the germline, where *glp-1* function is needed (Austin and Kimble, 1987).

RESULTS

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

We first identified a *lin-12* genomic clone that can rescue the sterility and vulval defects of hermaphrodites lacking endogenous *lin-12* activity [*lin-12(0)* mutants] (Figs 1 and 2; see also Materials and Methods). We then constructed different *lin-12/glp-1* gene chimeras that replace different amounts of the 3′ genomic region of *lin-12(+)* with the corresponding genomic region of the *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 *lin-12(0)* hermaphrodites, and the lethality of the *lin-12 glp-1* double mutant. As described below and summarized in Table 1, various chimeric genes, which encode the intact glp-1(+) protein or a lin-12/glp-1 chimeric protein under the control of *lin-12* regulatory sequences, have *lin-12(+)* activity by these assays. We also describe in detail the ability of glp-1(+) protein to mediate *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, *lin-12/glp-1* chimeric genes involved the replacement of *lin-12* intron and 3′ flanking sequences, and therefore of potential sequence elements required for *lin-12*-specific

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

Protein encoded by transgene	Phenotype in: *lin-12(n941)* Fertility[a]	*lin-12(n941)* Vulva[b]	*lin-12(n941) glp-1(q46)* Viability[c]	*lin-12(n941) glp-1(q46)* Glp[d]
none	−	−	−	ND
lin-12(+)	+	+	ND	ND
glp-1(+)	+	+	+	−
Chimera 1	+	+	+	−
Chimera 2	+	+	+	−
Chimera 3	+	+/−[e]	+	−

All animals examined were also homozygous for the chromosomal marker *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.

[a]*lin-12(n941)* hermaphrodites are sterile but have sperm and oocytes (Greenwald et al., 1983; Seydoux et al., 1990).

[b]*lin-12(n941)* hermaphrodites have a large vulval protrusion with characteristic morphology (Greenwald et al., 1983; Seydoux et al., 1993).

[c]*lin-12(n941) glp-1(q46)* animals arrest as larvae (Lambie and Kimble, 1991).

[d]Glp, germline proliferation defective; a few sperm are present (Austin and Kimble, 1987; Priess et al., 1987).

[e]5% of hermaphrodites lacked the characteristic large vulval protrusion (see Materials and Methods).

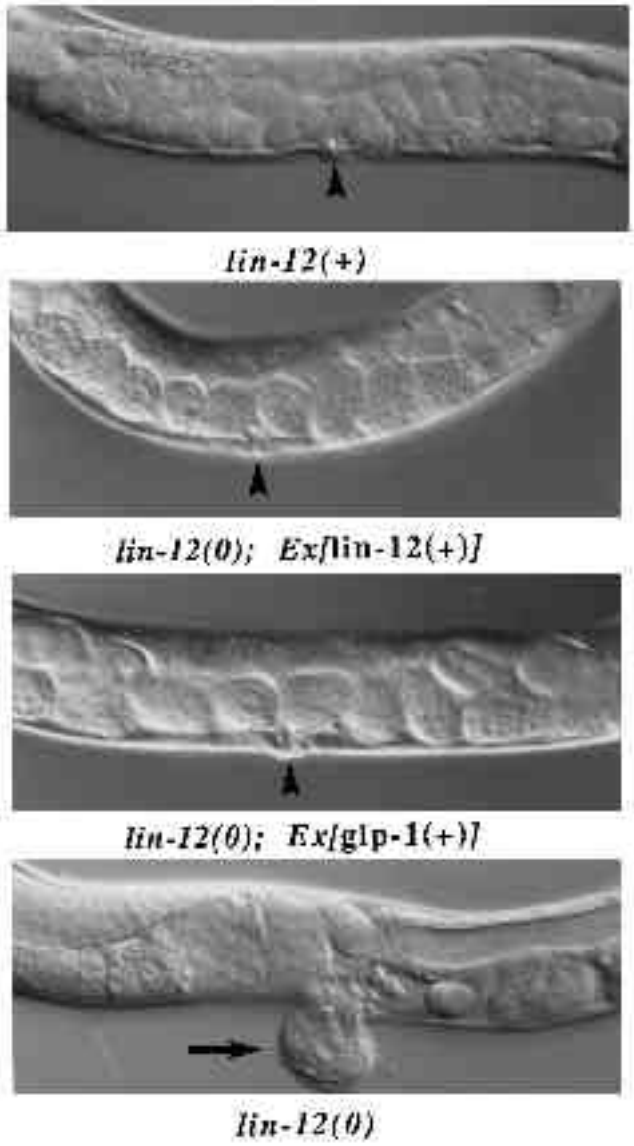

Fig. 2. General morphology of hermaphrodites of various genotypes. The *lin-12(+)* hermaphrodite is from the wild-type strain N2. The *lin-12(0); Ex[*lin-12(+)*]* hermaphrodite [complete genotype: *unc-36 lin-12(n941); arEx29*] is provided with lin-12(+) protein from *lin-12(+)* transgenes on the extrachromosomal array. The *lin-12(0); Ex[*glp-1(+)*]* hermaphrodite [complete genotype: *unc-36 lin-12(n941); arEx30*] expresses glp-1(+) protein from *lin-12/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 *lin-12(0)* hermaphrodite [complete genotype: *unc-36 lin-12(n941)*] is sterile and an arrow marks the characteristic large vulval protrusion.

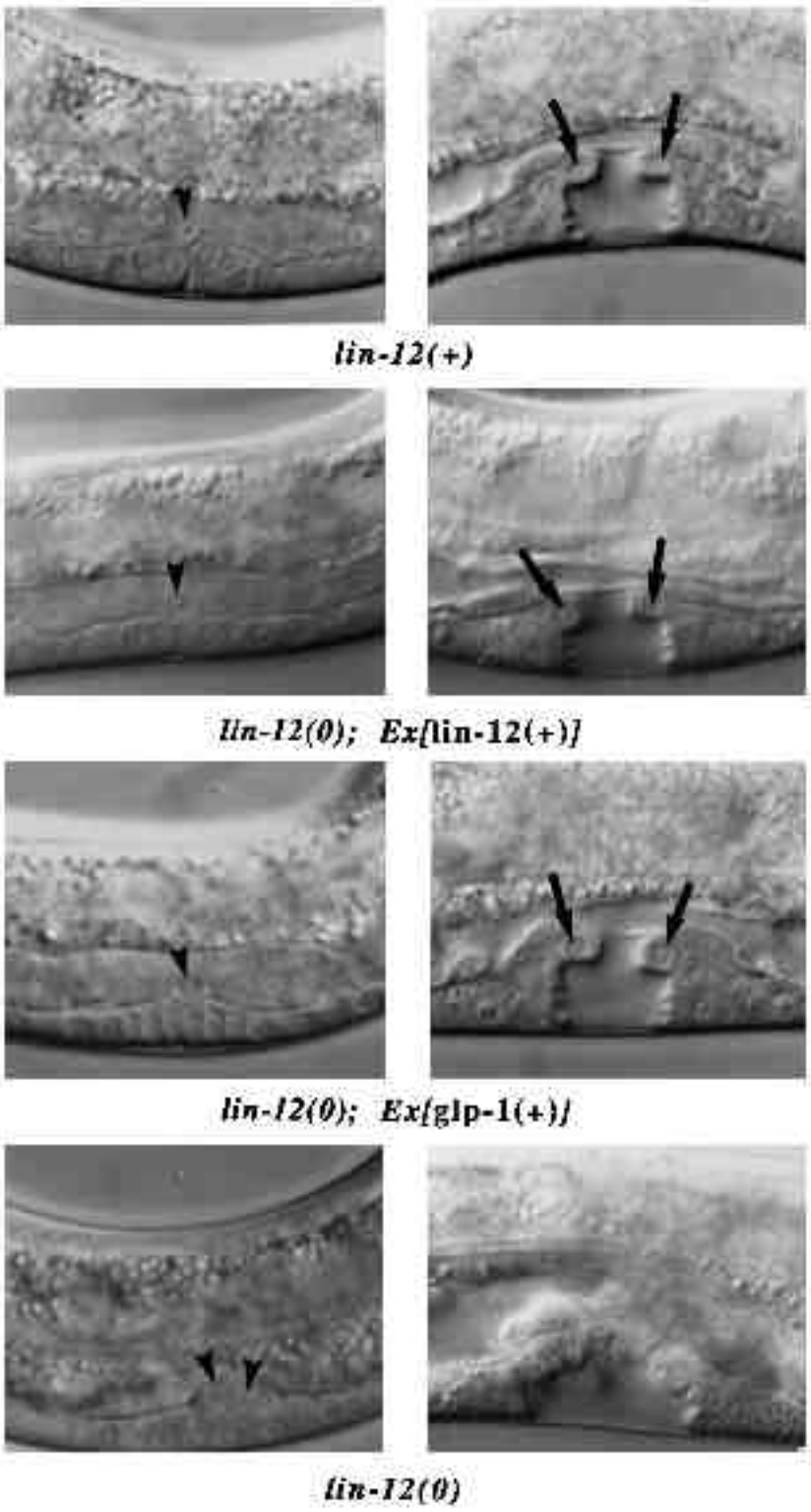

Fig. 3. Vulval development in hermaphrodites of various genotypes. For each genotype, the left panel shows an L3 hermaphrodite, with an AC indicated by an arrowhead. The right panel shows the vulval invagination in a mid-L4 hermaphrodite, with arrows marking cells that have the morphology and position 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.

expression, with sequences from the *glp-1* gene. We found at least one such *lin-12*-specific regulatory element in the first intron of *lin-12* and inferred the existence of another elsewhere in the gene (see Materials and Methods). Thus, minor differences in behavior of *lin-12/glp-1* chimeric genes may result from differences in gene expression rather than differences in protein function.

The ability of glp-1(+) protein to mediate cell fate choice during vulval development

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

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

Relevant Genotype	Hermaphrodites Z1.ppp/Z4.aaa No. of ACs	Hermaphrodites VPCs vulva	Hermaphrodites VPCs pseudovulvae	Males P9.p-P11.p No. of hooks
lin-12(d)	0	0	≤6	≤3
lin-12(+)	1	1	0	1
lin-12(0)	2	abn[a]	0	0
lin-12(0); Ex[(lin-12(+)*]*	1 (40/46)[b]	1 (40/46)[b]	0 (33/37)[e]	1 (8/10)[f]
*lin-12(0); Ex[*glp-1(+)*]*	1 (39/43)[c]	1 (50/51)[d]	0 (51/51)	1 (6/7)[g]

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 VPCs that do not receive an inducing signal due to absence of an anchor cell (Kimble, 1981).

[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.

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/glp-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/glp-1* chimeric genes expressing glp-1(+) protein (Fig. 4), implying that glp-1 can mediate choice of the 2° fate by a VPC.

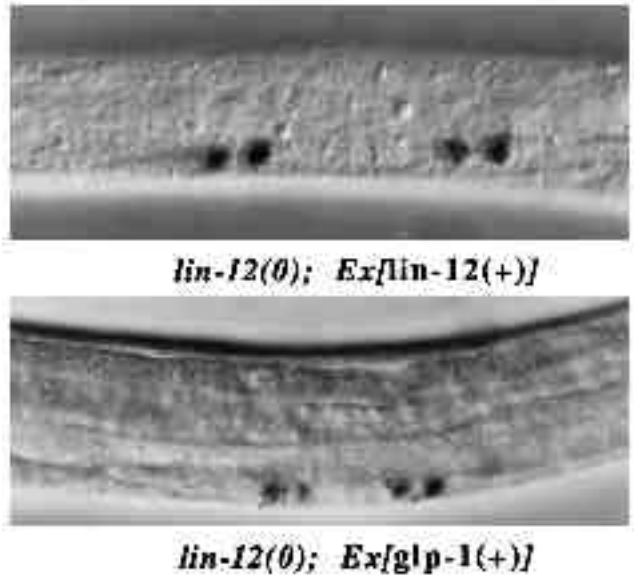

Fig. 4. Expression of a reporter gene for the 2° fate by VPC descendants in hermaphrodites of various genotypes. The *lin-11::lacZ* reporter gene is a marker for the choice of the 2° fate by a VPC; in wild type, two VPC descendants adopt the 2° fate, and two descendants of each of those cells stain (Freyd, 1991; G. Freyd and H. R. Horvitz, personal communication). The *lin-12(0); Ex[*lin-12(+)*]* hermaphrodite has the complete genotype *unc-36 lin-12(n941); nIs2; arEx31*. The *lin-12(0); Ex[*glp-1(+)*]* hermaphrodite has the complete genotype *unc-36 lin-12(n941); nIs2; arEx32*.

Ability of glp-1(+) protein to mediate cell fate choice during somatic gonadal development

We examined the ability of the glp-1(+) protein to mediate the best understood cell fate decision that is normally mediated by *lin-12*: the decision of two cells in the hermaphrodite gonad, Z1.ppp and Z4.aaa, between the anchor cell (AC) and ventral uterine precursor cell (VU) fates. In wild-type, interactions between Z1.ppp and Z4.aaa result in one choosing to become the AC and the other choosing to become a VU (Kimble and Hirsh, 1979; Kimble, 1981; Seydoux and Greenwald, 1989). In *lin-12(0)* mutants, both cells choose to become ACs; when *lin-12* is inappropriately activated, both choose to become VUs (Greenwald et al., 1983; Greenwald and Seydoux, 1990; Struhl et al., 1993). Most *lin-12(0)* hermaphrodites carrying lin-12(+) transgenes and most *lin-12(0)* hermaphrodites carrying *lin-12/glp-1* chimeric genes expressing glp-1(+) protein have a single AC

(Table 2, Fig. 3), implying that intercellular signalling between Z1.ppp and Z4.aaa occurs normally. None of the hermaphrodites carrying *lin-12/glp-1* chimeric genes expressing glp-1(+) protein exhibited the phenotype associated with inappropriate *lin-12* activation, the absence of an AC.

Ability of the glp-1(+) protein to mediate cell fate choice during male tail development

We examined the ability of glp-1(+) protein to mediate the choice of cell fate by the equivalent cells P9.p-P11.p in the male tail. In wild type, descendants of P10.p give rise to a 'hook', a distinctive sensory structure (Kimble and Hirsh, 1979). In *lin-12(0)* males, no hook is produced, while when *lin-12* is inappropriately activated, males have up to three hooks: the normal hook descended from P10.p and two additional ones, descended from P9.p and P11.p (Greenwald et al., 1983; Struhl et al., 1993). *lin-12(0)* males carrying either *lin-12(+)* transgenes or *lin-12/glp-1* chimeric genes expressing glp-1(+) protein produce a single normal hook structure (Table 2, Fig. 5). None of the males carrying *lin-12/glp-1* chimeric genes expressing glp-1(+) protein exhibited the ectopic hooks associated with inappropriate *lin-12* activity.

Ability of lin-12/glp-1 chimeric proteins to substitute for lin-12(+) protein

lin-12/glp-1 chimeric genes expressing chimeric proteins 1-3 (Fig. 1) 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. Chimera 1 contains the lin-12 extracellular and transmembrane domains and the glp-1 intracellular domain; chimera 2 contains the first EGF-like motif of lin-12, a hybrid 'T+Y' motif, and the remainder of the glp-1 protein; chimera 3 contains a hybrid first EGF-like motif and the remainder of the glp-1 protein. All three protein chimeras rescued the sterility defect of *lin-12(n941)* and the lethality of *lin-12(n941) glp-1(q46)* (but not the *glp-1*-specific germline defect) (Table 1). Chimeras 1 and 2 also rescued the vulval defect of *lin-12(n941)*; chimera 3 did not efficiently rescue the vulval defect (Table 1; see also Materials and Methods). It is unclear if inefficient rescue of the vulval defect of *lin-12(n941)* by chimera 3, which has a hybrid first intron, results from reduced protein function or reduced gene expression.

DISCUSSION

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(+) protein. 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

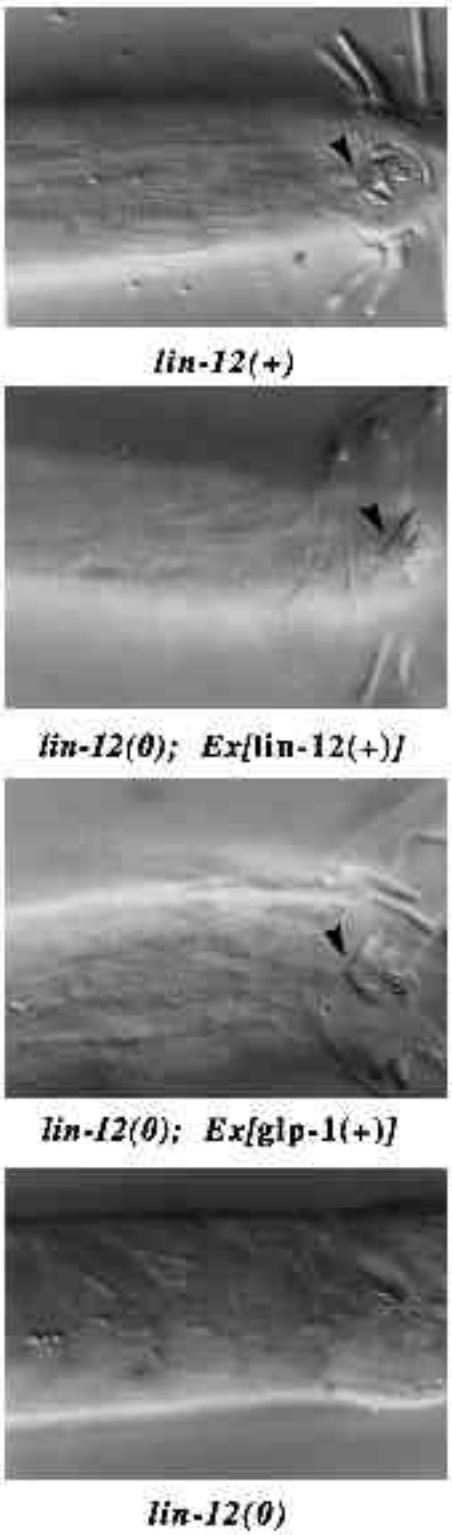

Fig. 5. Tail morphology in males of various genotypes. A normal-looking hook is marked by an arrowhead. The *lin-12(+)* male is from the wild-type strain N2. The *lin-12(0); Ex[*lin-12(+)*]* male has the complete genotype *unc-36 lin-12(n941); him-5; arEx33*. The *lin-12(0); Ex[*glp-1(+)*]* male has the complete genotype *unc-36 lin-12(n941); him-5; arEx34*. The *lin-12(0)* male shown is of genotype *unc-32 lin-12(n137 n720)*.

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(+) protein. 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(0)* 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 some 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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