Translational Control of Maternal *glp-1* mRNA Establishes an Asymmetry in the C. elegans Embryo

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Summary

In C. elegans, the *glp-1* gene encodes a membrane receptor that is required for anterior cell fates in the early embryo. We report that GLP-1 protein is localized to anterior blastomeres in 2- to 28-cell embryos. By contrast, *glp-1* mRNA is present in all blastomeres until the 8-cell stage. Furthermore, the *glp-1* 3′ untranslated region can restrict translation of a reporter mRNA to anterior blastomeres. Therefore, the translation of maternal *glp-1* mRNA is temporally and spatially regulated in the C. elegans embryo. The regulation of maternal *glp-1* mRNA has striking parallels to the regulation of maternal *hunchback* mRNA in the Drosophila embryo. Thus, the establishment of embryonic asymmetry in diverse organisms may involve conserved mechanisms of maternal mRNA regulation.

Introduction

The establishment of embryonic polarity is a fundamental event during development. Asymmetry in the embryo usually foreshadows the segregation of developmental potential and is thought to underlie the formation of the body axes. In many phyla, embryos display asymmetry at or near fertilization (Davidson, 1990). However, the molecular mechanisms that generate embryonic asymmetry are, for the most part, poorly understood. Furthermore, the extent to which these mechanisms are conserved among different species is unknown and is an intriguing problem, given the apparent diversity of developmental strategies used by different types of embryos.

In Drosophila, polarity is established in a large syncytium in which nuclei divide in the absence of cell division (for review see Nüsslein-Volhard, 1991). Gradients of morphogens are generated within this syncytium to create asymmetry. To form the anteroposterior axis, maternal mRNA encoding bicoid is localized to the anterior pole of the oocyte (Berleth et al., 1988), and maternal mRNA encoding nanos is localized to the posterior pole (Wang and Lehmann, 1991). Diffusion of bicoid and nanos proteins from the poles generates an anterior to posterior gradient of a transcription factor called hunchback. Subsequently, hunchback triggers a cascade of transcription factors that specify pattern along the anteroposterior axis (Driever and Nüsslein-Volhard, 1989, Struhl et al., 1989, 1992). To generate the hunchback gradient, bicoid activates zygotic transcription of *hunchback* in anterior nuclei (Driever and Nüsslein-Volhard, 1989, Struhl et al., 1989), while nanos represses translation of maternal *hunchback* mRNA in the posterior region (Tautz and Pfeifle, 1989; Wharton and Struhl, 1991). The regulation of *hunchback* by nanos is achieved through regulatory elements in the 3′ untranslated region of maternal *hunchback* mRNA (Wharton and Struhl, 1991).

Unlike Drosophila, C. elegans begins development in a much smaller zygote with a series of asymmetric cleavage divisions (Sulston et al., 1983; see Figures 1 and 2). In C. elegans, the first division generates two unequal cells: an anterior blastomere, called AB, and a smaller posterior blastomere, called P1, which have distinct developmental potentials (Figure 1) (Laufer et al., 1980; Priess and Thomson, 1987). At the 4-cell stage, the daughters of AB, called ABa and ABp, are developmentally equivalent, but cell–cell interactions cause them to follow distinct fates (Priess and Thomson, 1987; Priess et al., 1987; Wood, 1991; Bowerman et al., 1992b). Therefore, early patterning of the C. elegans embryo appears to be specified by the segregation of intracellular determinants and cell interactions between blastomeres.

The molecular mechanisms that generate polarity in the early C. elegans embryo have been elusive. Some maternal gene products are asymmetrically distributed in the embryo. For example, P granules segregate to the posterior end of the fertilized zygote by an actin-dependent mechanism (Strome and Wood, 1983; Hill and Strome, 1988). In addition, SKN-1 protein is localized to the posterior blastomeres EMS and P2 by the 4-cell stage, and is necessary for specifying the fate of EMS (Bowerman et al., 1992a, 1993). Mutations in some genes, such as *mex-1* and the *par* genes, disrupt localization of P granules, localization of SKN-1, and blastomere determination (Kemphues et al. 1988; Mello et al., 1992; Morton et al., 1992; Bowerman et al., 1993), but how they influence these early events is not known at the molecular level.

Early C. elegans embryogenesis is also dependent on *glp-1*, a gene crucial for cell interactions (Figure 1B; Priess et al., 1987; Austin and Kimble 1987). The *glp-1* protein, called GLP-1 (Yochem and Greenwald, 1989; Austin and Kimble, 1989), belongs to a conserved family of transmembrane proteins that includes LIN-12 in C. elegans (Yochem et al., 1988), Notch in Drosophila (Wharton et al., 1985; Kidd et al., 1986), and numerous homologs in vertebrates including humans (Coffman et al., 1990; Ellisen et al., 1991; Jhappan et al., 1992; Stifani et al., 1992). GLP-1 appears to function as a membrane receptor for extracellular signals (Austin and Kimble, 1987, 1989; Yochem and Greenwald, 1989; Roehl and Kimble, 1993). During early embryogenesis, GLP-1 is required between the 4- to 28-cell stages (Priess et al., 1987; Austin and Kimble, 1987). Emerging evidence indicates that GLP-1 is required for inductive interactions between posterior and anterior blastomeres to specify several anterior cell fates (Figure 1B)

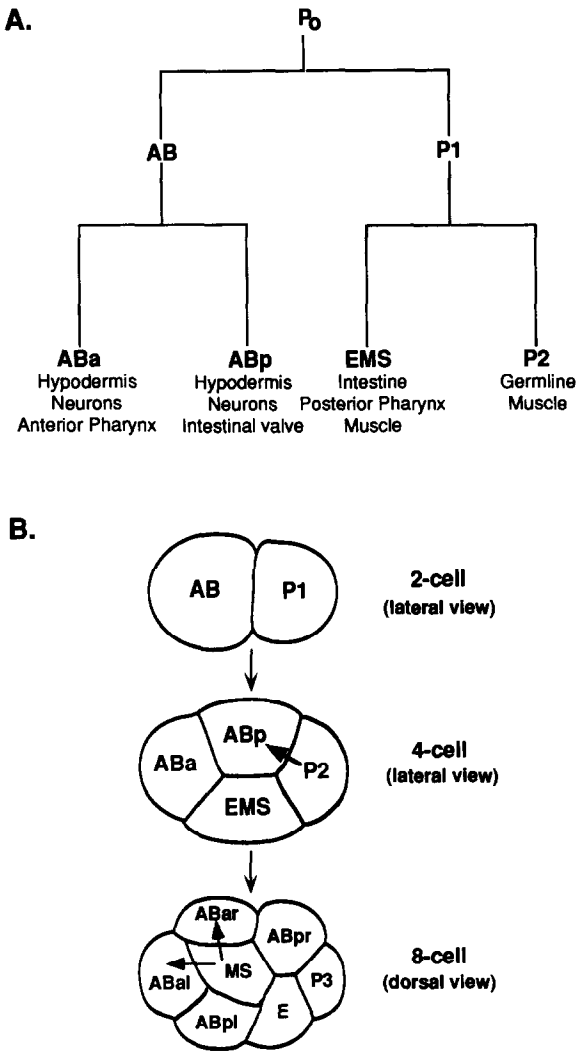

Figure 1. Early Embryogenesis in C. elegans

A) Diagram showing the cell lineage to the 4-cell stage, and some of the cell types generated by each blastomere of the 4-cell embryo (from Sulston et al., 1983). Anterior is to the left. P_0 is the fertilized zygote. (B) Schematic of 2-, 4-, and 8-cell embryos showing relative positions of individual blastomeres; arrows depict *glp-1*-dependent cell interactions. Anterior is to the left. The first cleavage division is asymmetric; AB is the large anterior daughter and P1 is the small posterior daughter. At the 4-cell stage, ABa and ABp are equivalent in developmental potential; a cell interaction (arrow) between P2 and ABp induces ABp to become different from ABa (Bowerman et al., 1992b; Mello et al., submitted). At the 8-cell stage, cell interactions (arrows) between MS, and daughters of ABa induce cells of the anterior pharynx (Priess and Thomson, 1987; Moscowitz et al., submitted; Mango et al., submitted). All of these cell interactions require maternal *glp-1* activity (Priess et al., 1987; Austin and Kimble, 1987; Mello et al., submitted). For the 8-cell embryo, ABal and ABar are the left and right daughters of ABa; ABpl and ABpr are the left and right daughters of ABp; MS and E are the daughters of EMS; P3 is one of the daughters of P2; the other P2 daughter, C, is not shown.

(Priess et al., 1987; Austin and Kimble, 1987: Mello et al., submitted; H. Hutter and R. Schnabel, submitted; I. Moscowitz, S. Gendreau, and J. Rothman, submitted).

The *glp-1* gene contributes a regulated maternal mRNA to the early embryo. GLP-1 function in the embryo is strictly dependent on maternal *glp-1* expression (Priess et al., 1987; Austin and Kimble, 1987). By Northern blot analysis, *glp-1* mRNA is present in both the germline and early embryo (Austin and Kimble, 1989; Ahringer et al., 1992). Furthermore, by in situ analysis, *glp-1* mRNA is present in maturing oocytes, whereas GLP-1 protein is not (S. C., E. Troemel, T. E., and J. K., submitted). Therefore, oocytes appear to contain a translationally inactive maternal mRNA that must be activated in early embryos to make GLP-1 for early cell interactions.

In this paper, we investigate the mechanisms that control GLP-1 production during early embryogenesis in C. elegans. We find that the translation of maternal *glp-1* mRNA is temporally and spatially regulated to restrict GLP-1 to anterior blastomeres of the early embryo. Furthermore, we find that sequences within the *glp-1* 3′ untranslated region direct repression of maternal mRNA translation in oocytes and embryos. The spatial regulation of *glp-1* mRNA has striking parallels to the translational control of maternal *hunchback* mRNA in the Drosophila embryo. Therefore, the establishment of anteroposterior asymmetry in organisms as diverse as nematodes and flies may rely on related molecular mechanisms that have been adapted to dramatically different strategies for early development.

Results

GLP-1 Protein Is Localized to Descendants of the Anterior Blastomere

To examine the distribution of GLP-1 in early embryos, we used three polyclonal antibodies raised to distinct regions of GLP-1 (see Experimental Procedures; Figure 2). No GLP-1 was detected in 1-cell zygotes (Figure 2A). In 2-cell embryos, GLP-1 was either not detected (53%, n = 17) or was faintly visible in the cytoplasm of the AB blastomere and in or near the membrane separating AB and P1 (47%, n = 17) (Figure 2B). At the 4-cell stage, GLP-1 was found in all embryos (n = 29), but only in descendants of the anterior blastomere: GLP-1 was associated with membranes between ABa and ABp, between ABa and EMS, and between ABp and P2, whereas no GLP-1 was observed in the membrane separating EMS and P2 (Figure 2C). Moreover, GLP-1 was found in the cytoplasms of ABa and ABp, but not in the cytoplasms of EMS or P2 (Figure 2C). This pattern indicates that GLP-1 expression is restricted to ABa and ABp. However, some of the surface staining could derive from EMS or P2. In contrast with GLP-1, actin was detected at the periphery of all blastomeres, indicating that all cells were accessible to antibodies (Figures 2E–2H).

After the 4-cell stage, GLP-1 continues to be restricted to AB descendants until it disappears from the early embryo (Figures 2D–2F). The distribution of GLP-1 among AB descendants appeared to be uniform for any given time from the 4-cell stage through the 28-cell stage. For example, at the 8-cell stage, all AB descendants are brightly stained, while at the 28-cell stage, all AB descendants are weakly stained (compare Figure 2D with Figure 2E). During the 12- to 28-cell stage, GLP-1 staining declined; cytoplasmic GLP-1 was no longer observed by the 12- to 16-cell stages, and membrane-associated GLP-1 was only faintly visible

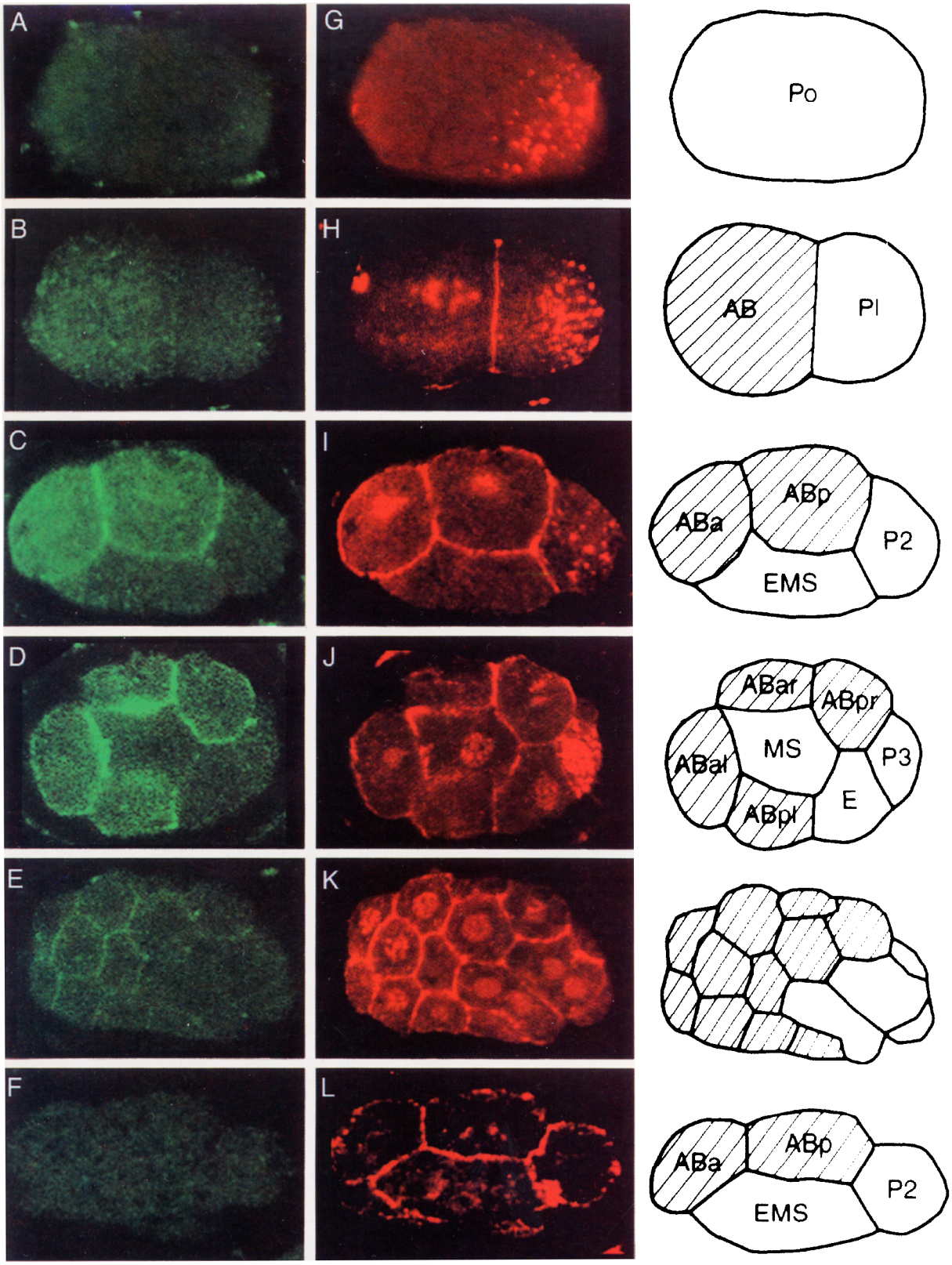

Figure 2. GLP-1 Is Localized to Descendants of the Anterior Blastomere AB in Early Embryos

Left column (A–F): embryos were stained for GLP-1 using an FITC-labeled secondary antibody:

(A) 1-cell zygote has no GLP-1.

(B) 2-cell embryo with faint cytoplasmic fluourescence detectable in AB and associated with the membrane separating AB and P1; not all 2-cell embryos contain GLP-1 (see text).

(C) 4-cell embryo; GLP-1 is present in both cytoplasm and membranes of ABa and ABp, but not in EMS or P2.

(D) 8-cell embryo; GLP-1 is associated with membranes and cytoplasms of AB descendants but not P1 descendants.

(E) 28-cell embryo; cytoplasmic GLP-1 disappears and membrane-associated GLP-1 is faint.

(F) A 4-cell embryo stained with a GLP-1 antibody directed against EGFL repeats 7–10 that had been preincubated with the EGF–(7–10) fusion protein (see Experimental Procedures).

Middle column (G–K): the same embryos as shown in left column stained for actin, DNA, and P granules using rhodamine-labeled secondary antibody. Actin outlines the surface of all blastomeres; DNA marks the nucleus; P granules identify the posterior blastomeres P1, P2, P3, and P4.

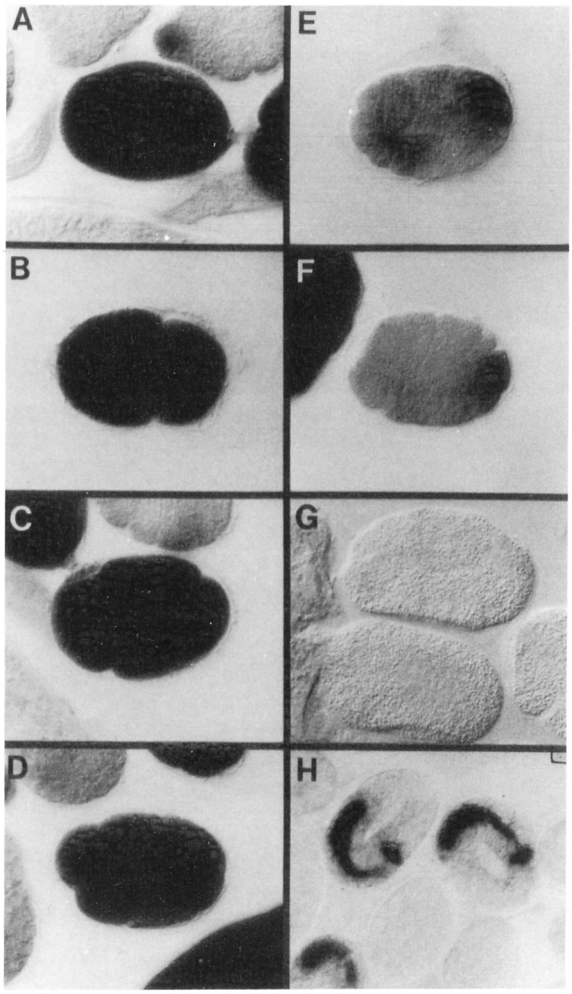

Figure 3. *glp-1* mRNA Is Uniformly Distributed in 1 to 8 Cell Embryos

Embryos were examined by in situ hybridization.

(A–F) Embryos were hybridized with antisense probe to *glp-1*: 1-cell (A), 2-cell (B), 4-cell (C), 8-cell (D), 12- to 16-cell (E), and 24-cell (F) embryos are shown.

(G) Embryos were hybridized with sense probe to *glp-1*: three embryos are shown (1-cell, 2-cell, and 3- to 4-cell).

(H) Embryos containing 200–800 cells were hybridized with probe to pharyngeal myosin; only the pharynx of late stage embryos stains. No *myo-1* hybridization was seen in early embryos under any condition tested (data not shown).

As in Figure 2, all embryos are oriented with anterior at left. Each micrograph in (A–F) is 80 μm wide; (G) is 75 μm wide, and (H) is 120 μm wide.

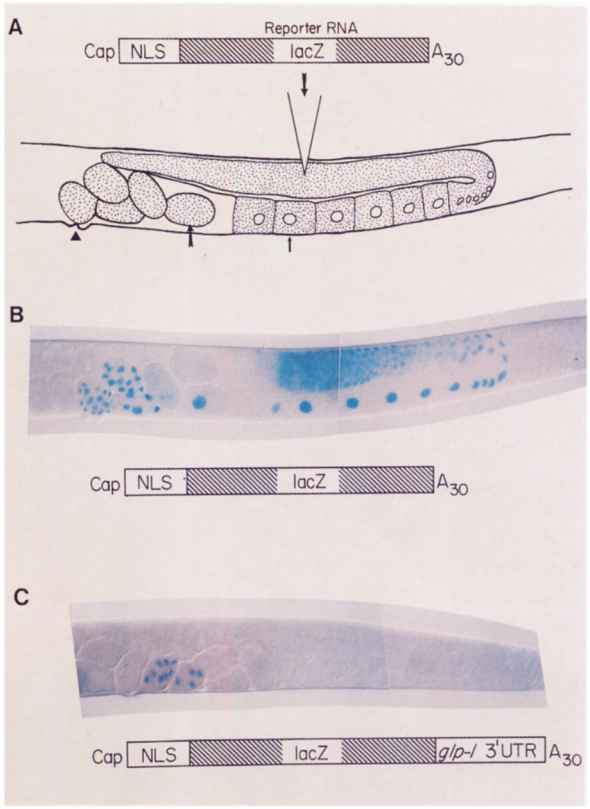

Figure 4. The *glp-1* 3′UTR Regulates Translation of Reporter RNA in Oocytes and Early Embryos

(A) Schematic of the reporter RNA microinjection assay. All reporter RNAs were capped (Cap) and polyadenylated (A_{30}), and all encoded β-galactosidase (LacZ) with a nuclear localization signal (NLS). Reporter RNAs were injected into the distal arm of the hermaphrodite gonad (stippled). The germline is a syncytium in which all nuclei share cytoplasm. The distal arm contains immature germ cell nuclei; as these nuclei move into the proximal arm, they mature into oocytes (small arrow); embryos (large arrow) develop in the uterus to about the 28- to 46-cell stage, and are then deposited through the vulva (arrowhead). (B and C) Adult hermaphrodites that were microinjected with reporter RNAs, incubated, and stained with X-Gal. (B) animal injected with control lacZ RNA. β-galactosidase is present in immature germ cells, oocytes, and embryos. (C) animal injected with lacZglp-1 RNA, which carries the entire *glp- 1* 3′UTR. No β-galactosidase is seen in oocytes; instead, β-galactosidase is found in two embryos (one 6- to 8-cell embryo and one 12- to 16-cell embryo).

by the 28-cell stage (Figure 2E). If cytoplasmic GLP-1 represents newly synthesized GLP-1 en route to the plasma membrane, as might be expected, the synthesis of GLP-1 may cease by the 12- to 16-cell stage. Later during embryogenesis, at about the 100-cell stage, GLP-1 reappeared in a few cells, which have not been identified (S. C., unpublished data).

glp-1 mRNA Is not Localized in the Early Embryo

The asymmetric distribution of GLP-1 could result from localization of *glp-1* mRNA, localization of translational controls, or localization of GLP-1 protein stability. To determine whether *glp-1* mRNA is localized in early embryos, we examined RNA distribution by in situ hybridization. We detected *glp-1* mRNA in 1- to 28-cell embryos following hybridization to a digoxygenin-labeled antisense RNA probe for *glp-1* (Figures 3A–3F). In 1- to 8-cell embryos, *glp-1* mRNA was observed in all cells with no reproducible difference in staining intensity between blastomeres (Figures 3A–3D). By the 16-cell stage, the level of *glp-1* mRNA declined (Figure 3E) and staining disappeared in most cells by the 28-cell stage (Figure 3F). The disappearance of *glp-1* mRNA was not uniform: *glp-1* mRNA reproducibly persisted longer in blastomeres in the posterior of the embryo (Figure 3F). Later in embryogenesis, *glp-1* mRNA

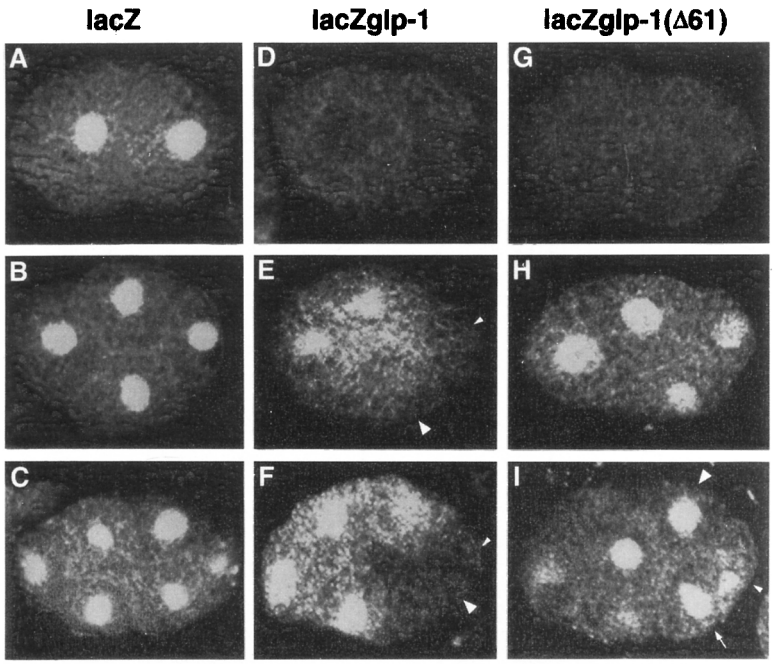

Figure 5. The *glp-1* 3′UTR Restricts Reporter RNA Translation to Anterior Cells

Embryos derived from injected animals were stained with anti-β-galactosidase antibody and examined by confocal microscopy.

Left column (A–C): embryos injected with lacZ RNA: (A) 2-cell, (B) 4-cell, and (C) 6-cell embryos are shown. All cells contain an eqivalent amount of β-galactosidase.

Middle column (D–F): embryos injected with lacZglp-1 RNA. (D), 2-cell embryo; no β-galactosidase is seen. (E), 4-cell embryo; β-galactosidase is greatly enriched in ABa and ABp, but some is detectable in EMS (larger arrow head) and possibly P2 (smaller arrowhead). (F), 8-cell embryo; all four descendants of the AB blastomere contain β-galactosidase, but no β-galactosidase is detected in the four descendants of P1: P3 (large arrowhead), C (small arrowhead), E, or MS (nuclei are out of focal plane).

Right column (G–I): embryos injected with lacZglp-1(Δ61) RNA, which is deleted for 61 nt (nucleotides 180–240) in the middle of the *glp-1* 3′UTR. (G), 2-cell embryo; no β-galactosidase is detected. (H), 4-cell embryo; (I), 8-cell embryo. All cells in (H) and (I) contain β-galactosidase. In the 8-cell embryo in (I), β-galactosidase is visible in the nuclei of ABpr (large arrowhead), P3 (small arrowhead), E (arrow), and MS (in middle of embryo); the nuclei of ABal, ABar, ABpl, and C are out of the focal plane, but contain a similar level of β-galactosidase. Each micrograph is approximately 60 μm wide.

reappeared in a pattern similar to that seen with GLP-1 antibodies, presumably a reflection of zygotic transcription (T. E., unpublished data). No staining was observed with a *glp-1* sense probe in any embryo (Figure 3G; data not shown). In addition, a probe for pharyngeal myosin mRNA stained only the pharynx of late embryos (Figure 3H), indicating that the staining pattern is probe-specific and that older embryos are accessible to RNA probes.

The *glp-1* mRNA observed in early embryos is probably maternal RNA that is made in the germline, provided to the embryo, and degraded early in embryogenesis. As noted above, *glp-1* function in the embryo is strictly dependent on maternal *glp-1* expression. In addition, the staining intensity of *glp-1* mRNA in 1- to 8-cell embryos (Figures 3A–3D) is comparable to that seen in oocytes (data not shown; S. C., E. Troemel, T. E., and J. K., submitted). This observation is consistent with previous Northern blot experiments, which showed that full-length *glp-1* mRNA is present in the germline and embryo, and declines after the 8-cell stage (Austin and Kimble, 1989, Ahringer et al., 1992). These results suggest that *glp-1* mRNA is delivered by the oocyte to the embryo, where it distributes to both anterior and posterior blastomeres. Therefore, the asymmetric localization of GLP-1 protein in anterior blastomeres depends on spatially- restricted translation of *glp-1* mRNA or posttranslational control of GLP-1 stability.

The *glp-1* 3′ Untranslated Region Confers Temporal and Spatial Regulation on Translation of a Reporter mRNA

If the translation of maternal *glp-1* mRNA is regulated, regulatory information must be present within the RNA. To test this and to explore the mechanism of control, the effect of the *glp-1* mRNA sequences on the translation of reporter mRNAs was assayed in vivo. Figure 4A depicts the assay. Capped and polyadenylated reporter mRNAs, which encode β-galactosidase that carries a nuclear localization signal, were synthesized in vitro and microinjected into the distal arm of the hermaphrodite germline (see legend to Figure 4). Injected animals were incubated to allow incorporation of reporter mRNAs into maturing oocytes and early embryos. The β-galactosidase made from these mRNAs was detected by histochemistry or indirect immunofluorescence. Using this assay, we tested the regulatory properties of the *glp-1* 3′ untranslated region (3′UTR). We focused on the *glp-1* 3′UTR because many maternal mRNAs are regulated by their 3′ UTRs (Wickens, 1992; Macdonald, 1992; Evans et al., 1992). For initial experiments, we synthesized two reporter mRNAs: a control mRNA called lacZ, which carries no *glp-1* sequence, and an experimental mRNA called lacZglp-1, which contains the entire 369 nt *glp-1* 3′ UTR.

The lacZglp-1 mRNA is temporally regulated much like endogenous *glp-1* mRNA. Animals injected with the control lacZ mRNA exhibited β-galactosidase throughout the germline, in oocytes, and in early embryos (Figure 4B; Figures 5A–5C; Table 1). In contrast, animals injected with lacZglp-1 mRNA contained little or no detectable β-galactosidase in oocytes, 1-cell, or 2-cell embryos, but did express reporter protein in 4- to 28-cell embryos (Figure 4C, Figures 5D–5F; Table 1). The lacZ mRNA expressed β-galactosidase strongly in oocytes over a 10-fold range of mRNA concentrations (10–100 nM in the microneedle), whereas lacZglp-1 mRNA was inactive over this same concentration range (data not shown). In embryos, β-galactosidase made from lacZglp-1 mRNA was first detected in

Table 1. Temporal and Spatial Control of Reporter RNA by the *glp-1* 3′UTR

RNA[a]	β-Galactosidase Stain in Oocytes (percentage of Gonads)[b] None	Weak	Strong		Localization of β-Galactosidase in Embryos (percentage of embryos)[c] Localized	Partially localized	Unlocalized	
lacZ —A_{30}	0	7	93	(n = 40)	0	0	100	(n = 52)
lacZ glp-1 3′UTR(wt) A_{30}	96	4	0	(n = 77)	78	11	11	(n = 45)
(175) A_{30}	91	9	0	(n = 22)	59	25	16	(n = 24)
(190) A_{30}	0	0	100	(n = 20)	0	0	100	(n = 27)
(125) A_{30}	0	13	87	(n = 31)	0	0	100	(n = 12)
(61) A_{30}	70	30	0	(n = 40)	0	0	100	(n = 40)

[a] Reporter RNAs containing no 3′UTR, the *glp-1* 3′UTR, or deletion derivatives of the *glp-1* 3′UTR were injected at 50 nM into the distal arms of hermaphrodites. The number of nucleotides deleted within the *glp-1* 3′UTR is bracketed (see Figure 7 for precise deletion endpoints).

[b] Animals were fixed and stained with X-Gal, and the number of gonads containing β-galactosidase in oocytes was determined. Diffuse staining that could only be detected in oocyte nuclei as thin blue slashes at 1000 × was defined as weak. Uniform blue staining that was readily visible in oocyte nuclei at 50–200 × was defined as strong. n is the number of gonads examined in 2–5 different experiments using different RNA preparations. Most oocytes within any given gonad showed the same level of stain.

[c] β-galactosidase was detected in embryos by immunofluorescence or by X-gal stain. Any 4- to 16- cell embryos in which β-galactosidase was detected at equivalent levels in all cells were scored as unlocalized (see Figures 5B and 5H); those in which β-galactosidase was enriched in AB descendants, but detected in posterior cells as well, were scored as partially localized (see Figure 5E); those in which β-galactosidase was only detected in AB descendants were scored as localized. (see Figure 5F). Only those embryos in which cell identity and β-galactosidase localization could be unambiguously assigned were scored. n is the number of 4- to 16- cell embryos scored.

4-cell embryos, as much as one cell cycle later than endogenous GLP-1. This difference could reflect the presence of additional regulatory information in endogenous *glp-1* mRNA, or it could result from experimental differences in the detection of β-galactosidase and GLP-1. Two observations suggest that RNA degradation is not responsible for the lack of lacZglp-1 mRNA activity in oocytes and 1- to 2-cell embryos. First, lacZglp-1 mRNA becomes active in early embryos (Figure 4C, Figures 5D–5F), suggesting that at least some fraction of the injected mRNA is intact and translationally activated in the embryo. Second, the staining intensities of lacZ and lacZglp-1 reporter mRNAs were similar following in situ hybridization (Figure 6). Therefore, we propose that the *glp-1* 3′UTR is required for the translational repression of *glp-1* maternal mRNA in oocytes and 1- to 2-cell embryos.

The translation of the lacZglp-1 mRNA is also spatially restricted in the embryo, much like endogenous *glp-1* mRNA. Only the descendants of the anterior blastomere AB contained detectable levels of β-galactosidase in most of the 4- to 16-cell embryos that carried the lacZglp-1 mRNA (Figures 5D–5F; Table 1). By contrast, β-galactosidase was detected in all cells of embryos carrying the control lacZ mRNA (Figures 5A–5C, Table 1), although this may include protein made during oogenesis. As observed for the endogenous *glp-1* mRNA, the lacZglp-1 mRNA was not localized in embryos; both the lacZ and lacZglp-1 mRNAs were detected in all embryonic blastomeres following in situ hybridization with a lacZ RNA probe (Figure 6).

The regulation of reporter mRNA translation by the *glp-1* 3′UTR in oocytes and embryos is sequence specific. A reporter mRNA carrying the 3′UTR of *unc-54*, a muscle myosin heavy chain gene (MacLeod et al., 1977), produces a β-galactosidase-staining pattern similar to that seen with the lacZ control (data not shown). In addition, a reporter mRNA carrying the 3′ UTR of a sex-determination gene, *tra-2*, produces strong β-galactosidase staining, but only in the most proximal oocytes and in embryos (Goodwin et al., 1993). Thus, different 3′UTRs affect the activity of lacZ mRNA in distinct patterns. In addition, these results show that the lacZ coding region is translatable in most, if not all, stages of oogenesis and embryogenesis (see below).

The glp-1 3′UTR Contains Regulatory Elements that Repress Translation

To begin identifying the elements that regulate *glp-1* mRNA, we have analyzed deletions within the *glp-1* 3′ UTR using the reporter RNA assay. Our results are presented in Table 1. Removal of the first 175 nt had no significant effect on reporter RNA regulation. Conversely, deletion of the last 190 nt (nucleotides 180–369) disrupted regulation: β-galactosidase was detected in oocytes and in all cells of early embryos. Thus, the 3′-most 194 nt (nucleotides 176–369) are necessary and sufficient for both temporal

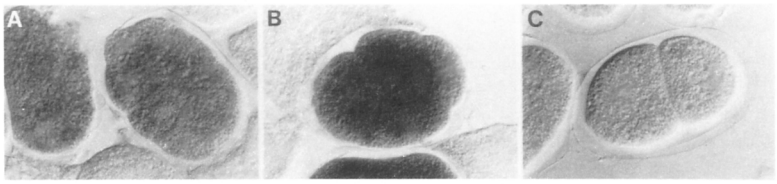

Figure 6. Reporter RNA Containing the *glp-1* 3′ UTR Is Present in All Blastomeres of Early Embryos

Animals were microinjected with reporter RNA and embryos were examined by in situ hybridization using a lacZ antisense probe.

(A) Two early embryos (4-cell and 8-cell) from a mother injected with lacZ reporter RNA.

(B) A 4-cell embryo from a mother injected with lacZglp-1 reporter RNA.

(C) A 2-cell embryo from a mother that was not injected; no hybridization was detected.

Each micrograph is approximately 85 μm wide.

and spatial control of reporter RNA translation. Removal of the last 125 nt of the *glp-1* 3′ UTR (nucleotides 245–369) similarly caused premature expression of β-galactosidase in oocytes. As discussed below, this region may be sufficient for translational repression in oocytes and zygotes. Therefore, temporal control of translation is mediated by sequences within the last 125 nt of the *glp-1* 3′ UTR.

A 61 nt region in the middle of the *glp-1* 3′UTR (nucleotides 180–240) is specifically required for spatial restriction of RNA translation to anterior descendants. When this sequence was removed, the localization of translation was disrupted, while the timing of translation was largely unaffected; β-galactosidase staining was equivalent in all blastomeres of embryos with four or more cells, but was not detected in most oocytes or 1- and 2-cell embryos (Figures 5G–5I; Table 1). Therefore, the 61 nt region between nucleotides 180–240 is required for repression of reporter RNA translation in descendants of the posterior blastomere P1. We do not know whether this region is sufficient to repress translation in posterior cells: RNAs that lack the 125 nt temporal control region (nucleotides 245–369) translate β-galactosidase in oocytes, which may persist in posterior cells of embryos even if translation is inactivated by the 61 nt region.

The 61 nt region (nucleotides 180–240) implicated in spatial control contains sequences with striking similarity to a regulatory element that spatially regulates *hunchback* translation in the embryos of Drosophila melanogaster (Figure 7). Within the *hunchback* 3′UTR are two sequence elements, called nanos response elements (NREs), that are necessary for repression of *hunchback* maternal mRNA translation by the localized nanos protein (Wharton and Struhl, 1991; see Discussion). Both elements are bipartite, consisting of the motifs GUUGU and AUUGUA, separated by 5 nt (Wharton and Struhl, 1991). These motifs and their spatial arrangement are conserved in the *hunchback* 3′UTR from Drosophila virilis, but the nucleotides between and surrounding them are not conserved (Treier et al., 1989). The *glp-1* 3′ UTR contains a similar bipartite sequence: GUUGU and AUUGAU, separated by 9 nt, and AUUGUA, which lies 8 nt upstream of the GUUGU motif (Figure 7B).

Discussion

Our results lead to three main conclusions about *glp-1* regulation in the early embryo. First, GLP-1 protein expression is both temporally and spatially restricted to anterior descendants of 2- to 28-cell embryos. Second, GLP-1 regulation is achieved by translational control of maternal *glp-1* mRNA. Third, two regions within the glp-1 3′ UTR mediate control of translation: a temporal control region represses translation until the 2- to 4-cell stage, and a

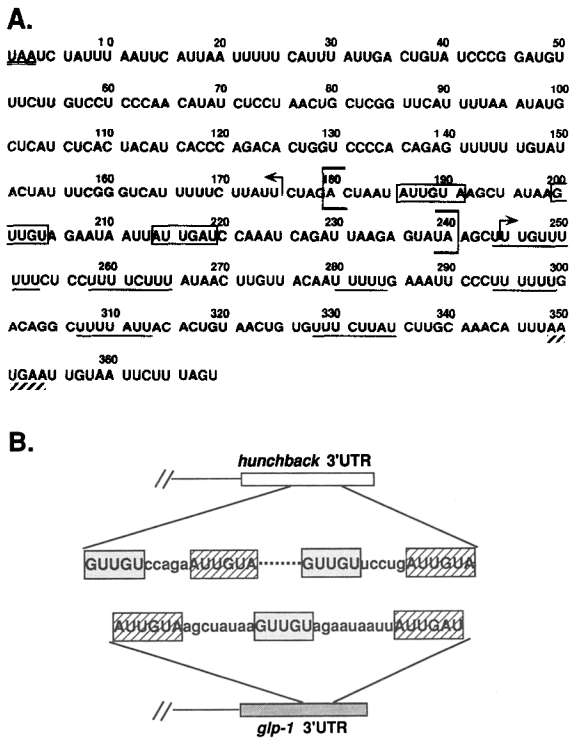

Figure 7. The *glp-1* 3′ UTR Contains NRE-Like Motifs

(A) Sequence of the *glp-1* 3′UTR in *glp-1* mRNA. Nucleotides are numbered from the first base in the termination codon to the last nucleotide found in cDNAs before the poly(A) tract (Yochem and Greenwald, 1989; V. K., unpublished data). The termination codon UAA is marked by a double underline; the predicted cleavage and polyadenylation signal AAUGAA is marked by a hatched underline. The 3′ endpoint of the deletion that removes the first 175 nt is marked by the arrow at position 175; the remaining nucleotides in this RNA are sufficient for both temporal and spatial control. The spatial control region removed in lacZglp-1(Δ61), nucleotides 180–240, is bracketed. Motifs within this region, which are similar to the conserved nanos response elements (NREs) of the Drosophila *hunchback* 3′ UTR, are boxed. The 5′ end of the deletion that removes the 125 nt temporal control region is marked by the arrow at position 245. Uridine-rich stretches within this 125 nt temporal control region are underlined.

(B) Comparison of NREs in the *hunchback* 3′ UTR to the NRE-like sequences within the spatial control region in the *glp-1* 3′ UTR.

spatial control region is required to repress translation in posterior blastomeres. The spatial control region in the *glp-1* 3′ UTR is structurally and functionally similar to the NREs in the 3′ UTR of the maternal mRNA *hunchback* from Drosophila. In the following sections, we discuss the implications of these results for early C. elegans embryogenesis and for the establishment of embryonic polarity throughout phylogeny.

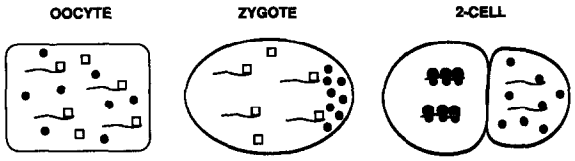

Figure 8. A Model for Translational Regulation of *glp-1* Maternal mRNA in Oocytes and Embryos

To keep *glp-1* mRNA (thin curved lines) silent until the 2-cell stage, a translational repressor (open squares) binds to the *glp-1* 3′UTR and blocks translation in oocytes and 1-cell embryos and is inactivated at the 2-cell stage. To restrict translation to anterior cells, a second translational repressor (closed circles) is localized to the posterior of the 1-cell zygote, and prevents translation in P1 and its descendants. Other models are also consistent with our results (see text).

GLP-1 and Cell–Cell Interactions in the Early Embryo

GLP-1 expression in early embryos both confirms predictions and raises questions about the embryonic function of this regulatory protein. Temperature-shift experiments suggested that GLP-1 acts in 4- to 28-cell embryos (Austin and Kimble, 1987; Priess et al., 1987). Indeed, we detected GLP-1 in 2- to 28-cell embryos. In addition, previous studies predicted that GLP-1 is a membrane-bound receptor in cells that receive inductive signals (Austin and Kimble, 1987, 1989; Priess et al., 1987; Yochem and Greenwald, 1989). We found that GLP-1 is associated with membranes of anterior cells, as expected for an integral membrane receptor.

The presence of GLP-1 in both the ABa and ABp lineages is consistent with the view that GLP-1 is widely involved in the development of anterior cell fates. At the 4-cell stage, an induction of ABp by P2 may resolve the eqivalence of ABa and ABp, and this induction relies on GLP-1 (Bowerman et al., 1992b; Mango et al., submitted; Mello et al., submitted; Moscowitz et al., submitted). Thus, GLP-1 may function in ABp to receive a P2 signal. At the 8- to 15-cell stage, GLP-1 is also required for interactions between the posterior cell MS and AB descendants that lead to pharyngeal induction and to left–right differences between AB descendants (Priess et al., 1987; Austin and Kimble, 1987; S. Mango, E. Lambie, and J. K., submitted; H. Hutter and R. Schnabel, submitted; S. Gendreau, I. Moscowitz, and J. Rothman, submitted). The continued presence of GLP-1 in AB descendants after the 4-cell stage supports the idea that GLP-1 receives the MS signal. Therefore, the role of GLP-1 in the specification of anterior fates is complex; GLP-1 may mediate communication between posterior and anterior blastomeres by participating in at least two distinct inductive events.

The localization of GLP-1 to anterior blastomeres suggests that the asymmetric distribution of this receptor protein is critical for embryogenesis. Inappropriate expression of GLP-1 in posterior cells may interfere with posterior determinants, or it might sabotage the highly ordered and specific cell interactions between posterior and anterior blastomeres. Because the early C. elegans embryo is small and contains few cells at the time of these inductions, the receptor may need to be localized to achieve localized activation of the signal transduction process. This mode of localization differs from the signaling events that control dorsal–ventral polarity and terminal development of the large Drosophila embryo, which involve the localized activation of evenly distributed membrane receptors (Casanova and Struhl, 1989; Stevens et al., 1990; Stein et al., 1991; Hashimoto et al., 1991).

The Translational Control of Maternal *glp-1* mRNA

Our results argue that the localization of GLP-1 protein to anterior cells of embryos is achieved by translational control of *glp-1* mRNA: *glp-1* mRNA is not localized in the early embryo, and the *glp-1* 3′ UTR confers regulation on a reporter mRNA encoding a heterologous protein. In the absence of control elements, reporter protein is expressed in oocytes and posterior blastomeres, suggesting that the *glp-1* 3′UTR mediates repression of GLP-1 translation. Translational repression could result from masking or sequestration of maternal mRNA away from the translational machinery, or it may reflect direct control of the translational machinery. In addition, it is possible that other mechanisms, such as protein stability, could also contribute to GLP-1 localization.

Reporter RNA experiments suggest that two regulatory regions reside within the *glp-1* 3′UTR. First, a 125 nt region at the 3′ end keeps the maternal mRNA translationally inactive until the 2- to 4-cell stage. Within this region are several uridine-rich sequences (Figure 7A), which are reminiscent of control elements that regulate the polyadenylation and translation of maternal mRNAs in frogs and mice (reviewed by Richter, 1991; Wickens, 1992). Perhaps, the temporal control of *glp-1* occurs by a similar mechanism. Second, a 61 nt region in the middle of the *glp-1* 3′UTR is required to repress translation in the descendants of the P1 blastomere. Within this region are motifs similar to the NREs in Drosophila maternal mRNAs.

The presence of two regulatory regions could indicate that *glp-1* mRNA is regulated by more than one factor. In one model (Figure 8), temporal regulation could be achieved by a translational repressor that binds within the 125 nt at the 3′ end of the *glp-1* 3′UTR to prevent translation of *glp-1* mRNA in oocytes and early embryos. This temporal repressor might then be inactivated between the 2- and 4-cell stages. To restrict translation spatially, a second translational repressor, or its mRNA, could be localized to the posterior end of the embryo sometime before the first cell division. This spatial repressor may interact with the NRE-like region to keep *glp-1* mRNA silent in posterior

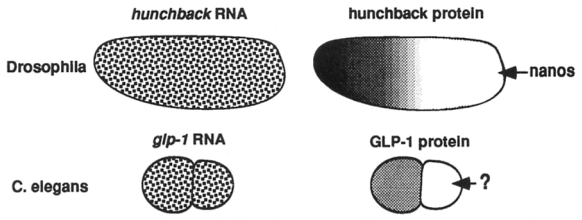

Figure 9. Similarities between the Regulation of Anterior–Posterior Asymmetry in C. elegans and in Drosophila melanogaster Embryos

Both *hunchback* and *glp-1* maternal RNAs are uniformly distributed in the early embryo and both *hunchback* and GLP-1 proteins are produced in the anterior of the early embryo. In both cases, the respective maternal RNAs are translationally repressed posteriorly. The regulator of *hunchback* translation in the posterior of the embryo requires activity of the *nanos* gene. The regulator of *glp-1* translation in the posterior blastomere is unknown.

cells. Of course, other models are consistent with our data. For example, a single translational repressor could be evenly distributed in the embryo, and inactivated by determinants that are localized to anterior blastomeres. Also, removal of the NRE-like region may alter the binding of a single factor such that it functions in oocytes but not in posterior cells. Regardless, we emphasize the need for both temporal and spatial control to restrict GLP-1 to anterior cells. Without temporal control, GLP-1 would be expressed in oocytes and zygotes, and could be inherited by posterior blastomeres. Similarly, some component of the regulatory system must be asymmetrically distributed to restrict translation to anterior cells.

Translational Control and the Establishment of Polarity in the C. elegans Embryo

Previous work, together with the results presented here, indicate that embryonic polarity in C. elegans arises from the localization of intracellular components soon after fertilization. By the time the pronuclei have fused in the 1-cell zygote, P granules have segregated to the posterior pole (Strome and Wood, 1983). By the 4-cell stage, SKN-1 protein is effectively localized to the posterior blastomeres EMS and P2 (Bowerman et al., 1993), and we have shown that the GLP-1 receptor is localized to anterior blastomeres by the 2- to 4-cell stages. It seems likely that the spatial restriction of these and other regulatory molecules will be crucial to development of the embryo.

To date, the molecular mechanisms controlling polarity of the C. elegans embryo have been poorly understood. Our results imply that localized translational control may play an important role in establishing or maintaining anteroposterior asymmetry. The translational regulators of *glp-1* mRNA could be specific for *glp-1* or they could regulate the translation of other maternal mRNAs to establish an elaborate pattern of molecular asymmetries. For example, the same translational controls that restrict GLP-1 to anterior cells could promote the posterior localization of SKN-1 by restricting the translation of a SKN-1 regulator to anterior blastomeres. In this regard, it is intriguing that other mRNAs expressed in the C. elegans germline possess NRE-like sequences in their 3′UTRs (Roussell and Bennett, 1993; M. Gruidl and K. Bennett, personal communication).

The Establishment of Embryonic Polarity May Involve Conserved Mechanisms of Translational Control

The regulation of maternal *glp-1* mRNA in C. elegans is similar to that of maternal *hunchback* mRNA in Drosophila. Both maternal mRNAs are translated only in the anterior due to translational repression in the posterior of their respective embryos (Figure 9). Furthermore, the cis-acting sequences that spatially control translation of *hunchback* and *glp-1* mRNAs are located in the 3′UTR and bear similar sequence motifs. In Drosophila, the repression of *hunchback* mRNA in the posterior is mediated, directly or indirectly, by nanos protein (Tautz and Pfeifle, 1989; Wharton and Struhl, 1991). Therefore, we speculate that the trans-acting factor that represses *glp-1* mRNA in the posterior cells of C. elegans embryos could be related to nanos.

In Drosophila, nanos protein is restricted to the posterior by the localization of maternal *nanos* mRNA to the posterior polar plasm (Wang and Lehmann, 1991). Perhaps the spatial regulator of *glp-1* mRNA is regulated in a similar fashion in C. elegans. In many ways, the polar granules of Drosophila are similar to the P granules of C. elegans. Like Drosophila polar granules, P granules reside in the posterior of the zygote, and eventually segregate to germ cell precursors during embryogenesis (Strome and Wood, 1983). In addition, a gene related to Drosophila *vasa*, a component of polar granules in flies (Hay et al., 1988), has been isolated from C. elegans (called *glh-1*; Roussell and Bennett , 1993) and its protein product may be localized to P granules (M. Gruidl and K. Bennett, personal communication). Therefore, P granules in C. elegans may localize posterior determinants. We suggest that one of these determinants could be the translational regulator of *glp-1*. If the mRNA for this translational regulator is localized to P granules, its translation at the 2-cell stage would provide regulator protein to all posterior descendants. Alternatively, the translational regulator itself might associate with P granules until the 2-cell stage, at which time it is released.

The apparent similarities in the control of embryonic polarity in Drosophila and C. elegans are striking, given their differences in embryogenesis. Drosophila embryos generate pattern within a large syncytium of nuclei, whereas C. elegans embryos are much smaller and develop by asymmetric cell cleavages. Perhaps, a conserved regulatory mechanism has been adapted to control different types of molecules that reflect different developmental strategies. For C. elegans, the asymmetric localization of GLP-1 may help establish communication between anterior and posterior cells, whereas, for Drosophila, the spatial restriction of *hunchback* establishes an anterior to posterior gradient of transcription factors in an embryonic syncytium.

How conserved are the mechanisms for establishing embryonic polarity? The control of maternal mRNAs

through 3′UTR sequences is emerging as a common theme in the establishment of early asymmetry in a number of organisms (Nüsslein-Volhard, 1991; Melton, 1991; Macdonald, 1992). Although the development of these organisms appears to be diverse, our results hint that some of the underlying mechanisms may be similar. Interestingly, a maternal mRNA in Xenopus, called Xcat-2, encodes a protein related to nanos, and is localized in the oocyte (Mosquera et al., 1993). Possibly, Xcat-2 controls important maternal mRNAs in frog embryos by a mechanism related to the control of *hunchback* and *glp-1* mRNAs. We predict that similar mechanisms will extend to many other organisms as well. The challenge for the future is to determine the degree to which these mechanisms are conserved, and how they have been altered to effect distinct patterns of development.

Experimental Procedures

Strains and Culture Conditions

The wild-type strain, N2, was used for all experiments. Animals were grown on agar plates as described (Brenner, 1974). For most immunofluorescence experiments with *glp-1* antibodies, animals were grown at 15°C. For all other experiments, animals were grown at 20°C.

Plasmids

Standard methods for manipulation of DNAs and plasmid growth were employed (Ausubel et al., 1987). Plasmid pJK350, which encodes the lacZ reporter RNA, has an SP6 promoter, the lacZ coding region (including the SV40 nuclear localization signal), a 3′ polylinker, and a poly(A) tract of 30 residues. To build pJK350, an XbaI to SpeI fragment from pPD16.43 (Fire et al., 1990) was cloned into the EcoRI site of pSP65 (Promega). A synthetic 3′polylinker and poly(A) tract were then cloned into the StuI and HindIII at the 3′ end of the lacZ fragment. The 3′ polylinker includes several unique sites: StuI, SalI, ApaI, BglII, and PstI. This polylinker is followed by a poly(A) tract of 30 residues and two sites, NsiI and HindIII, to linearize the template for in vitro transcription (described below). Plasmid pJK355, which encodes lacZglp-1 reporter RNA, was constructed by inserting the entire 369 *glp-1* 3′UTR into pJK350. The *glp-1* 3′ UTR was amplified by polymerase chain reaction from the *glp-1* genomic DNA vector pJK185 and was cloned into the SalI and BglII sites of pJK350. Deletions within the *glp-1* 3′ UTR were made essentially by restriction fragment removal and blunt-end ligation. To make pJK356, which encodes lacZunc-54 RNA, the 286 bp *unc-54* 3′ UTR plus 20 bp of 3′ flanking DNA, as recently defined (A. Aryana, P. Okkema, and A. Fire, personal communication), was polymerase chain reaction amplified from pPD29.59 (a gift from A. Fire) and cloned into the SalI and BglII sites of pJK350. The *unc-54* polymerase chain reaction fragment includes the 286 bp 3′ UTR plus 20 bp of 3′ flanking DNA. The plasmid pJK409, which was used to make RNA probes to *glp-1* for in situ hybridization, contains a 3.7 kb fragment of *glp-1* cDNA. To make pJK409, fragments from 3 independent cDNA clones were ligated into pGEM 7Zf(+) (Promega). pJK409 contains 85% of the *glp-1* coding region, from the BamHI site within the second epidermal growth factor (EGF) repeat, to the XbaI site within the 3′ UTR (see Yochem and Greenwald, 1989).

Antibodies and Immunostaining

The generation and properties of rat polyclonal antibodies to GLP-1 are described elsewhere (S. C., E. Troeml, T. E., and J. K., submitted). Three antibodies were used here: anti-EGFL and anti-LNG, which were raised against two distinct parts of the extracellular domain, and anti-ANK, which was raised against the intracellular domain. Three lines of evidence argue that the embryonic staining shown in Figure 2 reflects the distribution of endogenous GLP-1. First, the staining pattern was identical using the three different antibodies (data not shown). Second, staining was eliminated if GLP-1 antibodies were preincubated with excess protein fragments against which the antibodies were raised (Figure 2F; data not shown). Third, GLP-1 staining is eliminated in mitotic germ cells by a *glp-1* nonsense mutation (S. C., E. Troemel, T. E., and J. K., submitted). Because animals homozygous for a *glp-1* null mutation are sterile and produce no embryos (Austin and Kimble, 1987; Kodoyianni et al., 1992), we have not been able to stain null mutant embryos.

For immunofluorescence detection of GLP-1, embryos were fixed by a modification of the freeze–cracking protocol described previously (Strome and Wood, 1983; Albertson, 1984). In brief, gravid hermaphrodites were transferred to 5–10 μl of M9 buffer (or phosphate-buffered saline [PBS]) containing 0.25 mM levamisole on polylysine-subbed slides, and cut with a 25-gauge needle to dissect out embryos and germlines. A coverslip was added, pressure applied, and the slides were placed on dry ice for 10 min. After removal of the coverslip, slides were incubated for 7 min in cold methanol and then 7 min in acetone. Samples were then blocked in Tris-buffered saline or PBS with 0.5% bovine serum albumin (BSA) for 15–30 min, and stained with GLP-1 antibodies as described (S. C., E. Troemel, T. E., and J. K., submitted). Embryos were simultaneously costained with monoclonal antibody K76 to P granules (a gift of S. Strome), monoclonal antibody to actin (ICN), monoclonal antibody to tubulin, or monclonal antibody to DNA (MAb 030, Chemicon).

For immunofluorescence detection of β-galactosidase, embryos were dissected into 2.5 μl of PBS with 5 mM EGTA on polylysine coated slides, and 2.5 μl of 5% paraformaldehyde in PBS/EGTA was added. After applying pressure and freezing as described above, slides were placed in cold methanol for 5–10 min, air dried, and fixed in 2.5 % paraformaldehyde in PBS/EGTA for 10 min. Slides were washed four times with PBS/BSA for 5–10 min per wash. Rabbit anti-β-galactosidase (a gift from S. Carroll), at 1:1000 in PBS/BSA, was added and slides were incubated overnight at 4°C. Slides were washed again, and incubated in biotinylated Donkey anti-rabbit IgG (Jackson Labs), diluted 1:200, at 25°C for 2 hr. Slides were then washed and incubated in fluorescein (DTAF)-labeled strepavidin (Vector) at 1:200 for 1 hr. Slides were washed with 40 ng/ml DAPI added to the third wash. Most embryos were simultaneously costained with GLP-1 antibdies or the P granule antibody, K76. Rhodamine (LRSC)-labeled donkey anti-mouse or donkey anti-rat antibodies (Jackson Labs) were used to detect the costained markers. All slides were examined by epifluorescence, and by laser scanning confocal microscopy on a Bio-Rad MRC600 LSCM (Bio-Rad Microscience Division, Cambridge, Massachusetts) as described (Paddock et al., 1993). The identity of embryonic cells was determined by cell size and position, nuclear morphology and cell cycle position, and by staining for P granules or GLP-1 as cell markers.

In Situ Hybridization

The in situ hybridization protocol was adapted for use on C. elegans embryos from a published method (Tautz and Pfeifle, 1989). Embryos were dissected and frozen on polylysine-coated slides in 2.5% paraformaldehyde in PBS/EGTA, as described above, and then placed in cold methanol for 10 min. After air drying, slides were fixed for 25 min in 5% paraformaldehyde in PBS/EGTA. The fix solution was removed, and slides were placed in methanol for 5 min, washed twice in ethanol for 5 min, once in 50% ethanol/xylene for 5 min, and then in xylene for 1 hr. Slides were then washed in ethanol/xylene for 5 min, three times in ethanol , once in methanol, and then air dried. Slides were fixed again in 2.5% paraformaldehyde for 25 min, and then washed 4 times in PBS. For most experiments, slides were incubated in 5 μg/ml protease K (BRL) for 25 min at 20°C. For some experiments either no protease or 25 μg/ml protease K was used. For *glp-1* RNA detection, all conditions gave the same staining pattern except that staining was slightly weaker with no or 25 μg/ml protease K; also, at 25 μg/ml protease K, the staining was more uneven within cells. For *myo-1* RNA detection, 25 μg/ml protease K gave stronger staining than the other conditions. The resulting background was unaffected by the amount of protease used. After protease treatment, slides were washed in PBS with 2 mg/ml glycine, and then three times in PBS. Slides were fixed again in 2.5% paraformaldehyde for 20 min, and washed four times in PBS. Slides were then washed twice in Hyb buffer (5× SSC, 50% formamide, 1 mg/ml glycogen, 100 μg/ml herring sperm DNA, and 0.1% Tween-20), and then incubated in Hyb buffer at 55°C for 1 hr. Digoxygenin-labeled RNA probe was added (0.2–1 μg/ml in Hyb buffer) and slides were incubated at 55°C for 36 hr. Slides were then

washed in Hyb buffer twice for 20 min, once for 4–6 hr, and twice for 20 min, all at 55°C. Slides were then washed four times in PBS with 0.5% BSA at room temperature. Alkaline phosphatase-linked anti-digoxygenin antibody (Boehringer Mannheim) was added at 1:2000 in PBS/BSA and slides were incubated at 4°C overnight (or at room temperature for 2 hr). The slides were then washed four times in PBS and developed as described in the Genius kit from Boehringer Mannheim.

Digoxygenin-labeled RNA probes were synthesized essentially according to the Genius kit from Boehringer Mannheim with the following modifications. Labeled RNAs were treated with DNAase I for 30 min at 37°C, and the unincorporated nucleotides were removed by gel filtration on Sephadex G-50 resin. The labeled RNAs were then partially hydrolyzed in 20 mM sodium carbonate buffer (pH 10.2) for 40 min at 65°C, precipitated in ethanol, and resuspended in dH20. Both sense and anti-sense probes for *glp-1* were made using pJK409 as a template.

Reporter RNA Synthesis and Microinjection

Capped and polyadenylated reporter RNAs were synthesized as described (Krieg and Melton, 1987) with modifications. DNA templates, all derivatives of pJK350 (see above), were linearized with NsiI, extracted three times with phenol/chloroform and two times with chloroform, and precipitated in ethanol. DNA templates linearized with NsiI yield RNA with only 30 adenosines at the 3′ end. RNAs were synthesized in a 40 μl reaction containing 15 U of SP6 polymerase in SP6 buffer (BRL) with ATP, CTP, and UTP (all at 0.5 mM); GTP at 0.1 mM; 7-methyl-G(5′)ppp(5′)GTP (New England Biolabs) at 1.0 mM; dithiothreitol at 10 mM; 20 U of RNasin (Promega); and 0.2–1.0 μg of DNA template. Included as a tracer for quantitation of RNA yields was 15 nCi of [α-^{32}P]UTP (800 Ci/mmol, Amersham). Reactions were treated with DNAase I, and unincorporated nucleotides removed by gel filtration. Full-length polyadenylated RNA was purified by chromatography on oligo-dT cellulose (Pharmacia). Excess oligo-dT was removed by centrifugation, and reporter RNAs were extracted with phenol/chloroform and chloroform, and precipitated in ethanol. Reporter RNAs were resuspended in 5 mM Tris at pH 7.5.

Microinjections were performed essentially as described (Fire, 1986), except that injection pressure was applied with N_2 gas using a Narashige IM-200 microinjector (Fryer Company) to control injection duration and pressure. Microneedles were pulled with a Flaming/Brown P 87 micropipet puller (Sutter Instruments) using borosillicate glass capillaries of 1.0 mm OD and 0.75 mm ID (Dagan Corporation). Animals were injected with reporter RNAs in the distal arm (in the middle to distal end of the core region) of one or both gonads (see Figure 4A and legend). RNA concentrations for injection were 10 nM to 100 nM in the microneedle. Injection volumes were estimated to be 10–20 pl, based on comparison to the injection method of Mello et al. (1991). Animals were then incubated in recovery buffer (Fire, 1986) for 15–30 min, washed with M9 buffer, transferred to seeded plates, and incubated at 20°C for 5.5 hr. To detect β-galactosidase protein, animals were either fixed and stained histochemically with X-Gal (Fire et al., 1990), or they were dissected, and embryos were fixed and stained with β-galactosidase antibodies as described above. To detect reporter RNAs by in situ hybridization, injected animals were dissected, and embryos were fixed and hybridized, as desribed above, to an anti-sense digoxigenin-labeled probe to lacZ RNA (a gift of G. Panganiban).

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Note Added in Proof

The data listed as Mello et al., submitted, is now published: Mello, C. C., Draper, B. W., and Priess, J. R. (1994). The maternal genes *apx-1* and *glp-1* and establishment of dorsal–ventral polarity in the early C. elegans embryo. Cell *77*, 95–106.