Transcript Analysis of glp-1 and lin-12, Homologous Genes Required for Cell Interactions during Development of C. elegans

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Summary

The glp-1 and lin-12 genes mediate several cell interactions during C. elegans development. We have identified the glp-1 gene in a region about 20 kb from lin-12. In collaboration with Yochem and Greenwald (1989; see accompanying paper), we show that a sequence identified by its similarity to lin-12 is in fact glp-1. We find a single 4.4 kb glp-1 transcript and a distinct 4.6 kb lin-12 transcript. Expression of the glp-1 transcript during development differs from that of lin-12. As expected from genetic analyses, glp-1 RNA is primarily in the germline while lin-12 RNA is primarily in the soma. Unexpectedly, we find that glp-1 RNA is also expressed in larval somatic tissues and that lin-12 RNA is abundant in early embryos. We suggest that glp-1 and lin-12 may play broader roles in development than previously thought.

Introduction

Cellular interactions are critical to metazoan development. In the nematode Caenorhabditis elegans, cellular interactions influence multiple processes of development including proliferation, differentiation, and morphogenesis (Sulston and White, 1980; Kimble, 1981; Kimble and White, 1981; Sulston et al., 1983; Priess and Thomson, 1987). Two genes of C. elegans that appear to regulate cell-cell interactions during development have been identified: glp-1 and lin-12. The glp-1 gene is required for interactions that induce proliferation in the germline and development of the embryonic pharynx (Austin and Kimble, 1987; Priess et al., 1987). Specifically, mitotic divisions in the germline depend on an interaction between the germline tissue and a somatic regulatory cell, the distal tip cell (Kimble and White, 1981). In addition, generation of the anterior half of the pharynx depends on an interaction between blastomeres in early embryos (Priess and Thomson, 1987). The lin-12 gene is required for the specification of fate in equivalent cells that choose one of two fates by cell interactions (Greenwald et al., 1983; Ferguson and Horvitz, 1985). The lin-12 gene has been cloned; its predicted amino acid sequence indicates that the lin-12 protein is a transmembrane protein with EGF-like repeats in its putative extracellular domain (Greenwald, 1985; Yochem et al., 1988). In this paper, we report the cloning of glp-1. Independently, a gene was cloned by virtue of its sequence similarity to lin-12; this gene mapped close to glp-1 (Yochem and Greenwald, 1989). Our data together with those of Yochem and Greenwald show that the lin-12 homolog is glp-1. In addition, we identify the glp-1 transcript and compare the patterns of glp-1 and lin-12 expression during development.

Results

Molecular Identification of the glp-1 Gene

Our strategy for cloning glp-1 exploited the proximity of glp-1 to a previously cloned gene, lin-12, and the availability of cosmids containing lin-12 and neighboring DNA (Greenwald, 1985; Greenwald et al., 1987). glp-1 maps 0.02% to the right of lin-12 on chromosome III (Austin and Kimble, 1987). Because a genetic map unit is equal to approximately 830 kb in the lin-12 region (Greenwald et al., 1987), we expected glp-1 to be 15–20 kb to the right of lin-12.

To locate glp-1, we hybridized probes from the lin-12 region to DNAs that had been isolated from glp-1 mutants, digested with one of several restriction enzymes, and transferred to a filter (Figure 1). We first examined eight ethyl methane sulfonate–induced glp-1 mutants. When these mutants were probed with a cosmid containing lin-12 (T26E12), the wild-type pattern of restriction fragments was observed in all eight (data not shown). However, when these mutants were probed with a cosmid to the right of lin-12 (ZK506), a novel 1.2 kb EcoRI restriction fragment was detected in glp-1(q172) DNA (Figure 1A). A single 1.5 kb EcoRI fragment of ZK506, pJK139, detects the novel 1.2 kb EcoRI fragment in glp-1(q172) DNA (Figure 1). pJK139 also detects alterations of the wild-type pattern in two gamma ray–induced alleles of glp-1, qD2, and glp-1(q339) (Figure 1B). Further analysis has shown that glp-1(q172) is a 300 bp deletion, that qD2 is a larger deletion with its left endpoint in glp-1, and that glp-1(q339) is a complex rearrangement (Figure 1 and data not shown). The evidence that three glp-1 mutations are associated with alterations in the same 1.5 kb EcoRI fragment strongly suggests that this region of DNA is necessary for normal glp-1 function.

glp-1 is Homologous to lin-12

In an independent study, genes with sequence similarity to lin-12 were identified by hybridization of lin-12 probes to a C. elegans genomic library; one such gene mapped to the cosmid ZK506 (Yochem and Greenwald, 1989). To test whether this lin-12 homolog was glp-1, we probed glp-1(q172), qD2, and glp-1(q339) DNAs with a phage containing the homolog (GS1#6-2, kindly provided by J. Yochem). GS1#6-2 hybridized to the novel restriction fragments associated with the three glp-1 mutations (Figure 1C). The wild-type 1.5 kb EcoRI fragment identified as necessary for glp-1 function is located in the middle of the lin-12 homolog contained in GS1#6-2 (Figure 1D), confirming that the homolog is glp-1. Subsequent sequence analysis has shown that glp-1 and lin-12 are similar in both sequence and overall organization (Yochem and Greenwald, 1989).
Figure 1. Molecular Identification of glp-1
Southern blots of wild-type and glp-1 mutant DNAs digested with EcoRI. Arrowheads indicate novel bands associated with specific mutations of glp-1. In each case, wild-type bands are also seen because the animals contained a wild-type copy of glp-1. 32P-labeled probes were prepared by nick translation.
(A) The ZK506 cosmid hybridizes to many EcoRI restriction fragments in wild-type (+) DNA. In glp-1(q483); qDp3 DNA (and in DNAs of six other glp-1 mutants, data not shown), only the wild-type pattern of restriction fragments is observed. In glp-1(q72); qDp3 DNA, a novel 1.2 kb band is also found.
(B) pJK139 hybridizes to a single EcoRI restriction fragment in wild-type (+) DNA, but it also detects novel fragments in glp-1(q72); qDp3, glp-1(q339); qDf1, and qDf2; qDp3 DNAs.
(C) GS#1.6-2 hybridizes to several bands in wild-type (+) DNA; in gdp-1(q72); qDp3, gdp-1(q339); qDf1, and qDf2; qDp3 mutant DNAs, it hybridizes to the wild-type restriction fragments minus the novel bands associated with gdp-1 mutants.
(D) Simplified map of the lin-12-gdp-1 region. Probes used for identification of gdp-1 are below the map and alterations associated with gdp-1 mutations are above the map.

Identification of a 4.4 kb gdp-1 Transcript
To identify gdp-1 transcript(s), we hybridized Northern blots of total RNA from wild-type adult hermaphrodites with probes from the gdp-1 region (Figure 2). The 1.5 kb EcoRI fragment used to identify gdp-1 (pJK139, Figure 1, and fragment C, Figure 2C) hybridizes to a single 4.4 kb transcript; the direction of transcription was determined using single-stranded RNA probes (Figure 2A). This same transcript is detected by probes spanning 9 kb of genomic DNA centered around pJK139 (Figure 2C; data not shown). Comparison of the bound and unbound fractions of oligo(dT)-selected RNA indicates that this transcript is polyadenylated (Figure 2B).

We wanted to compare the expression of the 4.4 kb gdp-1 transcript with that of the lin-12 transcript. Our lin-12 probe hybridized to a 4.6 kb RNA (Figure 3B), which agrees with an earlier estimate of 4.5 kb (Yochem et al., 1988). Because the gdp-1 and lin-12 transcripts are similar in size as
Figure 3. Specificity of glp-1 and lin-12 Probes
Northern analysis of RNAs isolated from wild-type, glp-1(q172); qDp3, and lin-12(n1376;2032); qDp3 embryos and hybridized with glp-1 and lin-12 probes. The glp-1(q172) mutation carries a 300 bp deletion in glp-1. The lin-12(n1376;2032) mutation carries a Tct insertion in lin-12 (Greenwald, 1985). Each lane contains 10 μg of total RNA. Antisense RNA probes were synthesized by T7 transcription using [α-32P]CTP.

The glp-1 probe synthesized from pJK139 contains the 1.5 kb EcoRI fragment shown as fragment C in Figure 2C. The lin-12 probe synthesized from pJK149 contains the 2.9 kb PstI fragment from the center of lin-12 as described in Greenwald (1985). Arrows indicate the glp-1 and lin-12 wild-type transcripts.

(A) Using a glp-1 probe, one band of 4.4 kb is observed in wild-type (+) and lin-12(n1376;2032) RNAs, but a smaller 4.2 kb transcript is also observed in glp-1(q172) RNA.

(B) Using a lin-12 probe, one band at 4.6 kb is observed in wild-type (+) and glp-1(q172) RNA, but novel transcripts of 2.1 kb and 6.5 kb are observed in lin-12(n1376;2032) RNA. At this exposure the 2.1 kb transcript is very faint, but it is clearly visible in longer exposures.

(C) Using both glp-1 and lin-12 probes, a broader band extending from 4.4 kb to 4.6 kb includes the glp-1 and lin-12 transcripts.

Expression of glp-1 and lin-12 Transcripts during Development
To investigate glp-1 and lin-12 expression during development, we examined their transcripts in staged wild-type hermaphrodites. Figure 4A shows our result for glp-1 using a probe from the center of the gene (fragment C, Figure 2C). Similar results are also observed using probes from the 5' or 3' portions of the gene. The relative level of the 4.4 kb glp-1 transcript changes dramatically during development. The glp-1 transcript is abundant in embryos, present at a low but increasing level during larval development, and abundant again in adults. Figure 4B shows our result for lin-12. Here, we find that the lin-12 transcript is abundant in embryos and present at a lower level during larval development. In contrast to glp-1, no increase in expression of lin-12 is seen in adults.

Both glp-1 and lin-12 transcripts are abundant in RNA isolated from embryos (Figure 4). To determine more accurately when glp-1 and lin-12 transcripts are present during embryogenesis, RNAs were isolated from synchronized embryos at early (0–2 hr), middle (3–8 hr), or late (7–13 hr) stages of embryogenesis (see Experimental Procedures). When these staged embryonic RNAs were probed with glp-1, we found that the glp-1 transcript is abundant in early embryos but barely detectable in middle and late embryos (Figure 5A). The lin-12 transcript is most abundant in early embryos, but a substantial amount is seen in middle stage embryos as well (Figure 5B).

Expression of glp-1 and lin-12 Transcripts in Germline and Soma
We previously showed by analysis of genetic mosaic animals that glp-1 is required in the germline but not in the
soma for continued germline mitoses (Austin and Kimble, 1987). Therefore, we suspected that glp-1 transcription might be limited to the germline. Consistent with this idea was the steady increase in glp-1 RNA during postembryonic development (Figure 4A) that paralleled the increase in number of germline nuclei from L1 to adult (Kimble and White, 1981; Table 1). Conversely, because lin-12 is required in the soma during postembryonic development (Greenwald et al., 1983), we expected lin-12 RNA to be present in the soma.

To distinguish between transcripts present in germline and soma, we compared the levels of glp-1 and lin-12 RNAs in wild-type hermaphrodites to those in mutant hermaphrodites with a reduced number of germ cells. Table 1 shows the number of germ line descendants present in wild-type animals and animals homozygous for a temperature-sensitive mutation, SS104(bn2), that markedly reduces the number of germ cells. No other defects associated with this mutation have been observed. The difference between the number of germ line descendants in wild-type and mutant animals becomes striking only in the second half of larval development; therefore, we made our comparisons using L3, L4, and adult hermaphrodites.

The levels of glp-1 RNA in wild-type and SS104(bn2) animals are shown in Figure 6A; those for lin-12 RNAs are shown in Figure 6B. In wild type, the amount of glp-1 transcript increases from L3 to adult. In contrast, in SS104(bn2) hermaphrodites, which contain few germ cells, the level of glp-1 transcript does not increase. This result indicates that the marked increase in the level of glp-1 RNA during late larval development observed in wild-type animals is due to an increase of glp-1 transcript in the germline tissue. However, this result also indicates that glp-1 RNA is made in the soma during larval development: glp-1 RNA is observed in SS104(bn2) hermaphrodites at each stage examined. Of particular note is the similarity in levels of glp-1 RNA in wild-type and mutant L3 animals despite the order-of-magnitude difference in the number

Table 1. Number of Germline Descendants in Wild-Type and SS104(bn2) Hermaphrodites

<table>
<thead>
<tr>
<th>Developmental Stage</th>
<th>Wild-Type</th>
<th>SS104(bn2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>2–12</td>
<td>2–6</td>
</tr>
<tr>
<td>L2</td>
<td>16–50</td>
<td>5–9</td>
</tr>
<tr>
<td>L3</td>
<td>60–130</td>
<td>6–13</td>
</tr>
<tr>
<td>L4</td>
<td>180–400</td>
<td>6–13</td>
</tr>
<tr>
<td>Adult</td>
<td>~1500</td>
<td>7–16</td>
</tr>
</tbody>
</table>

All animals were grown at 25°C.

* L1–L4: germ cells were counted at both the beginning (lower number) and end (higher number) of each larval stage (N = 2). Adult: germ cells were counted in young adults (N = 2).

+ and !SS104(bn2) probes were prepared as described in Figure 3. Arrows indicate the glp-1 (A) and lin-12 (B) transcripts. Faint additional signals observed at 3.0 kb and 1.8 kb are due to the presence of ribosomal RNA (see Experimental Procedures).
of germ cells in these animals (Table 1). This comparison suggests that most of the glp-1 RNA in L3 is present in some somatic tissue. For lin-12, during L3 and L4, the amount of RNA is similar in wild-type and SS104(brn2) hermaphrodites, indicating that lin-12 RNA is primarily present in the soma. In adults, the lin-12 signal is stronger in wild-type than in mutant hermaphrodites. This may indicate expression of lin-12 in the adult germline.

Discussion

In this paper we report the molecular identification of glp-1. The glp-1 gene lies about 20 kb to the right of lin-12 on chromosome III. This location was predicted from the map position of glp-1 and the known relationship between genetic and physical distances in this region of the chromosome. Our identification of glp-1 is based on the association of three glp-1 mutations with physical alterations of a restriction fragment in the predicted vicinity of glp-1. Independently, Yochem and Greenwald (1989) have identified a lin-12 homolog close to the location predicted for glp-1. We find that this lin-12 homolog contains the restriction fragment identified as part of glp-1. Further analysis has shown that this fragment includes the sequence encoding multiple EGF-like repeats in the putative extracellular domain of the lin-12 homolog (Yochem and Greenwald, 1989). Recently, a plasmid carrying the region defined as glp-1 has been used to rescue a glp-1 mutation by genetic transformation (J. Priess and A. Fire, personal communication.) The lin-12 homolog is therefore clearly glp-1. The proximity of these two genes and their sequence similarity suggest a common ancestry.

The similarity in amino acid sequence of the predicted glp-1 and lin-12 proteins (Yochem and Greenwald, 1989) corresponds to a similarity in function that has been deduced from genetic studies. Both glp-1 and lin-12 are required for cell interactions that regulate cell fate during C. elegans development (Austin and Kimble, 1987; Priess et al., 1987; Greenwald et al., 1983). Further, both genes function in the receiving cell rather than in the signaling cell. Activity of glp-1 is needed in the germline for continued germline mitoses, but not in the somatic distal tip cell (Austin and Kimble, 1987). Similarly, in the interaction between the pair of cells that will become the anchor cell (AC) and the ventral uterine precursor (VU), one of the two cells receives a signal that results in its commitment to the VU fate. Seydoux and Greenwald (1989) have shown that lin-12 activity is required for the reception of this signal in the cell that will become VU. Therefore, the two genes may carry out parallel functions in different tissues.

Although the glp-1 and lin-12 functions are similar, several lines of evidence indicate that they are not identical. First, the two genes mediate different types of interactions. glp-1 is needed for inductive interactions that occur between cells with very different developmental potentials (e.g., somatic distal tip cell vs. germline). In contrast, lin-12 is needed for interactions that occur between cells with equivalent developmental potential. Second, the functions unique to glp-1 are specific to the germline; these include control of germline growth and production of a maternal product for embryonic development (Austin and Kimble, 1987; Preiss et al., 1987). In contrast, the functions unique to lin-12 occur in somatic tissues (Greenwald et al., 1983). Germline development in lin-12(0) homozygotes is abnormal [T. Scheld, personal communication], but this defect is due to a lack of lin-12 activity in the soma rather than in the germline (G. Seydoux and I. Greenwald, personal communication.) Finally, we have tested extragenic suppressors of glp-1 for suppression of lin-12; those tested to date do not suppress lin-12 (Maine and Kimble, 1989, and unpublished data). The differences between glp-1 and lin-12 make it clear that these two homologous genes have evolved to carry out different functions during development.

To understand the function of glp-1 and lin-12 in mediating regulatory cell interactions, we have begun an analysis of their products during development. In this paper we address questions about their transcripts. We find that glp-1 makes a 4.4 kb poly(A) RNA whereas lin-12 makes a 4.6 kb poly(A) RNA. We were interested in the possibility of transcripts that might be present in addition to the full-length transcripts corresponding to the predicted membrane proteins (Yochem et al., 1988; Yochem and Greenwald, 1989). For example, a smaller glp-1 transcript containing only the EGF-like repeats of glp-1 might encode the distal tip signal. However, we found no evidence of additional transcripts for either glp-1 or lin-12. To search for such transcripts we used probes covering the entire glp-1 gene (data not shown) and a single probe covering a central region of lin-12 that encodes many of its EGF-like repeats. Based on these results, we suggest that each of glp-1 and lin-12 produces a single major transcript.

The glp-1 and lin-12 transcripts are differentially expressed during development. Both transcripts are present at a high level in early embryos. However, while glp-1 RNA is barely detectable during later stages of embryogenesis, lin-12 is observed at a moderate level. The embryonic profile of glp-1 RNA is consistent with the presence of a maternal message used early in embryogenesis and then degraded. This possibility corresponds well with the known maternal requirement for glp-1 and with the early embryonic temperature-sensitive period of glp-1 (Priess et al., 1987; Austin and Kimble, 1987). Analysis of lin-12 mutants has suggested that lin-12 activity is required during embryogenesis only for the determination of a single pair of cells (Greenwald et al., 1983). The surprising abundance of lin-12 RNA in early embryos suggests that this gene may have additional roles in embryonic determination. During postembryonic development, the level of glp-1 RNA increases steadily whereas the level of lin-12 RNA is low but relatively constant. These differences in steady-state levels of glp-1 and lin-12 RNA indicate that there are differences in the control of transcription or message stability for these two homologous genes.

As a first step in examining the tissue specificity of glp-1 and lin-12 RNAs, we compared animals with and without germlines to look for differences in transcript levels between the germline and somatic tissues. As predicted from genetic analyses (Austin and Kimble, 1987), glp-1 is primarily expressed in the germline during late larval
stages and adulthood. However, in addition to its expression in the germline, we find that gfp-1 is expressed in the larval soma. Although unexpected, this finding is consistent with an unusual somatic phenotype of one gfp-1 allele: many gfp-1(q35) heterozygotes and homozygotes produce pseudovulvae (Austin and Kimble, 1987).

The expression of lin-12 by somatic tissues was predicted from genetic analyses (Greenwald et al., 1983; Seydoux and Greenwald, 1989) and is confirmed here by the presence of somatic lin-12 RNA. In addition, our data suggest that lin-12 is expressed in the germline of adults. However, the level of lin-12 transcripts in adults is low compared with that seen in embryos, and there is the possibility that our adult RNA preparations contain some early embryonic RNA. For this reason, we conclude only that lin-12 RNA is present in somatic tissues during postembryonic development. The possibility that lin-12 RNA is present in the adult germline is contributed to the embryo by the mother must await other experiments. Although the experiments reported here clearly show somatic expression of gfp-1 and lin-12, they do not provide the cellular resolution required to identify which cells produce each RNA. Of particular interest is whether the same cells produce both gfp-1 and lin-12 during development of the larval soma.

In summary, we report the molecular identification of the gfp-1 gene, its identity with a lin-12 homolog isolated by Yochem and Greenwald (1989), and an analysis of the RNAs produced by these homologous genes during development. The difference found in both temporal and spatial expression of the gfp-1 and lin-12 RNAs underscores the known differences between the two genes. However, the unexpected presence of lin-12 RNA in early embryos and gfp-1 RNA in the larval soma raises the possibility that these genes have roles in development that are as yet unknown. In particular, we suggest the possibility that gfp-1 and lin-12 have overlapping functions. Analysis of the phenotypes of gfp-1 or lin-12 single mutants has identified unique functions of each gene, but these single mutant phenotypes would not reveal functions for which either the gfp-1 or lin-12 gene product is sufficient. Any common functions of gfp-1 and lin-12 would be observed only in the double mutant. Preliminary results indicate that the lin-12 gfp-1 double mutant does exhibit developmental defects not seen in either single mutant (J. Kimble, unpublished data). An understanding of the redundant activities of gfp-1 and lin-12 may shed light on the original function of their common ancestor.

**Experimental Procedures**

**Worm Strains and Culture**
The wild-type parent of all strains in this study is C. elegans var. Bristol, strain N2 (Brenner, 1974). The mutations used were: L1175, unc-32(e1893); L1176, unc-32(e1899); L1178, lin-12(n73(e2032); L1179, glp-1(35); L1180, gfp-1(46); L1181, glp-1(756); L1182, gfp-1(231), and gfp-1(224). The gfp-1 mutations are described in Austin and Kimble (1987); L1201(n73(e2032) is described in Greenwald (1985); L1202 is provided by P. Strome; other mutations are described in Swanson et al. (1984). In addition, we used the free duplication, qDpD3(III,IV) (Austin and Kimble, 1987) and the translocation of glp-1(V) (Rosenbluth and Baillie, 1981). Routine maintenance of C. elegans cultures was as described by Brenner (1974).

**Isolation of Gamma Ray-Induced Mutations in gfp-1**
unc-32(e1893); glp-1(35); unc-32(e1899); glp-1(756) were isolated from individual petri dishes and scored for presence of Dpy and Unc progeny. For parents with sterile Dpy or Unc progeny, complementation tests were performed with gfp-1(q46). In addition, a glp-1 complementation test was carried out for any animal that did not segregate Dpy or Unc progeny because it might carry a glp-1 mutation associated with a lethal event. qDpD3 was isolated as a lethal mutation linked to unc-32(e1893); also, gfp-1(q339) was isolated as a mutation with a sterile phenotype linked to dpy-19(e1259).

**Cloning gfp-1**
Preparation of nematode DNA was as described in Emmons and Yesner (1984). Wild-type DNA was isolated from N2 hermaphrodites. DNA from glp-1 mutants was isolated from strains of the type glp-1(x); glp-1(x); dpy-19(e1259); glp-1(q339); qDpD3 is inviable; dpy-19(e1259); glp-1(q339). 3P-labeled probes were prepared by nick translation. Hybridizations were carried out at 65°C-68°C. Cosmids used for identification of gfp-1 were provided by J. Nicass and A. Coulston; the phage GS51.6-2 was provided by J. Yochem and I. Greenwald. Restriction fragments of ZK506 and GS51.6-2 were subcloned into pBlues (International Biotechnologies, Inc.), a vector that contains T7 and SP6 polymerase promotors for transcription of insert DNA.

**Preparation of Synchronized Nematodes**
Gravid hermaphrodites were placed on 100 mm NCM plates heavily seeded with C600 and allowed to lay eggs overnight. After gentle rinsing with M9 to remove adult animals, embryos left on the plates were collected and treated with hypochlorite (Emmons et al., 1979) to sacrifice remaining adults. Embryos were placed on fresh 100 mm NGM plates and allowed to develop. Unless noted otherwise, animals were grown at 25°C. After a period of growth, animals were rinsed off plates, collected by centrifugation, and rinsed three times with M9. Samples were taken to calculate the total number of animals present and to determine their developmental age. Animals were staged by examining the development of the somatic gonad and vulva using Nomarski differential interference contrast microscopy as described in Kimble and Hirsh (1979), Sulston and Horvitz (1977), and Sulston et al. (1983).

Early embryos were prepared by digesting gravid hermaphrodites with hypochlorite and immediately harvesting the embryos. Middle and late stage embryos were prepared by allowing hermaphrodites to lay eggs on seeded NGM plates for 4 hr at 20°C, removing the hermaphrodites, and harvesting the embryos after 3 hr (middle) or 7 hr (late). Embryos were staged using Nomarski microscopy as described in Sulston et al. (1983).

**Isolation and Analysis of RNA**
Pellets of synchronized worms were frozen in a dry ice-ethanol bath and stored at -70°C. Total RNA was prepared as described (Rosenquist and Kimble, 1986). Briefly, frozen worms were broken open in the presence of 4 M guanidinium isothiocyanate, 100 mM Tris (pH 7.5), 10 mM Sarcosyl, and 10 μg/ml aurin tricarbonylic acid. The lysate was extracted three times with phenol-chloroform and once with chloroform. RNA was precipitated with 0.75 vol of ethanol and 0.025 vol of 1 M acetic acid overnight at -20°C. Precipitated RNA was resuspended in distilled water treated with diethyl pyrocarbonate and the concentration was determined spectrophotometrically. Average yields of total RNA from developmentally staged wild-type hermaphrodites (mg/10 animals) were as follows: embryos, 0.6; L1, 0.6; L2, 1.6; L3, 2.9; L4, 4.5; and adult, 10.2.

Northern blots were prepared by electrophoresing RNAs on 15% agarose-formaldehyde gels followed by transfer to nylon membranes (Nytran). All samples consisted of 10 μg of total RNA (RNA concentration determined by absorbance at 260 nm). Total RNA was used to avoid sample variation in the efficiency of pol(A)+ RNA selection. RNAs were fixed on membranes by baking at 65°C for 1 hr. 32P-labeled probes were prepared by transcription using T7 polymerase.
Hybridizations were performed at 66°C–68°C in 50% formamide, 0.8 M NaCl, 5x Denhardt's solution, 1 mM EDTA, 0.2% SDS, 50 mM NaPO₄, 200 μg/ml denatured herring sperm DNA. A 3-fold volume of each RNA sample was prepared; one-third of the sample was loaded on each of three different gels. The first two gels were blotted and subsequently hybridized with 10⁶ cpm/ml of either the glp-1 or the lin-12 probe. The third gel was stained with ethidium bromide to visualize the ribosomal bands and ensure that an equivalent amount of total RNA was present in each sample. As an independent assessment of the amount of RNA loaded, we rehybridized filters with an act-1 probe (pT773 18–103, a gift from M. Krause), which is specific for one of the muscle actin genes of C. elegans (Files et al., 1985), and also with a myo-1 probe, which hybridizes to the transcripts of both C. elegans pharyngeal myosin genes (Miller et al., 1986). RNA sizes were determined by comparison to RNA size standards (Bethesda Research Laboratories, Inc.).

In addition to the bands of intact glp-1 and lin-12 transcript observed in the Northern blots shown in this paper, two smaller bands can be seen that we believe are due to the presence of ribosomal RNA. First, there is a sharp line of hybridization that comigrates with the bottom of the upper ribosomal band at approximately 3.0 kb. This line appears to be due to a concentration of degraded glp-1 or lin-12 RNA that is excluded from the upper ribosomal band; generally, it is proportional in intensity to the bands above it. Second, there is a band of hybridization at 1.8 kb that comigrates with the lower ribosomal band; the strength of this band depends on the stringency of hybridization and is not observed in oligo(dT)-selected RNA.

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