Genes Required for GLP-1 Asymmetry in the Early Caenorhabditis elegans Embryo

Sarah L. Crittenden,* David Rudel,† Joe Binder,* Thomas C. Evans,‡,¹ and Judith Kimble,*‡,§,²

*Howard Hughes Medical Institute, Departments of †Biochemistry and §Medical Genetics, and ‡Laboratory of Molecular Biology, University of Wisconsin - Madison, Madison, Wisconsin 53706

The translation of maternal glp-1 mRNA is regulated both temporally and spatially in the early Caenorhabditis elegans embryo (T. C. Evans, S. L. Crittenden, V. Kodoyianni, and J. Kimble, Cell 77, 183-194, 1994). To investigate the control of embryonic glp-1 expression, we have examined the distribution of GLP-1 protein in selected maternal effect mutants that affect pattern or fate in the early embryo. We find that mutants that disrupt anterior-posterior asymmetry in the early embryo (par-1–par-6, emb-8, Par(q537)) disrupt the spatial but not temporal control of GLP-1 expression: GLP-1 is observed at the normal stage of embryogenesis in par-like mutants; however, it is uniformly distributed. In contrast, mutants that alter blastomere identity (skn-1, pie-1, mex-1, apx-1) do not affect the normal GLP-1 pattern. We conclude that genes controlling the asymmetry of cellular components, including P granules, also control GLP-1 asymmetry in the early embryo. The finding that mutants that disrupt anterior-posterior asymmetry translate GLP-1 in all blastomeres suggests that loss of embryonic asymmetry causes translational activation of GLP-1 in the posterior.

INTRODUCTION

The specification of cell fates during metazoan development requires the precise temporal and spatial control of regulatory proteins. GLP-1, a transmembrane receptor related to Drosophila Notch, mediates several inductive interactions in the early Caenorhabditis elegans embryo (Priess et al., 1987; Austin and Kimble, 1987; Yochem and Greenwald, 1989; Austin and Kimble, 1993; Hutter and Schnabel, 1994, 1995; Mango et al., 1994a; Mello et al., 1994; Moskowitz et al., 1994; Shelton and Bowerman, 1996; see Fig. 1). The expression of GLP-1 protein from maternal glp-1 mRNA is controlled both temporally and spatially (Evans et al., 1994; see Fig. 1). Whereas glp-1 mRNA is uniformly distributed in both oocytes and early embryos (1 to 4 cells), GLP-1 protein is first seen at the 2-cell stage, in the anterior AB but not the posterior P1 blastomere; GLP-1 continues to be expressed in AB descendants through the 28-cell stage.

Both temporal and spatial aspects of the maternal GLP-1 pattern rely on translational control (Evans et al., 1994). Reporter mRNAs carrying the glp-1 3’ untranslated region (UTR) are expressed in a pattern similar to that of GLP-1. Furthermore, a small deletion of only 61 nt from the middle of the glp-1 3’UTR eliminated spatial control but left temporal regulation intact: the reporter was expressed at the correct stage but not in the right place (Evans et al., 1994). Because reporter RNA regulated by this mutant 3’UTR was expressed ectopically in posterior P1 descendants, we predicted the existence of a trans-acting repressor localized in the P1 blastomere (Evans et al., 1994).

We have begun to investigate the genetic control of GLP-1 asymmetry in the early C. elegans embryo. Such genes might regulate glp-1 mRNA specifically or they might be required more generally for establishing embryonic polarity. In either case, genes regulating the early embryonic pattern of GLP-1 are predicted to act maternally because GLP-1 asymmetry is visible early in embryogenesis, at the 2-cell stage. Furthermore, mutations in such genes are predicted to cause lethality because release of glp-1 mRNA from control by its 3’UTR results in lethality (T.E., unpublished).
In addition to GLP-1 asymmetry, several other asymmetries are established soon after fertilization in the early C. elegans embryo. The point of sperm entry determines the future posterior of the embryo (Goldstein and Hird, 1996). After fertilization, cytoplasmic rearrangements occur (Hird and White, 1993; Hird, 1996) and P granules, putative germ-line determinants, are localized to the posterior end of the zygote (Strome and Wood, 1982, 1983). Disruption of the actin cytoskeleton at this point has profound effects on the ability of the cell cortex to cleave properly and to produce specific tissue types (Hill and Strome, 1988, 1990). This period of cytoplasmic rearrangement preceding first cleavage is likely to be crucial for the asymmetric distribution of many regulatory proteins, including GLP-1. The first cleavage division splits the fertilized zygote into a larger anterior daughter (AB) and a smaller posterior daughter (Pp) (Fig. 1). AB and Pp differ in their molecular components as well as in their patterns of division and the types of tissues they generate (Laufer et al., 1980; Sulston et al., 1983; Cowan and Mclntosh, 1985; Priess and Thomson, 1987; see Guo and Kemphues, 1996, for review).

The maternal effect par genes (for partitioning defective) play a central role in controlling the earliest embryonic asymmetries (Kemphues et al., 1988; Kirby et al., 1990; Morton et al., 1992; Cheng et al., 1995; Guo and Kemphues, 1996). The phenotypes of par-1 to par-4 include disruption of the symmetry of the actin cytoskeleton, failure to localize P granules to the posterior, and loss of the normal asymmetric cleavage pattern. par-1 also disrupts the localization of a posterior determinant, SKN-1 (Bowerman et al., 1993). Three par genes have been characterized at the molecular level. PAR-1 is a serine/threonine kinase (Guo and Kemphues, 1995), PAR-2 protein contains a putative ATP-binding site and a zinc binding domain of the “RING finger” class (Levitan et al., 1994), and PAR-3 is a novel cytoplasmic protein (Etemad-Moghadam et al., 1995). PAR-1, PAR-2, and PAR-3 are localized in the 1-cell embryo soon after fertilization: both PAR-1 and PAR-2 localize to the posterior cortex (Guo and Kemphues, 1995; Boyd et al., 1996), while PAR-3 is found in the anterior cortex. PAR-3 is restricted to the anterior by PAR-2 (Cheng et al., 1995; Etemad-Moghadam et al., 1995), and PAR-2 and PAR-3, in turn, are required for localization of PAR-1 to the posterior (Etemad-Moghadam et al., 1995). The asymmetric distribution of PAR proteins in the cell cortex of the zygote is thought to play an important role in polarizing the embryonic cytoskeleton, which establishes multiple cellular and molecular asymmetries (Guo and Kemphues, 1996).

While the par genes play a broad role in specifying anterior–posterior asymmetry, a separate set of maternal genes specify the identities of individual blastomeres. SKN-1, a transcription factor found predominantly in the P1 blastomere at the 2-cell stage, is required for proper specification of one of the daughters of P1, called EMS (Bowerman et al., 1992, 1993; Blackwell et al., 1994). Localization of SKN-1 to P1 depends on both par-1 and mex-1 activity: in both mutants, SKN-1 is found ectopically in AB descendants (Bowerman et al., 1993; Mello et al., 1992). Activity of the pie-1 gene is required for specification of the P2 blastomere (Mello et al., 1992, 1996). In addition, GLP-1 signaling is abberant in both skn-1 and pie-1 mutants (Bowerman et al., 1992; Mello et al., 1994a; Shelton and Bowerman, 1996). Finally, APX-1, a transmembrane protein and potential ligand for GLP-1, is required for signaling from P2 to ABp (Mello et al., 1994a; Mello et al., 1994a; Mickey et al., 1996; Shelton and Bowerman, 1996).

To identify genes involved in regulating GLP-1 asymmetry, we analyzed GLP-1 in a broad spectrum of maternal effect lethal mutant embryos. GLP-1 is mislocalized in par mutants, but not in mutants with altered blastomere identity or defective GLP-1 signaling. In addition, we identified

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**FIG. 1.** GLP-1 translation is regulated both temporally and spatially in the early C. elegans embryo. Thick lines represent GLP-1 in the membrane, shading represents GLP-1 in the cytoplasm. GLP-1 translation is repressed in oocytes and 1-cell embryos (temporal repression). GLP-1 translation is repressed in Pp, ABp, and ABar descendants from the 2-cell stage (spatial repression). At the 4-cell stage, GLP-1 in ABar is thought to interact with APX-1 in Pp to promote ABp and ABar descendants to interact with an unknown ligand in MS to promote development of the anterior pharynx (Gen-dreau et al., 1994; Hutter and Schnabel, 1994; Moskowitz et al., 1994; Mickey et al., 1996; Shelton and Bowerman, 1996). At the 12-cell stage, GLP-1 in ABal descendants and ABar descendants is thought to interact with an unknown ligand in MS to promote development of the anterior pharynx (Gendreau et al., 1994; Hutter and Schnabel, 1994; Mango et al., 1994a; Mango et al., 1994b; Moskowitz et al., 1994). Arrows indicate inductive interactions.
C. elegans Strains and Maintenance

C. elegans strains were maintained as described by Brenner (1974). Mutants and strains used in this study were as follows: LGI, emb-6(hc65), emb-12(g5), emb-20(g27), zyg-2(b10), par-6(ze170)unc-13(e459)/ht2; LGII, emb-21(g31), emb-23(g99), zyg-9(b244), emb-7(g48), zyg-1(b1), rol-1(e91)mel-1(ze121)/mc1, rol-1(e91)mel-1(zu120)/mc1; LGIII, emb-5(hc61), emb-8(hc69), emb-13(h6), emb-16(g19), emb-25(g45), emb-30(g53), emb-33(g60), emb-1(hc62), emb-7(hc66), par-2(iz23)unc-45(e952)/sc1, par-2(iz5s), ion-1(e185)par-3(iz71)q1, ple-1(ze154)unc-25(e156)/q1; LGIV, emb-3(hc59), emb-11(g1), emb-26(g77), emb-31(g55), par-5(it122)unc-22(e96)/dn7t1, skn-1(ze67)/dn7t1; LGV, emb-4(ze60), emb-18(g21), rol-4(ze8)par-1/b274/dn7t1, dpy-21(e1428)par-4(it133)/oz7f2, par-4(it75s), apx-1(or-3)dpv-11(e224)/dn7t1. See text for references. Temperature sensitive mutants were grown at 15°C to maintain stocks; other strains were grown at 20°C unless noted otherwise.

Immunofluorescence

To detect GLP-1, we used a mixture of anti-EGFL, anti-LNG, and anti-ANK polyclonal antibodies (Crittenden et al., 1994); to detect P granules, we used either OICID4 or K76 monoclonal antibodies (Strome and Wood, 1983). Embryos and germ lines were stained as described (Evans et al., 1994). Secondary antibodies were labeled with FITC, LRC5, Cy3, or Cy5. Images were collected on Bio-Rad MRC1000 and 1024 laser scanning confocal microscopes. To stain temperature-sensitive mutants, L4 hermaphrodites were grown at 25°C for 16–24 hr after which embryos were processed for staining.

RESULTS

To identify genes required for proper GLP-1 expression in the early embryo, we examined a variety of maternal effect lethal (Mel) mutant embryos. In the wild-type adult germ line, GLP-1 is not detected in meiotic cells or maturing oocytes (Crittenden et al., 1994); in wild-type embryos, GLP-1 is not observed at the 1-cell stage and is found in AB descendants by the 4-cell stage (Evans et al., 1994). Some 2-cell embryos possess weak GLP-1 staining that also is restricted to AB, suggesting that translation of maternal glp-1 mRNA first occurs at the 2-cell stage in AB blastomeres. After the 4-cell stage, strong GLP-1 staining persists in AB descendants until the 28-cell stage (Evans et al., 1994), while weak GLP-1 is detected in membranes between certain P1 descendants beginning at the 8-cell stage (between E and MS and between E and P3, data not shown; R. Feichinger and R. Schnabel, personal communication).

To examine GLP-1 expression in Mel mutants, we stained mutant embryos derived from homozygous mutant hermaphrodites with anti-GLP-1 and anti-P granule antibodies. We refer to such embryos as “x embryos” where x represents the gene. For example, a par-1 embryo refers to a homozygous par-1 mutant embryo derived from a homozygous par-1 hermaphrodite.

To score spatial regulation of GLP-1, we examined GLP-1 in 4- to 15-cell embryos. Mutant embryos were scored as having a wild-type GLP-1 pattern if the levels of GLP-1 in AB and P1 descendants were similar to those in wild-type. Embryos were scored as having mislocalized GLP-1 if GLP-1 was present in P1 descendants at a level higher than that in wild-type embryos (see Materials and Methods for more details). To score temporal regulation of GLP-1, we looked for the presence or absence of GLP-1 in oocytes and 1-cell embryos, when it is not normally detected.

Mutations in par-1 and par-4 Always Disrupt GLP-1 Asymmetry

In par-1 embryos, the first cleavage division is symmetrical and P granules are not localized to P1, but instead are found in both AB and P1 (Kemphues et al., 1988; Guo and Kemphues, 1995). In par-4 embryos, the first cleavage often generates large AB and small P1 blastomeres as in wild-type, but P granules are distributed to both AB and P1 (Kemphues...
et al., 1988; Morton et al., 1992). We stained embryos homozygous for a strong par-1 allele, par-1(b274) (Kemphues et al., 1988) or for a strong par-4 allele, par-4(it33). All par-1 embryos (n = 30) and par-4 embryos (n = 30) mislocalized GLP-1.

In 100% of both par-1 and par-4 mutant embryos we detected GLP-1 in all blastomeres (Fig. 2). For par-1, all embryos had approximately equal levels of GLP-1 expression in all blastomeres (Fig. 2B). In embryos where P granules could be scored, they were distributed equally among the blastomeres (n = 4; Fig 2B; see also Kemphues et al., 1988; Guo and Kemphues, 1995). Adult germ lines (n = 9), oocytes (n = 9), and 1-cell embryos (n = 8) had a normal GLP-1 pattern. For par-4, 90% (27/30) had similar levels of GLP-1 in all blastomeres, while three 4-cell embryos had noticeably lower levels of GLP-1 in two blastomeres. P granules stained weakly and were present in all blastomeres (n = 4; Fig 2C; see also Kemphues et al., 1988). Adult germ lines (n = 20), oocytes (n = 12), and 1-cell embryos (n = 5) had a normal GLP-1 pattern.

Thus, both par-1 and par-4 are required for spatial but not temporal regulation of GLP-1 asymmetry. The time of onset of GLP-1 translation appears to be normal in these mutant embryos, and the presence of GLP-1 in P1 descendants is likely to reflect ectopic translation there.

**Mutations in par-2 and par-3 Often Disrupt GLP-1 Asymmetry**

Many par-2 and par-3 embryos segregate P granules properly to P1, at the first division; however, all of them exhibit synchronous and symmetric first cleavage divisions. The second cleavage planes differ between the two mutants (Kemphues et al., 1988; Cheng et al., 1995).

We stained embryos homozygous for the nonsense mutation par-2(lw32) (Leviton et al., 1994). Thirty-seven percent (12/32) mislocalized GLP-1 (Fig. 2F). Among embryos mislocalizing GLP-1, three 4-cell embryos had lower levels of GLP-1 in P1 descendants. In embryos where P granules could be scored, 86% (12/14) had P granules localized to P1 descendants (Fig. 2E), while 14% (2/14) had P granules in AB as well as P1 descendants (Fig. 2D; see also Kemphues et al., 1988). One embryo that had GLP-1 only in AB descendants also had a large number of P granules in both AB and P1 descendants (Fig. 2D), raising the possibility that anterior localization of GLP-1 may not depend on proper posterior localization of P granules. Adult germ lines (n = 7), oocytes (n = 7), and 1-cell embryos (n = 3) had normal distributions of GLP-1.

We stained embryos homozygous for the null mutant par-3(iit71) (Etemad-Moghadam et al., 1995). Seventy-seven percent (23/30) mislocalized GLP-1 (Figs. 2G, 2H, and 2I). Among embryos mislocalizing GLP-1, five 4-cell embryos had lower levels of GLP-1 in P1 descendants (Figs. 2G and 2H). In embryos where P granules could be scored, 75% had P granules in both AB and P1 descendants (Figs. 2G and 2H; see also Kemphues et al., 1988), while 25% had P granules only in P1 descendants. We also noticed two par-3 embryos with a very low level of GLP-1 in P1 descendants, but with P granules distributed equally in all four blastomeres (Fig. 2G). Adult germ lines (n = 14), oocytes (n = 13), and 1-cell embryos (n = 2) had normal distributions of GLP-1.

Thus, in par-2 and par-3 mutant embryos, GLP-1 is mislocalized in less than 100% of the embryos, indicating that the activities of PAR-2 and PAR-3 are not essential for GLP-1 asymmetry.

We also stained weak alleles of two par genes that are less well characterized. In par-5(ii121) mutant embryos, which have a phenotype similar to that of par-2 (K. Kemphues, personal communication), 14% (1/7) had mislocalized GLP-1. In par-6(zeu170) mutant embryos, which have a phenotype similar to that of par-3 (Watts et al., 1996), 5% (1/20) had mislocalized GLP-1.

**emb-8 Is Required for both GLP-1 and P Granule Asymmetry**

In an attempt to identify other Mel mutants affecting GLP-1 asymmetry, we screened a collection of 24 previously isolated temperature-sensitive strict Mel mutants (Wood et al., 1980; Miwa et al., 1980; Cassada et al., 1981) with anti-GLP-1 and anti-P granule antibodies. Of these only one, emb-8(hcr69) mislocalized GLP-1 (see below). Four other mutants arrested at the 1-cell stage with no detectable GLP-1 (emb-1(hc62), n = 3; emb-7(hc66), n = 3; emb-27(g48), n = 75; zyg-1(b1), n = 49), indicating that temporal control of GLP-1 expression is normal. However, since embryos arrested at the 1-cell stage, the asymmetric distribution of GLP-1 could not be scored. Finally, 19 localized GLP-1 properly (emb-3(hc59), n = 3; emb-4(hc60), n = 1; emb-5(hc61), n = 16; emb-6(hc65), n = 25; emb-11(g1), n = 26; emb-12(g5), n = 4; emb-13(g6), n = 6; emb-16(g19), n = 42; emb-18(g21), n = 4; emb-20(g27), n = 9; emb-21(g31), n = 9; emb-23(g39), n = 8; emb-25(g45), n = 22; emb-26(g47), n = 3; emb-30(g53), n = 2; emb-31(g55), n = 2; emb-33(g60), n = 10; zyg-2(b10), n = 16; zyg-9(b244), n = 13).

For emb-9(hc69) embryos, 68% (13/19) had a normal distribution of GLP-1 (Figs. 3A, 3B, and 3E). Thirty-two percent (6/19) of the embryos stained for GLP-1 in both AB and P1 descendants at the 4- to 12-cell stages (Figs. 3C and 3E). In all six of the 4-cell embryos that mislocalized GLP-1, the level of GLP-1 was higher in two of the four blastomeres, indicating that some asymmetry still exists (Fig. 3C). In those emb-8(hc69) embryos scored for both GLP-1 and P granules, 70% (7/10) had P granules only in P1 descendants (Fig. 3A) and 30% (3/10) had P granules in both AB and P1 descendants (Figs. 3B and 3E). Interestingly, in several 4-cell embryos, P granules were found either mostly in EMS (Fig. 3A) or in all blastomeres except EMS (Fig. 3B; also observed by L. A. Khan and S. Siddiqui, personal communication). In two 4-cell embryos, GLP-1 was localized properly to AB descendants even though P granules were present in
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Control of GLP-1 Asymmetry

GLP-1 P granules

A

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 & \text{% with GLP-1 in AB and P1 descendants} & \text{% with P granules in AB and P1 descendants} \\
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\text{emb-8} & 32 & 30 \\
 & n=19 & n=10 \\
\text{q537} & 39 & 37 \\
 & n=38 & n=27 \\
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GLP-1 P granules

A

B

C

D

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both AB and P$_1$ descendants (Fig. 3B). All blastomeres of many 4-cell emb-8 embryos had similar sizes and nuclei appeared to be at the same stage of the cell cycle, suggesting that emb-8 embryos may have synchronous and symmetrical early divisions like par mutants.

Screen for New Genes That Control GLP-1 Asymmetry

To identify new genes that control GLP-1 asymmetry, we screened for strict Mel mutants and stained them to examine GLP-1 expression. To determine whether mutants affected other embryonic asymmetries, we also stained with anti-P granule antibodies and noted relative size and orientation of blastomeres in the stained embryos.

We screened 4734 haploid genomes and isolated 182 strict maternal effect lethal mutants. GLP-1 was detectable in all 182 strict Mel's. Therefore, we did not identify any candidate activators of GLP-1 translation. However, 3/182 mutants expressed GLP-1 in both AB and P$_1$ descendants at the 4-cell stage. All three new mutants also mislocalized P granules and appeared to have altered early cleavage patterns (Fig. 3D and data not shown). GLP-1 was not detected in oocytes or 1-cell embryos of any of these mutants. Two of the three new mutants, q514 and q516 are new alleles of par-4 since they map to the approximate position of par-4 on chromosome V and fail to complement par-4.

The other mutant, q537, appears to be an allele of a new par gene. Par(q537) maps to chromosome II; none of the 6 known par genes is found on chromosome II. For Par(q537), 39% (23/38; Fig. 3E) had GLP-1 in both AB and P$_1$ descendants. Of these, 10 had less GLP-1 in P$_1$ descendants than in AB descendants (Fig. 3D). In those embryos in which P granule staining was scored, 37% (10/27) had P granules in both AB and P$_1$ descendants. P granules were often clustered in the middle of the embryo (Fig. 3D).

The lack of mutants that specifically disrupt GLP-1 asymmetry can be interpreted in several ways. One possibility is that only one or a few genes can mutate to this specific phenotype and that our screen was not large enough to recover these mutants. Alternatively, such a regulator may have a zygotic phenotype if it is also required for the proper regulation of GLP-1 (or other regulatory proteins) at other times or in different tissues.

Genes That Affect Blastomere Identity and/or GLP-1-Mediated Inductions Do Not Affect GLP-1 Level or Distribution

A number of maternal effect genes affect the identity of individual blastomeres at the 4-cell stage. In skn-1 and pie-
1 mutants, fates of the two P1 descendants, EMS and P2, are altered; in mex-1 mutants, AB fates are altered at least in part due to mislocalization of SKN-1. In addition, P granules are not segregated normally in mex-1 embryos (Melio et al., 1992; Schnabel et al., 1996). We stained 4- to 12-cell skn-1(zu67) embryos (n = 18; Fig. 4A), pie-1(zu154) embryos (n = 27; Fig. 4B), and embryos from two alleles of mex-1, mex-1(zu121) (n = 17; Fig. 4C), and mex-1(zu120) (n = 18; data not shown) and found that GLP-1 staining was similar to wild-type. Thus, the correct fates of AB and P1 descendants are not essential for asymmetric GLP-1 expression.

The apx-1 gene encodes a signaling ligand for GLP-1 that is used in P2 to induce ABp at the 4-cell stage (Mello et al., 1992). APD DB 8413 / 6x16h$$541 12-16-96 21:34:07 dbas

The Initial Establishment of Polarity Is Linked with the Establishment of GLP-1 Asymmetry

We have found that the activity of genes regulating many aspects of anterior-posterior asymmetry in the early embryo, par-1, par-6, emb-8, and Par(r537), that influence GLP-1 asymmetry in the early embryo and have shown that many others do not affect GLP-1 expression. Our results have a number of implications for the control of GLP-1 asymmetry in the early embryo, which are discussed below.

DISCUSSION

Evans et al. (1994) showed that maternal glp-1 mRNA is translationally regulated by elements in its 3′UTR to achieve an asymmetric expression of GLP-1 protein in the early embryo. In this paper, we have begun to identify genes required for GLP-1 asymmetry. Specifically, we have identified eight genes, par-1, par-6, emb-8, and Par(r537), that influence GLP-1 asymmetry in the early embryo and have shown that many others do not affect GLP-1 expression. Our results have a number of implications for the control of GLP-1 asymmetry in the early embryo, which are discussed below.

The Initial Establishment of Polarity Is Linked with the Establishment of GLP-1 Asymmetry

We have found that the activity of genes regulating many aspects of anterior-posterior asymmetry in the early embryo, par-1, par-6, emb-8, and Par(r537), are required for the proper spatial but not temporal regulation of maternal GLP-1 expression. In par mutant embryos, we observe extra GLP-1 expression in posterior blastomeres, indicating that GLP-1 can be translated in all blastomeres in the absence of par activity. In contrast, we have found that GLP-1 distribution in the early embryo is independent of both GLP-1-mediated signaling and the proper specification of anterior and posterior blastomere identity.

Several Individual Aspects of Embryonic Polarity Can Be Uncoupled from the Establishment of GLP-1 Asymmetry

Phenotypic differences among the par mutants indicate that GLP-1 asymmetry is not coupled to all early embryonic asymmetries. Par-1 and par-4 which strongly affect GLP-1 and P granule asymmetry only weakly affect cytoplasmic streaming, the position of pronuclear fusion, and the asymmetric distribution of actin in the 1-cell embryo (Kemphues et al., 1988; Kirby et al., 1990). In addition, the first division in par-4 mutants is asymmetric (Kemphues et al., 1988; Morton et al., 1992), indicating that proper placement of the first cleavage plane is not sufficient for proper GLP-1 asymmetry. In contrast, par-2 and par-3, which have a weaker effect on GLP-1 and P granule asymmetry, have strong effects on the other early embryonic asymmetries that par-1 and par-4 only affect weakly (Kemphues et al., 1988; Kirby et al., 1990). Thus, par-1 and par-4 play a central role in controlling the asymmetric distribution of proteins, including GLP-1, within the early embryo. In contrast, par-2 and par-3 appear to play a central role in cytoskeletal asymmetry.

A pathway for par gene activity has been proposed in which PAR-2 and PAR-3 are required for the proper localization of PAR-1 (Etemad-Moghadam et al., 1995). However, in contrast to PAR-1, PAR-2 and PAR-3 are not absolutely required for GLP-1 asymmetry. This dependence on PAR-1 suggests that sufficient PAR-1 activity remains in many par-2 and par-3 mutant embryos to allow proper regulation of GLP-1 asymmetry. Consistent with this, it has been proposed that PAR-1 localization may not be absolutely required for its activity (Boyd et al., 1996).

Temporal Regulation Can Be Separated from Spatial Regulation in the Early Embryo

The glp-1 3′UTR mediates both temporal and spatial regulation of GLP-1 translation in the early embryo (Evans et al., 1994). Deletion analysis of the 3′UTR raised the possibility that temporal and spatial regulation were mediated by different regions of the 3′UTR, possibly with separate regulators binding each element. Consistent with this idea, GLP-1 appears to be translated at the normal time in par mutants; however, its spatial asymmetry is lost. Alternatively, a single element in the 3′UTR may direct both temporal and spatial regulation, but temporal and spatial regulation may be controlled by distinct factors.

Translational Regulation of glp-1 in the Early Embryo: Repression or Activation?

Two simple ways to create GLP-1 asymmetry are (1) presence of a translational activator in the anterior of the embryo, or (2) presence of a translational repressor in the posterior of the embryo (see Fig. 5). In addition, temporal control of GLP-1 translation is necessary to ensure that GLP-1 protein is not synthesized in a 1-cell embryo and subsequently inherited by all embryonic blastomeres. The role of the par genes in GLP-1 translational control is not understood; however, because par gene activity is required for a wide range of embryonic asymmetries, it is likely that the main
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ules. We have also observed several embryos in which GLP-1 is properly localized, but P granules are found in both AB and P1 descendants. These results raise the possibility that proper P granule localization may not be absolutely required for proper regulation of GLP-1 asymmetry.

**Signaling Is Not Required for GLP-1 Expression in the Early Embryo**

Positive feedback does not appear to play a role in maintaining GLP-1 expression levels in the early embryo: neither active ligand nor active GLP-1 itself (S.C., unpublished) is required for proper expression. In contrast to the embryo, glp-1 activity is required for continued GLP-1 expression in the germ line (S.C., unpublished), and positive autoregulation has been suggested (Kodoyianni et al., 1992; Christensen et al., 1996; Berry and Schedl, in press). Positive feedback has also been proposed to maintain expression of a C. elegans GLP-1 homolog, LIN-12, in the larval somatic gonad (Seydoux and Greenwald, 1989; Wilkinson et al., 1994). While positive feedback does not appear to control levels of GLP-1 in 2- to 28-cell embryos, activity of maternal GLP-1 appears to activate LIN-12 expression in AB descendants later in embryonic development (Moskowitz and Rothman, 1996).

**Blastomere Identity Is Established after GLP-1 Asymmetry**

GLP-1 asymmetry is established early and requires the activity of the par genes. However, genes that establish blastomere identity do not affect GLP-1 asymmetry. For example, the presence of GLP-1 in mex-1(zu120) AB blastomeres indicates that even though these blastomeres contain SKN-1 (Bowerman et al., 1993) and produce tissue types typical of MS (Mello et al., 1992), they still regulate GLP-1 in an AB-specific manner. In addition, SKN-1 is neither necessary nor sufficient to repress GLP-1 expression. Therefore, SKN-1 specifies EMS identity after GLP-1 asymmetry is established and establishment of blastomere identity is not closely linked to regulation of GLP-1 asymmetry in the early embryo.

**Is There a Separate Pathway for Regulation of GLP-1 Asymmetry?**

The mechanisms for generating the asymmetric localization of certain regulators in the early embryo can be separated genetically. Thus, mex-1(zu120) affects SKN-1 but not GLP-1 asymmetry (Bowerman et al., 1993; this paper), and mex-3 affects PAL-1 asymmetry but not GLP-1, SKN-1, or APX-1 asymmetries (B. Draper and J. Priess, 1996; C. Hunter and C. Kenyon, 1996). Furthermore, par-2–6 may not affect GLP-1 and SKN-1 asymmetry to the same extent (B. Bowerman, personal communication; this paper). Thus, there are both global regulators of embryonic asymmetry, such as...
as par-1, and regulators affecting a more restricted group of asymmetries, such as mex-3. Specific regulators of GLP-1 asymmetry have not yet been identified, and their existence remains an open question for future investigation.

ACKNOWLEDGMENTS

We thank Deena Schuster, Phil Balandyk, Kim Oas, and Eileen Durkin for technical assistance, Ken Kemphues, Bruce Bowerman, Susana Guedes, Craig Mello, and past and present members of the Kimble lab for helpful discussions, Lisa Kadyk, Maria Gallegos, and the reviewers for comments on the manuscript, and Bruce Bowerman, Laura Berry, Tim Schell, Richard Fechtinger, Ralf Schnabel, L. A. Khan, and S. Siddiqui for sharing unpublished data. We also thank Ken Kemphues, Lynn Boyd, Bruce Bowerman, and especially the Caenorhabditis Genetics Center for sending strains. Anti-P granule monoclonal antibodies K76 and OICID4 were provided by the Developmental Studies Hybridoma Bank maintained by a contract from NICHD (N01-HD-2-3144). This work was supported by grants from NIH and NSF to J.K. and Wisconsin Alumni Research Foundation and NIH Molecular Biosciences Training grant fellowships for D.R. J.K. is an investigator with the Howard Hughes Medical Institute.

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Received for publication August 23, 1996
Accepted September 23, 1996