Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9839–9842, October 1995
Developmental Biology

Inaugural Article: This is the first contribution by Judith Kimble, who was elected a Member of the National Academy of Sciences on April 25, 1995.

APX-1 can substitute for its homolog LAG-2 to direct cell interactions throughout *Caenorhabditis elegans* development

(signal transduction/GLP-1/LIN-12)

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Contributed by Judith Kimble, June 30, 1995

ABSTRACT **The homologous LAG-2 and APX-1 membrane proteins are putative signaling ligands in the GLP-1/LIN-12 signal-transduction pathway in *Caenorhabditis elegans*. Normally, LAG-2 and APX-1 mediate distinct cell interactions. Here, we demonstrate that APX-1, which normally interacts with GLP-1 in the early embryo, can substitute for LAG-2 throughout development. When expressed under control of the *lag-2* promoter, an *apx-1* cDNA can completely rescue a *lag-2* null mutant. To substitute for LAG-2, APX-1 must be able to interact with both GLP-1 and LIN-12 receptors and to mediate a variety of cell interactions during development. Therefore, APX-1 and LAG-2 are essentially equivalent in their ability to influence receptor activity. On the basis of this result, we suggest that the existence of multiple-signaling ligands in the LIN-12/GLP-1 signal transduction pathway does not reflect the evolution of functionally distinct proteins but rather the imposition of distinct controls of gene expression upon functionally similar proteins. Finally, we propose that the specification of distinct cell fates by the LIN-12/GLP-1 signal-transduction pathway relies on activities functioning downstream of the ligand and receptor, rather than on specific ligand–receptor interactions.**

During the development of multicellular organisms, cell–cell interactions ensure the adoption of specific pathways of differentiation in particular patterns. The homologous membrane receptors LIN-12 and GLP-1 of *Caenorhabditis elegans* and Notch of *Drosophila* play a prominent role in regulating cell interactions that control cell fate (see refs. 1 and 2 for reviews). However, the mechanism by which signals are transduced by this class of membrane receptors is not understood.

This paper focuses on the role of LAG-2 and APX-1 in the LIN-12/GLP-1 signal-transduction pathway. The LAG-2 and APX-1 membrane proteins are required for LIN-12/GLP-1 signaling (3–7). LAG-2 and APX-1 are members of a small family of proteins that include *Drosophila* Delta and Serrate (3, 6, 7) dubbed the DSL family, for its founding members Delta, Serrate, and LAG-2 (Fig. 1*A*). This class of proteins has recently been extended to vertebrates (8). In the extracellular domain of each DSL protein resides a cysteine-rich DSL motif, which is essential for function and may be a key motif for signal transduction (3) (Fig. 1*B*).

Various lines of evidence suggest that DSL proteins may be signaling ligands for LIN-12/GLP-1/Notch receptors. *In vivo*, Delta functions nonautonomously (9), and LAG-2 is specifically expressed in the signaling cell of an inductive interaction (3). In tissue culture, cells bearing Delta or Serrate selectively adhere to cells bearing Notch (10, 11). Furthermore, contact between cells expressing Jagged, a rat DSL protein, and cells expressing vertebrate Notch1 alters both transcription and differentiation (8). Although binding of purified proteins has not yet been demonstrated, the cumulative evidence supports the idea that DSL proteins may be ligands for the class of receptors characterized by LIN-12, GLP-1, and Notch.

Although the LAG-2 and APX-1 proteins are similar in sequence, each controls particular cell interactions during development. LAG-2 mediates embryonic cell interactions thought to rely on either LIN-12 or GLP-1, as well as larval cell interactions that rely specifically on GLP-1—e.g., induction of germ-line mitoses by the distal tip cell—or LIN-12—e.g., lateral signaling between anchor cell and ventral uterine precursor (4). By contrast, APX-1 is only known to be involved in one embryonic inductive cell interaction, which relies on GLP-1 as its receptor (5, 6).

Here, we ask whether APX-1 can substitute for its homolog LAG-2 during development. This experiment directly tests one possible source of specificity in the GLP-1/LIN-12 signal-transduction pathway. On the basis of our results, we suggest that specificity in the GLP-1/LIN-12 pathway does not reside in the molecular interaction of ligand with receptor. Instead, we propose that specificity relies on activities functioning downstream of the ligand and receptor.

MATERIALS AND METHODS

Worm Strains and Alleles. Nematode strains were cultured by standard techniques (12). N2 was the wild-type strain; *lag-2(q420ts)* and *lag-2(q411null)* (3) were used for rescue experiments (see below); and *smg-1(r861)* (13) was used to test for aberrant effects of the APX-1::β-galactosidase fusion protein (Table 1).

Plasmids. Plasmid pJK520 was constructed as follows: 3 kb from the 5′ flanking region of *lag-2*, extending from −10 bp to about −3 kb (3), was amplified by PCR with primers carrying *Bam*HI restriction sites, and the PCR product was inserted at the *Bam*HI site of pPD21.28 (kindly provided by A. Fire, Carnegie Institution of Washington, Baltimore). pPD21.28 includes a 42-bp synthetic intron and the *lacZ* coding sequence followed by *unc-54* 3′ sequences. Plasmid pJK521, containing the *apx-1::lacZ* fusion gene, was constructed by amplifying full-length *apx-1* cDNA by PCR with primers carrying *Kpn* I restriction sites and then ligating the PCR product into *Kpn* I-digested pJK520. In pJK521, *apx-1* cDNA is fused in frame with the *lacZ* coding region (see Fig. 2). Translation of the predicted fusion protein should start with the *apx-1* ATG initiation codon and terminate at the *lacZ* TAA stop codon.

Abbreviations: DSL, Delta, Serrate, and LAG-2; EGF, epidermal growth factor.

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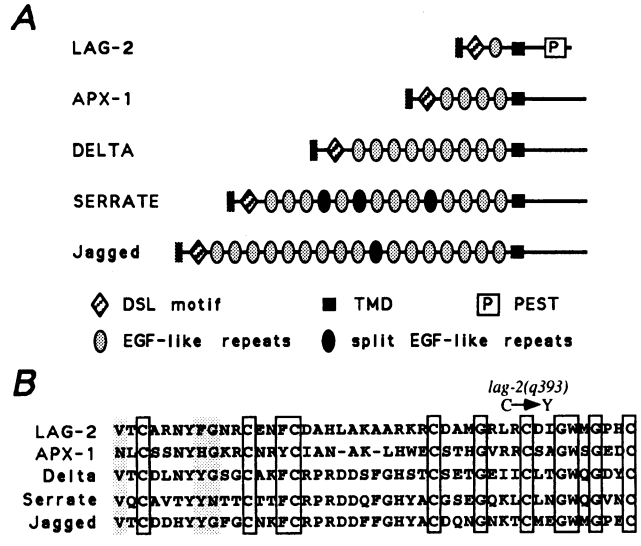

FIG. 1. Molecular architecture of DSL proteins. (*A*) All members of the DSL family have a predicted leader sequence (black bar), a family-specific DSL motif (striped diamond), one or more epidermal growth factor (EGF)-like repeats (shaded ovals), and a transmembrane domain (TMD) (solid square). The intracellular domains bear no resemblance to each other or to other proteins, with the exception of a putative PEST motif in LAG-2 (square with internal P). (*B*) The DSL motif. Comparison of diverse DSL motifs reveals a relatively low sequence similarity. The *lag-2(q393)* missense mutation alters a single cysteine residue in the DSL motif to a tyrosine (3); this DSL defect is associated with a phenotype that is virtually identical to that of the nonsense mutation *lag-2(q411)* and a *lag-2* deletion.

***lag-2* Rescue Experiments.** Transgenic animals were generated by injecting hermaphrodites with a DNA mixture of pRF4 (90 μg/ml) and pJK521 (*apx-1::lacZ*; 25 μg/ml) as described (14). Initial transformants carry transgenic DNA as an extrachromosomal array; chromosomal integration of the array was obtained by γ-irradiation (3500 rads). Rescue of *lag-2(q420ts)* and *lag-2(q411*null) was done as described (3), except that pJK521 (*apx-1::lacZ*) was used.

Detection of APX-1::β-Galactosidase Fusion Protein and GLP-1. β-galactosidase and GLP-1 were examined in dissected gonads as described (15).

RESULTS

To determine whether APX-1 could substitute for LAG-2 during development, we constructed a plasmid in which a full-length *apx-1* cDNA was placed under control of the *lag-2* promoter (Fig. 2). This *apx-1* cDNA was fused in frame to *lacZ* to monitor the protein in transgenic animals. We introduced *apx-1::lacZ* into wild-type animals and into two *lag-2* mutant strains, along with a coinjected dominant marker to detect transformants (see *Materials and Methods*). One mutant, *lag-2(q411)*, is a putative null, as it has a stop codon in the LAG-2 N-terminal region prior to the DSL motif; the other mutant, *lag-2(q420)*, is temperature sensitive and carries a single nucleotide change in a splice acceptor (3). Henceforth, we refer to *lag-2(q411)* as *lag-2(0)*, and *lag-2(q420)* as *lag-2(ts)*.

Normally, *lag-2(0)* and *lag-2(ts)* mutants die soon after hatching with a constellation of defects: characteristic bumps in head and tail, lack of an excretory cell and rectum, and a twisted "nose" (4) (Fig. 3*A*). Strains were generated carrying *apx-1::lacZ* either as an extrachromosomal array [*lag-2(0); qExAPX* and *lag-2(ts); qExAPX*] or as a chromosomal insertion [*lag-2(0); qIsAPX*]. From the original transformants, homozygous *lag-2* mutants were obtained, and their identity was confirmed by PCR (Fig. 3*B*). We found that the *apx-1::lacZ* transgene rescued both *lag-2(0)* and *lag-2(ts)* mutants (Fig. 3*C* and Table 1). Specifically, *lag-2(0)* mutants carrying the *apx-1::lacZ* transgene developed to adulthood with apparently wild-type morphology: the head and tail lack the characteristic

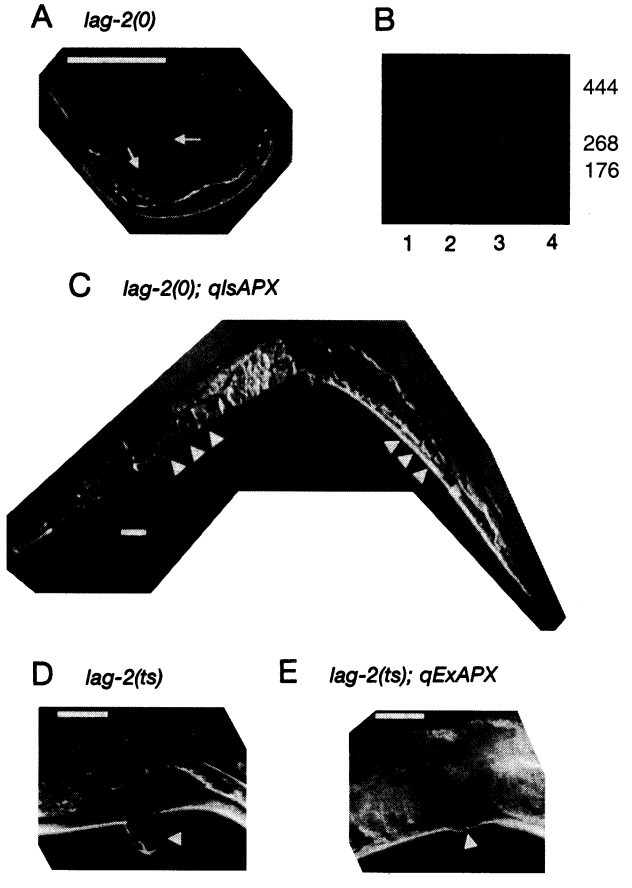

FIG. 3. *apx-1::lacZ* rescues *lag-2* mutant. (Bar = 20 μm.) (*A*) *lag-2(0)* phenotype. Animals lacking *lag-2* activity die as L1 larvae with characteristic bumps on head and tail (arrows). (*B*) PCR confirmation of *lag-2* mutants. The *lag-2(q411)* mutation eliminates a *Bbv* I restriction site in *lag-2*. Worm DNA was amplified by PCR with primers DG55 and DG56 (3), and products were digested with *Bbv* I. In this way, we proved that animals thought to be homozygous for *lag-2(q411)* were indeed of that genotype. Lane 1, *lag-2(0); qExAPX*; lane 2, *lag-2(0); qIsAPX*; lane 3, *lag-2(+)*; and lane 4, *lag-2(0)*. The positions of size standards (in bp) are given on the right. (*C*) *lag-2(0)* is rescued by *apx-1::lacZ*. An adult with genotype *lag-2(q411); qIsAPX*. Body size and morphology are normal; organs (gonad, vulva) are normal; head and tail are normal; hermaphrodites are self-fertile and lay eggs normally. Arrowheads point to oocytes. Embryos are visible as ovals in the animal's midsection. (*D*) *lag-2(ts)* vulva. A *lag-2(ts)* mutant raised at the restrictive temperature (25°C) from the first larval stage possesses a protruding vulva (arrowhead). (*E*) *lag-2(ts); qExAPX* vulva. A *lag-2(ts); qExAPX* animal raised at 25°C has normal vulval morphology (arrowhead).

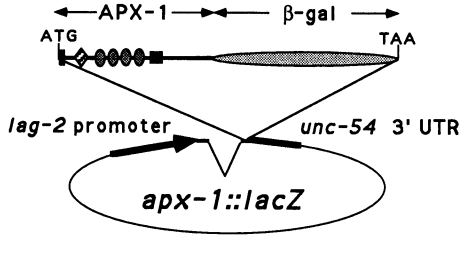

FIG. 2. The *apx-1::lacZ* plasmid. A full-length *apx-1* cDNA was placed under control of the *lag-2* 5′ flanking region and fused in frame to *lacZ*. β-gal, β-galactosidase; UTR, untranslated region.

Table 1. *apx-1::lacZ* rescues *lag-2* mutants

Genotype*	*t*, °C	% non-Lag†	% Lag‡	*n*§
lag-2(ts)	25	5	95	1760
lag-2(ts); qExAPX	25	74	26	3040
lag-2(0)¶	20	0	100	N/A
lag-2(0); qExAPX	20	71	29	1300
lag-2(0); qIsAPX	20	100	0	1240
lag-2(+); qExAPX	20	100	0	>1000
lag-2(+); qIsAPX	20	100	0	>1000
smg-1; lag-2(0); qIsAPX	20	100	0	>1000
smg-1; lag-2(+); qIsAPX	20	100	0	>1000

*qExAPX is *qEx108*, an extrachromosomal array carrying *apx-1::lacZ*. *qIsAPX* is *qIs7*, an insertion of *qEx108* on chromosome X.
†Animals are scored as nonLag if they reach adulthood.
‡Animals are scored as Lag if they die as L1 larvae. These animals are detectably Lag by the presence of bumps in head and tail and their flat, transparent appearance, when viewed through a dissecting microscope.
§*n* = number of animals scored; specific numbers were counted as total self-progeny from 20 hermaphrodites; >1000 describes progeny from many different parents.
¶See ref. 4.

Lag bumps, the excretory cell and rectum appear normal, and viability is restored.

In addition to embryonic function, *lag-2* acts postembryonically (4). When *lag-2(ts)* L1 larvae are shifted from the permissive temperature (15°C) to the restrictive temperature (25°C), their germ lines fail to proliferate, their vulvas protrude abnormally (Fig. 3*D*), and their tails often have defects leading to posterior rupture and larval death. None of these postembryonic defects was observed in either *lag-2(0); qIsAPX* or *lag-2(ts); qExAPX* transgenic animals (Fig. 3 *C* and *E*). Furthermore, aberrant development suggestive of unregulated LIN-12 or GLP-1 activity (16–18) was not observed.

We next asked whether ectopically expressed APX-1 might interfere with wild-type LAG-2. To this end, we examined *lag-2(+); qExAPX* and *lag-2(+); qIsAPX* for embryonic lethality, larval lethality, sterility, or vulval defects. None was observed (Table 1; data not shown). In a further attempt to observe any aberrant effect of *qIsAPX* on wild-type development, we placed *lag-2(+); qIsAPX* into a *smg-1* mutant background. *smg* mutants fail to degrade aberrant mRNAs (13). Even in a *smg-1* mutant background, development was normal (Table 1; data not shown). Therefore, coexpression of LAG-2 and APX-1 in the same cell is apparently not deleterious.

Finally, we examined APX-1::β-galactosidase fusion protein in the distal tip cell–germ line inductive interaction (Fig. 4). The distal tip cell is required for induction of germ-line tissue and establishment of the normal pattern of germ-line cell fates (19). Normally, GLP-1 is the germ-line receptor in this inductive interaction (20–22), and LAG-2 is the putative signaling ligand (3, 4, 7). Previous work showed that *lag-2* mRNAs are detected only in the signaling distal tip cell and not in the receiving germ-line tissue (3). In contrast to *lag-2* mRNAs, LAG-2::β-galactosidase reporter protein was found in both distal tip cells and the germ line (3). Since LAG-2::β-galactosidase rescues a *lag-2* null mutant, we suggested that LAG-2 may normally be taken up by the receiving tissue during signal transduction. To ask whether APX-1::β-galactosidase was similarly distributed, we examined gonads from *lag-2(0); qExAPX* animals. We found that APX-1::β-galactosidase, like LAG-2::β-galactosidase, was abundant in the distal tip cell and could be detected within the germ line (Fig. 4). This distribution of APX-1::β-galactosidase is consistent with its role as a GLP-1 ligand in the distal tip cell–germ line interaction and with uptake into the receiving tissue during signal transduction.

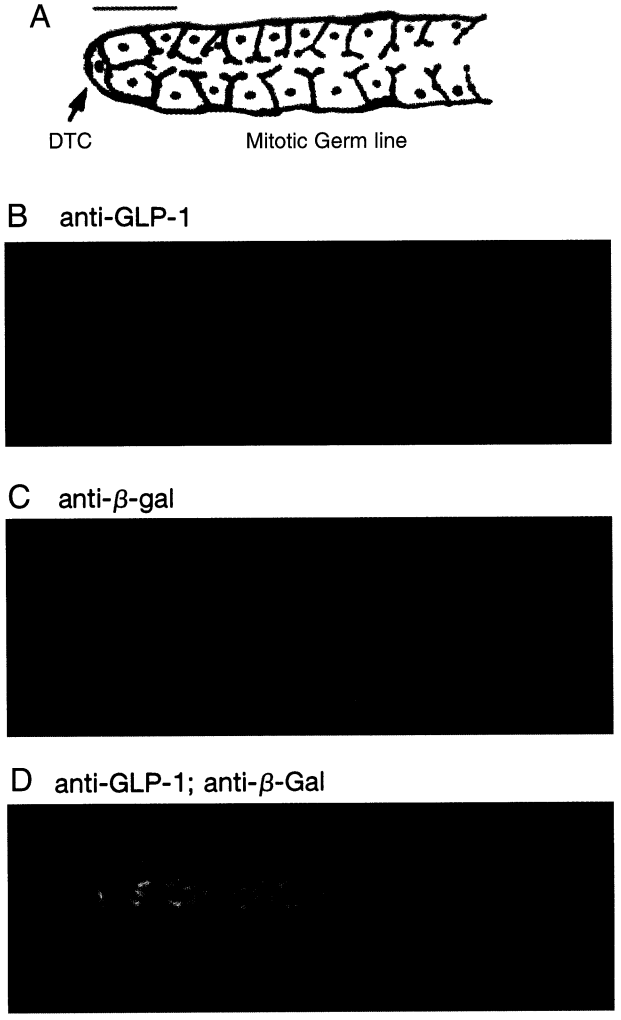

FIG. 4. Distribution of APX-1::β-galactosidase in a dissected gonad from *lag-2(0); qExAPX* animal. (Bar = 20 μm.) (*A*) Schematic of distal adult germ line. The signaling distal tip cell (DTC) is indicated by an arrow; the germ line is adjacent. (*B–D*) Confocal images of dissected gonad from *lag-2(0); qExAPX* with double staining for β-galactosidase (red) and GLP-1 (green). (*B*) GLP-1 is present in germ-line membranes, but not in the DTC. (*C*) APX-1::β-galactosidase fusion protein is present in both the DTC and the germ line. (*D*) Simultaneous detection of GLP-1 and β-galactosidase by merging fluorescein and rhodamine confocal images.

DISCUSSION

In this paper, we demonstrate that the APX-1 protein can substitute for LAG-2 throughout *C. elegans* development: animals lacking *lag-2* are viable and morphologically normal if *apx-1::lacZ* is present. Normally, APX-1 and LAG-2 control different cell interactions. APX-1 is required for one GLP-1-mediated inductive interaction in the early embryo (5, 6), while LAG-2 mediates interactions at multiple times during development (4). The ability of APX-1 to substitute for LAG-2 indicates that APX-1 must be capable of the same spectrum of functions as LAG-2. Therefore, APX-1 must be able to direct many different cell interactions, to interact with both LIN-12 and GLP-1, and to initiate the regulation required for lateral signaling between equivalent cells.

LAG-2 and APX-1 are similar in overall organization (Fig. 1*A*), but they bear a relatively low sequence identity: 26% in the N-terminal domain, 40% in the DSL domain (Fig. 1*B*), 30% of the single, full EGF repeat in LAG-2 to any one of the EGF-like repeats in APX-1, 30% in the transmembrane domain, and 15% in the intracellular domain (3, 6, 7). Given their roles in distinct cell interactions and their low sequence identity, one might have imagined *a priori* that LAG-2 and APX-1 each would have a distinct molecular function. Indeed, their biochemical properties—e.g., differences in binding af-

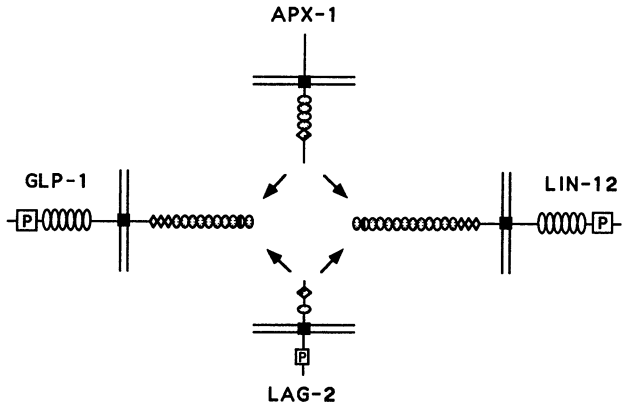

FIG. 5. Lack of specificity in ligand–receptor interactions. Both APX-1 and LAG-2 can interact with both LIN-12 and GLP-1 to control diverse cell fates. No specificity seems to derive from a given ligand or a given receptor. Motifs in APX-1 and LAG-2 follow the same convention shown in Fig. 1; motifs in LIN-12 and GLP-1 follow the convention set in ref. 15. In addition to the EGF-like motifs (shaded circles), family-specific LNG (LIN-12, Notch, GLP-1) repeats (diamonds) and ANK (ankyrin) repeats (ovals) are found in receptors.

finity or turnover rates—may differ. However, the results reported here suggest that LAG-2 and APX-1 proteins can function interchangeably.

A similar conclusion has recently been drawn for Delta and Serrate, two putative Notch ligands in *Drosophila*: when placed under control of a heat shock promoter, Serrate can replace Delta during embryonic neuroblast segregation (23). Because expression by a heat shock promoter clearly does not mimic endogenous gene expression, interpretation of the Serrate results is somewhat limited, but the apparent conclusion is the same as that reported here.

The ability of APX-1 to interact with both LIN-12 and GLP-1 suggests that specificity does not reside in the interaction of ligand with receptor. Just as the receptors LIN-12 and GLP-1 appear to be interchangeable (4, 16, 24), so are the ligands (Fig. 5). Since specificity does not appear to rely on specific ligand–receptor interactions, we suggest that it depends on the response to the ligand–receptor interaction, which is likely to be programmed within particular cells to achieve distinct fates.

If LAG-2 and APX-1 are indeed equivalent, why are there two ligands? One advantage is to exert distinct controls of gene expression upon the two proteins. Indeed, APX-1 is required maternally for induction in the early embryo, while LAG-2 is required zygotically in diverse somatic tissues. Perhaps *apx-1* maternal mRNA, like *glp-1* (25), is stringently controlled at the posttranscriptional level to ensure expression at the correct time and place in early embryos. By contrast, the somatic expression of *lag-2* is subjected to cell-specific transcriptional controls (3), as well as transcriptional feedback necessary for lateral signaling (26). Therefore, regulation of expression is clearly distinct for *lag-2* and *apx-1*, which may dictate the existence of two genes.

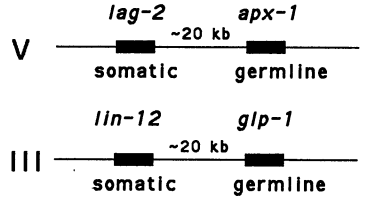

FIG. 6. Gene arrangement for *lag-2* and *apx-1* and for *lin-12* and *glp-1*. See text for explanation.

Finally, we note that the arrangement of *lag-2* and *apx-1* on chromosome V bears a remarkable and intriguing similarity to that of *lin-12* and *glp-1* on chromosome III (Fig. 6). The two pairs of homologous genes map close to each other and are likely to have been generated by gene duplication, each pair of proteins appears to be functionally interchangeable, and each pair has one gene that is predominantly expressed maternally and one that is expressed for the most part in somatic tissues (3–6, 21). One speculative possibility is that a regulatory cassette was inserted between the duplicated genes of each pair; such a regulatory cassette might effect germ-line expression from one gene and somatic expression from the other. As more protein families become identified and characterized, it will be exciting to learn whether a similar situation is found in other genes in *C. elegans*, in other invertebrates or indeed in vertebrates.

Note Added in Proof. Since this paper was submitted, the DSL family has now been extended to *Xenopus* (27), chick (28), and mouse (29).

We thank our colleagues in the J.K. lab (especially Sam Henderson), Joel Rothman, and Marv Wickens for stimulating discussions during the course of this work and for their critical reading of the manuscript. Craig Mello and Jim Priess kindly provided the *apx-1* cDNA and Andrew Fire provided the *lacZ* expression vector. We are also grateful to Leanne Olds for help with figures and Anne Helsley-Marchbanks for assistance with the manuscript. This work was supported by grants from the National Institutes of Health and the Council for Tobacco Research. J.K. is an investigator with the Howard Hughes Medical Institute.

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