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An inductive interaction in 4-cell stage C. elegans embryos involves APX-1 expression in the signalling cell

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SUMMARY

During the 4-cell stage of C. elegans embryogenesis, the P₂ blastomere provides a signal that allows two initially equivalent sister blastomeres, called ABa and ABp, to adopt different fates. Preventing P₂ signalling in wild-type embryos results in defects in ABp development that are similar to those caused by mutations in the glp-1 and apx-1 genes, which are homologs of the Drosophila genes Notch and Delta, respectively. Previous studies have shown that GLP-1 protein is expressed in 4-cell stage embryos in both ABa and ABp. In this report, we show that APX-1 protein is expressed in the P₂ blastomere and that a temperature-sensitive apx-1 mutant has a temperature-sensitive period between the 4-cell and 8-cell stages. We propose that APX-1 is part or all of the P₂ signal that induces ABp to adopt a fate different than ABa.

Key words: APX-1, GLP-1, cell-cell interactions, cell-fate specification, Caenorhabditis elegans

INTRODUCTION

The glp-1/l-in-12/Notch genes encode transmembrane proteins that function as receptors in many different cell-cell interactions in C. elegans and Drosophila and related genes have now been found in a large number of animal species, reviewed in Artavanis-Tsakonas et al., 1995). Activation of the receptor NOTCH in Drosophila appears to occur through cell signalling by a second transmembrane protein called DELTA (Vassin et al., 1987; Kopczynski et al., 1988; reviewed in Simpson, 1995). The lag-2 gene of C. elegans encodes a transmembrane protein that is similar to DELTA; the LAG-2 protein appears to function as a ligand for both GLP-1 and LIN-12 in certain cell-cell interactions (Lambie and Kimble, 1991; Henderson et al., 1994; Tax et al., 1994; Wilkinson et al., 1994).

The cell-cell interactions mediated by these proteins can involve inductive signals between dissimilar cells, or lateral signals between initially equivalent cells. In C. elegans for example, glp-1 mediates the response of proliferating germ cells to an inductive signal from the distal tip cell (DTC) (Kimble and White, 1981; Austin and Kimble, 1987). In this interaction, glp-1 is expressed only in the responding germ cells and lag-2 is expressed only in the DTC (Crittenden et al., 1994; Henderson et al., 1994). lin-12 has been shown to mediate lateral signalling between two initially equivalent cells that then adopt distinct fates (AC or VU); the lin-12 and lag-2 genes initially are expressed in both of the interacting cells (Wilkinson et al., 1994).

During the first four cleavages of the C. elegans embryo, GLP-1 functions in at least two distinct cell-cell interactions (Priess et al., 1987; Hutter and Schnabel, 1994; Mello et al., 1994). The first interaction begins at the 4-cell stage, when there are two pairs of sister blastomeres called ABa and ABp, and EMS and P₂ (Fig. 1). In normal development, each of these blastomeres has a distinct pattern of cleavage and differentiation (Sulston et al., 1983). However, when ABa and ABp are born they initially are equivalent and interchangeable (Priess and Thomson, 1987). The P₂ blastomere appears to provide a signal during the 4-cell stage that causes ABp to adopt a fate that is different from ABa (Bowerman et al., 1992; Hutter and Schnabel, 1994; Mello et al., 1994; Mango et al., 1994; Moskowitz et al., 1994). A second interaction begins at the 12-cell stage when a daughter of EMS, called MS, provides a signal that influences the development of neighboring ABa descendants (Priess and Thomson, 1987; Hutter and Schnabel, 1994; Mango et al., 1994). We will refer to these stage-specific events as the 4-cell stage interaction and the 12-cell stage interaction.

GLP-1 is expressed on the surfaces of ABa and ABp and their descendants during the 4-cell stage and 12-cell stage interactions (Evans et al., 1994). Analysis of temperature-sensitive (ts) glp-1 mutants has demonstrated requirements for glp-1(+) activity during each of the two interactions (Hutter and Schnabel, 1994; Mello et al., 1994). Thus, the P₂ and MS blastomeres could function as signalling cells by expressing ligands for the GLP-1 receptor. Alternatively, ABp and ABa, or their descendants, might express GLP-1 and a GLP-1 ligand simul-
taneously; lateral interactions between these blastomerces could be influenced by unrelated signalling molecules on P2 or MS.

The apx-1 gene was identified in screens for maternal-effect lethal mutants (Mango et al., 1994; Mello et al., 1994). Mutations in apx-1 result in defects in ABp development that are very similar to those seen in wild-type embryos following the removal of P2, or in glp-1(ts) mutants exposed to restrictive temperature during the 4-cell stage interaction (Mello et al., 1994). The apx-1 gene encodes a transmembrane protein similar to the NOTCH ligand, DELTA, and the LIN-12/GLP-1 ligand, LAG-2 (Mello et al., 1994). These results have suggested that the APX-1 protein may function as a GLP-1 ligand during the 4-cell stage interaction. Only two mutations have been described that specifically affect the 12-cell stage interaction; these are both missense mutations in the glp-1 gene itself (Priess et al., 1987; Kodoyianni et al., 1992).

In this paper, we address the possible roles of apx-1 in the 4-cell stage and 12-cell stage interactions. We show a temperature-sensitive mutant has a single temperature-sensitive period (TSP) that coincides with the 4-cell stage interaction and the first embryonic TSP for glp-1(ts) mutants. We show that in 4-cell stage embryos apx-1 RNA is present in all blastomerces, while the APX-1 protein accumulates only in the signalling cell, P2. We have not detected APX-1 protein in the MS blastomere, which is the signalling cell for the 12-cell stage interaction. These results provide evidence that APX-1 is part or all of the inductive P2 signal in the 4-cell stage interaction. These results also suggest that MS signalling at the 12-cell stage does not involve APX-1.

MATERIALS AND METHODS

Strains and alleles

Strain N2 was used as the standard wild-type strain (Brenner, 1974). Genetic markers and balancers used were: linkage group III (LGGIII): pie-1(zu154), unc-25(e156), qCl. LGGV: unc-5(e53), him-8(ec56), LGG: apx-1(zu183), apx-1(zu347ts), dpy-11(e224), nT1. LGX: lon-2(e678).

Isolation of apx-1(zu347ts)

apx-1(zu347ts) was identified as a mutant with defective pharyngeal development and body morphogenesis in a collection of conditional embryonic lethals generated by K. Harris, M. Morrison and M. Roth (personal communication). apx-1(zu347ts) was mapped to LGV and was shown not to complement the transposon-induced allele apx-1(zu183::TcI) using the strain apx-1(zu183) dpy-11(e224) nT1. For the complementation test, apx-1(zu183) dpy-11(nT1) males were mated to homozygous apx-1(zu347ts) hermaphrodites at the permissive temperature. F1 progeny were shifted individually to 26°C at the L4 stage and scored for the production of viable progeny. Approximately one half of the F1 animals produced dead eggs with an apx-1 phenotype. To confirm that these F1 animals were cross-progeny, animals that segregated apx-1 dead eggs at 26°C were shifted down to the permissive temperature and allowed to lay viable progeny; about 1/4 of these F2 progeny were DPY, indicating that their parents were heterozygotes for dpy-11(e224).

Temperature-shift analysis

The temperature-sensitive period of apx-1(zu347ts) was determined by placing hermaphrodites homozygous for apx-1(zu347ts) dpy-11(e224) at the permissive (23°C) or non-permissive temperature (26°C) at the L4 stage for about 18 hours, during which time the larvae became adults and produced embryos. Embryos were then isolated and mounted on microscope slides, scored for cell number, then temperature-shifted to 23°C or 26°C. The embryos were allowed to develop and then scored for hatching.

The postembryonic temperature-sensitivity of apx-1(zu347ts) was examined by shifting newly hatched larvae to the permissive or non-permissive temperature on plates and then scoring them for the ability to produce eggs.

In situ hybridization

Whole-mount in situ hybridizations on embryos were performed as described (Seydoux and Fire, 1994, 1995). Digoxigenin (DIG)-labeled single-stranded DNA probes were synthesized by multiple cycles of primer extension in the presence of DIG-dUTP using an apx-1 cDNA clone (pJP606) as template as described by Patel and Goodman (1992) with the following modifications. Following synthesis, sense and anti-sense probes were purified using a Qiagen nucleotide removal kit and were eluted in 50 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) before dilution in 300 μl hybridization buffer (see Seydoux and Fire, 1995). No signal was detected with the sense probe (data not shown).

Generation of APX-1 antisera

A full-length apx-1 cDNA was cloned into pET16B (Novagen) and transformed into BL-21 (Novagen) cells. The APX-1 fusion protein was isolated on a polyacrylamide gel and the induced APX-1 band was gel purified. Rabbits from the Jackson laboratory were immunized with the purified APX-1 fusion protein, boosted monthly and bled 2 weeks after each boost. The affinity purification was achieved using nitrocellulose-bound antigen. Briefly, the APX-1 fusion protein was isolated on a polyacrylamide gel and blotted to nitrocellulose. The region of nitrocellulose containing the APX-1 fusion protein was identified by Ponceau S staining. The APX-1 fusion protein was identified by Ponceau S staining. The APX-1 antisera was absorbed onto the nitrocellulose overnight at 4°C. The APX-1-specific antibodies were removed from the nitrocellulose using 100 mM glycine, pH 2.5, and then dialyzed against phosphate-buffered saline (PBS), pH 7.4.

Immunofluorescence

Embryos were processed for staining with APX-1 antisera in the following manner. Adult hermaphrodites were cut open on polylysine-coated slides in 20 μl of PBS to release the gonads and embryos. The PBS was then replaced with 20 μl of fixative (4% paraformaldehyde, 60 mM Pipes, 25 mM Hepes [pH 6.8], 10 mM EGTA, 2 mM MgCl2) and the embryos were squashed with a coverslip as described previously (Bowerman et al., 1993). After 5 minutes in a moist chamber, the slides were frozen on a block of dry ice and left for 10 minutes. The coverslips were then removed and slides were placed in methanol at −20°C for 5 minutes, followed by 5 minutes in acetone at −20°C. The embryos were air dried and then blocked in Tris-Buffered saline (PBS), pH 7.5, 200 mM NaCl, 0.1% Tween. 3% BSA (bovine serum albumin) for 30 minutes at room temperature. Affinity-purified APX-1 antisera was diluted 1:20 in the blocking solution and 10 μl was added to the embryos. Slides were incubated with the primary antibody for 2-4 hours at room temperature followed by 6 hours or overnight at 4°C. The embryos were then incubated with rhodamine-conjugated goat anti-rabbit secondary antibody for 1-2 hours at room temperature. Following each antibody incubation, embryos were washed in Tris-Tween 3 times, 5 minutes each; the last wash contained 20 ng/mL DAPI (4′, 6-diamidino-2-phenylindole) to stain chromosomal DNA. Embryos were mounted in 70% glycerol for viewing with epifluorescence.

In addition to the APX-1-specific staining, APX-1 antisera also appeared to stain chromosome-associated structures in all embryonic cells in a cell-cycle-dependent manner (data not shown). This staining pattern persists in apx-1(zu183) mutant embryos, which lack all other staining. The zu183 mutation is an insertion of the transposon TcI into the 3′UTR of the apx-1 transcript and thus does not affect the apx-1-coding sequences.
Cortical granule tracings
To characterize the segregation of membranes during the early cleavages, granules associated with these membranes were observed in living embryos. N2 embryos were mounted on microscope slides at the 2-cell stage and video-recorded under Nomarski differential interference optics using a Hamamatsu CCD C2400 camera. Recordings were played back in real time and individual cortical granules were marked at the 4-cell stage. These granules were traced back to the 2-cell stage on acetate sheets mounted to the video screen. Granules that remained visible throughout the 2-cell to 4-cell cleavages were chosen for tracing.

RESULTS
An apx-1 (ts) mutant has a TSP between the 4-cell and 8-cell stages
apx-1(zu347ts) is a temperature-sensitive allele of apx-1 identified in a screen for conditional embryonic lethal mutations. At 23°C, the permissive temperature, adults homozygous for apx-1(zu347ts) produce viable embryos. At 26°C, the non-permissive temperature, these adults produce all inviable embryos. These inviable embryos are morphologically indistinguishable from those produced by non-conditional apx-1 mutants described previously (Mello et al., 1994; Mango et al., 1994).

Because APX-1 has been proposed to be a ligand for the GLP-1 receptor, we wanted to compare the embryonic TSP of the apx-1(zu347ts) mutant with the previously determined TSPs of glp-1(ts) mutants. glp-1(ts) mutants show two TSPs during embryogenesis, one between the 4-cell and 8-cell stages (corresponding to the 4-cell stage interaction) and a second between the 12-cell and 28-cell stages (corresponding to the 12-cell stage interaction) (Mello et al., 1994). To determine the embryonic TSP for apx-1(zu347ts), embryos from homozygous mothers were up-shifted (permissive to non-permissive) or down-shifted (non-permissive to permissive) at various times between the 1-cell and 28-cell stages of embryogenesis and later scored for hatching (Fig. 2; Table 1). We found that none of the embryos up-shifted at the 2-cell stage hatched and that these embryos had morphological abnormalities similar to those of non-conditional apx-1 mutant embryos. In contrast, 94% of the embryos that were up-shifted at the 12-cell stage hatched. In reciprocal experiments, 82% of the embryos down-shifted at the 2-cell stage hatched, while only 3% of the embryos down-shifted at the 12-cell stage hatched. These results define a single TSP for apx-1(zu347ts) between the 4-cell and 8-cell stages of embryogenesis (Fig. 2).

The TSP of apx-1(zu347ts) thus differs from the 4-cell stage interaction and that embryonic transcription of apx-1 is not detected in either the signalling or responding cells during the 12-cell stage interaction.

APX-1 protein is localized to the signalling cell
Rabbit polyclonal antiserum was generated against a bacterially expressed, full-length apx-1 fusion protein and affinity-purified. The staining patterns that we describe below were not observed with preimmune serum, nor were they observed in embryos from mothers homozygous for apx-1(zu183::Tc1), a transposon-induced allele. We thus believe that these staining patterns represent the distribution of the APX-1 protein.

APX-1 protein is detected first in late 2-cell stage embryos at the anterior periphery of P1, where P1 contacts the AB blastomere (Fig. 4A). Because APX-1 has a predicted membrane-spanning domain and is closely related to the membrane protein DELTA in Drosophila, we will describe this peripheral staining as representing plasma membrane localization. In approximately 75% of the embryos, APX-1 appears to be present uniformly across the anterior membrane of P1. However, the remainder of embryos show a gap in the staining pattern in one sector of the anterior P1 membrane. We do not know the significance of this non-uniform staining, nor whether the position of the gap is the same in all embryos, relative to the future position of blastomeres at the 4-cell stage. In addition to the peripheral, presumably membranous, staining pattern, APX-1 also is detected in small, numerous, punctate structures underlying the P1 membrane (Fig. 4A). We will describe this as cortical staining. A few punctate structures also are visible throughout the P1 cytoplasm. Similar punctate structures have been observed in Drosophila embryos stained with an antibody that recognizes the DELTA protein and may represent ligand-containing vesicles going to or from the membrane (Kooh et al., 1993).

In embryos where AB has completed division into ABA and ABp, and P1 is in the middle of division, the punctate APX-1-containing structures remain visible near the surface where P1 contacts ABA and ABp. However, membrane staining is visible only where P1 contacts ABp (Fig. 4D). In early 4-cell stage embryos, APX-1 staining becomes prominent in P2 at the anterior membrane where P2 contacts ABp and in punctate structures underlying this membrane (Fig. 4G). APX-1 also is detected as punctate structures in the cytoplasm of EMS (data not shown); these structures are no longer detected in slightly
Fig. 1. Early embryonic stages. The 2-cell stage embryo consists of an anterior blastomere, called AB, and a slightly smaller, posterior blastomere, called P1. At the next cleavage cycle, AB divides before P1, such that the AB daughters have separated by the time P1 is in metaphase. The AB spindle initially is oriented perpendicular to the long axis of the egg. As the AB spindle elongates, it pushes against the eggshell surrounding the embryo and is forced to rotate. This rotation causes the AB daughters, ABa and ABp, to make asymmetrical contacts with the two P1 daughters, EMS and P2; at the 4-cell stage, EMS contacts both ABa and ABp, while P2 (shown in red) contacts only ABp. By the 12-cell stage, ABa and ABp have undergone two synchronous divisions to produce the eight AB great-granddaughters. The anterior daughter of EMS is the MS blastomere undergone two synchronous divisions to produce the eight AB great-granddaughters. The anterior daughter of EMS is the MS blastomere. Arrows indicate the GLP-1-mediated 4-cell and 12-cell interactions. AB and its descendants are outlined in green to represent the localization of the receptor GLP-1 (Evans et al., 1994).

Fig. 2. The temperature-sensitive period of apx-1(zu347ts). The temperature-sensitive period of apx-1(zu347ts) was determined by up-shift (23°C to 26°C, solid line) and down-shift (26°C to 23°C, dashed line) experiments on embryos from mothers homozygous for apx-1(zu347ts) (See also Table 1). The TSPs for glp-1(ts) mutants are between the 4-cell and 8-cell stages and between the 12-cell and 28-cell stages (Hutter and Schnabel, 1994; Mello et al., 1994). apx-1(zu347ts) has a TSP very similar to the first TSP for glp-1(ts).

Table 1. Temperature-shift analysis of apx-1 (zu347ts)

<table>
<thead>
<tr>
<th>Stage</th>
<th>23°C→26°C</th>
<th>26°C→23°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-cell</td>
<td>0/27</td>
<td>27/33*</td>
</tr>
<tr>
<td>2-cell</td>
<td>0/34</td>
<td>26/35*</td>
</tr>
<tr>
<td>4-cell</td>
<td>7/48</td>
<td>30/44</td>
</tr>
<tr>
<td>8-cell</td>
<td>40/50</td>
<td>5/39</td>
</tr>
<tr>
<td>12-cell</td>
<td>30/32</td>
<td>1/36</td>
</tr>
<tr>
<td>28-cell</td>
<td>31/31</td>
<td>1/35</td>
</tr>
</tbody>
</table>

Embryos were isolated from mothers homozygous for apx-1(zu347ts) at 23°C (permissive) or 26°C (non-permissive), scored for embryonic stage, and shifted to 26°C or to 23°C, respectively. The embryos were allowed to develop, then scored for hatching.

*The few down-shifted 1-cell and 2-cell stage embryos that did not hatch did not resemble previously described apx-1 mutants. These embryos were nearly normal but appeared to have morphogenesis defects late in embryogenesis. The basis for this defect is not known.

Blastomere contacts during the 2-cell to 4-cell division

Several models could explain the relationship between APX-1 expression in the P1 and P2 blastomeres. For example, membrane-associated APX-1 in the P1 blastomere could be segregated during division to the nascent P2 blastomere. Alternatively, APX-1 in P1 could be degraded during division and re-synthesized only in P2. At present, we cannot follow the movement of APX-1 directly in the P1 membrane during division. However, it is possible to characterize the movement of granules closely associated with the membrane using video light microscopy (Hird and White, 1993). We define a blastomere’s cortex to be the cytoplasm underlying its surface membrane to a depth of approximately 1 μm, corresponding to a thickness of 2-3 cytoplasmic granules. We filmed dividing early embryos and numbered individual cortical granules at the 4-cell stage (Fig. 4C,F,I). Granules 1-6 in Fig. 4I correspond to the cortex of EMS that is adjacent to ABa and ABp, and granules 7-9 correspond to the cortex of P2 that is adjacent to ABp. These granules were then traced back to the 2-cell stage embryo (Fig. 4C). We found that granules 1-6 were originally in the P1 cortex adjacent to the AB blastomere and that granules 7-9 were originally in a region of the P1 cortex that was not adjacent to AB. Because the relative positions of granules at the 4-cell stage from mothers homozygous for pie-1(zu154) were fixed and stained for APX-1.

Table 2. Detection of APX-1 in P2 at the 4-cell stage in pie-1 mutants

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Hatching/total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>2/30</td>
</tr>
<tr>
<td>Cytoplasmic only</td>
<td>8/50</td>
</tr>
<tr>
<td>Faint membrane</td>
<td>4/30</td>
</tr>
<tr>
<td>Staining absent</td>
<td>16/30</td>
</tr>
</tbody>
</table>

Embryos at the 4-cell stage from mothers homozygous for pie-1(zu154) were fixed and stained for APX-1.
AB and P₁ interface remain fairly constant during division, we believe the cortical regions of AB and P₁ that were adjacent in the 2-cell embryo remain adjacent in 4-cell embryos as the junction between EMS and the AB daughters. Thus the cortical APX-1 staining in the P₂ blastomere appears to occur at a site on the cortex where no staining was visible in earlier, 2-cell stage embryos. In contrast, the cortical regions that stained positively for APX-1 in 2-cell stage embryos do not appear to stain in late 4-cell stage embryos. Therefore we suggest that most, if not all, of the APX-1 associated with the cortex of P₁ is partitioned to EMS at division and that APX-1 expression in P₂ may represent new synthesis or membrane targeting (see Discussion).

**pie-1 mutations affect APX-1 expression**

Previously described mutations in the **pie-1** gene have incompletely penetrant defects in ABp development, such that ABp can fail to produce the cell types that normally result from P₂ signalling (Mango et al., 1994; C. C. Mello, unpublished results). **pie-1(+)** activity could be required in ABp for the GLP-1-mediated response to P₂ signalling. However, **pie-1(+)** activity is known to be required for the correct specification of the fate of the P₂ blastomere and thus **pie-1** mutations could cause abnormal ABp development by disrupting P₂ signalling. Since our present study suggests that APX-1 is the P₂ signal, we used the APX-1 antiserum to stain embryos from mothers homozygous for **pie-1(zu154)** to determine if APX-1 was expressed and localized correctly at the 4-cell stage.

We found that in 93% (n=30) of the 4-cell stage embryos examined, APX-1 was either not detectable in P₂ or was present at reduced levels relative to wild-type embryos (Fig. 5, Table 2). In several of the 4-cell stage **pie-1(zu154)** mutant embryos (8 of 30), APX-1 was present in the cytoplasm of the P₂ blastomere in punctate granules similar to those observed in a wild-type EMS blastomere early in the 4-cell stage (Table 2). In most 2-cell stage **pie-1(zu154)** mutant embryos, APX-1 was not localized to the P₁ anterior membrane but could be detected faintly in the cytoplasm (data not shown). While it is unclear how **pie-1** affects the expression or localization of APX-1, we can conclude that the ABp defect in **pie-1(zu154)** mutants is likely to be the result of a disruption in the expression or maintenance of APX-1.

**DISCUSSION**

During the 4-cell stage of *C. elegans* embryogenesis, the ABp blastomere becomes different from its sister, ABa, through interactions with the P₂ blastomere. The specification of the ABp fate requires the wild-type activities of **glp-1**, which encodes a transmembrane receptor protein, and **apx-1**, which encodes a presumptive GLP-1 ligand (Austin and Kimble, 1989; Yochem and Greenwald, 1989; Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). In 4-cell stage embryos, GLP-1 protein is present in both ABp and ABa (Evans et al., 1994). In this study, we have shown that the APX-1 protein is present only in the P₂ blastomere, which contacts ABp but not ABa. Our analysis of an **apx-1(ts)** mutant indicates that this mutant has a single TSP between the 4-cell and 8-cell stages of embryogenesis. This TSP is very similar to the first embryonic TSP of **glp-1(ts)** mutants and corresponds to the
During the division of P₁ in the middle of division, the P₂ signals ABp. Finally, we have shown that pie-1 mutants, which can fail to specify the fate of ABp, have defects in the expression and localization of APX-1. These results together provide strong evidence that APX-1 is part or all of the P₂ signal.

**APX-1 localization**

At the 2-cell stage, APX-1 protein is present predominantly at the anterior periphery of the P₁ blastomere, which is the parent of both P₂ and EMS. We propose that most or all of this protein is partitioned to EMS and subsequently is degraded during the 4-cell stage. We have observed the anterior cortex of P₁, and the adjacent posterior cortex of AB, in living embryos during the division of P₁. We find that this region of contact appears to be maintained as P₁ divides, and that it becomes the junction between EMS and the AB daughters in 4-cell stage embryos. This model of cortical segregation is consistent with previous observations on the division of AB and P₁. During division of the AB blastomere, the confining eggshell forces ABp posteriorly, altering the cleavage plane of P₁. If the eggshell is removed, AB divides transversely and P₁ undergoes a simple anterior-posterior division; in such embryos, the anterior half of P₁ becomes EMS and the posterior half becomes P₂ (Hyman and White, 1987). Many of these initially ‘T-shaped’ embryos...
eventually establish contact between the ABp and P2 blastomeres, and develop normally (Wood and Kershaw, 1991; Schierenberg and Junkersdorf, 1992). Thus we consider it likely that most, or all, of the APX-1 protein at the anterior cortex of P1 can be partitioned to the EMS blastomere without affecting development. We observe numerous, punctate APX-1-containing granules in EMS that disappear during the 4-cell stage, consistent with the hypothesis that APX-1 is degraded in EMS.

During the 4-cell stage the level of APX-1 staining in the P2 blastomere increases although apx-1 mRNA is present uniformly in all 4-cell stage blastomeres. Therefore the asymmetric distribution of APX-1 protein may involve regulation at the level of translation, membrane targeting, or protein stability. It is possible that apx-1 mRNA is not translated in the EMS, ABa and ABp blastomeres, or that APX-1 protein is synthesized but degraded in these blastomeres before it accumulates to detectable levels.

Maternally expressed glp-1 mRNA also is present in all 2- and 4-cell stage blastomeres, although GLP-1 protein is detected only in AB and its daughters, ABa and ABp (Evans et al., 1994). Thus GLP-1 and APX-1 have nearly reciprocal patterns of expression. GLP-1 protein has been shown to be restricted to AB and AB descendants, by specific elements of the glp-1 mRNA (Evans et al., 1994). It will be of interest to determine how APX-1 is restricted to P1 descendants and whether this mechanism is coupled to, or independent from, the factors that regulate GLP-1 expression.

APX-1 asymmetry within the P lineage

APX-1 is localized asymmetrically at the anterior peripheries of the P1 and P2 blastomeres. This asymmetry may result from an intrinsic anterior-posterior polarity in these blastomeres. The par genes play a role in establishing the anterior-posterior polarity of the embryo and encode gene products that are asymmetrically distributed in the embryo (Cheng et al., 1995; Kemphues et al., 1988; Levitan et al., 1994; Morton et al., 1992). PAR-3 protein has been shown to be localized to the anterior cortex of the 1-cell embryo and to the anterior cortices of the P1 and P2 blastomeres in 2-cell and 4-cell stage embryos, respectively (Etemad-Moghadam et al., 1995). In contrast, the PAR-1 protein is localized to the posterior cortex of the 1-cell embryo and at the posterior cortices of P1 and P2 (Guo and Kemphues, 1995). APX-1 localization in P1 and P2 is similar to that of the PAR-3 protein and could be controlled by similar mechanisms.

Alternatively, the anterior localization of APX-1 within the P1 and P2 blastomeres could result from interactions with neighboring blastomeres. For example, GLP-1 protein in AB could play a role in localizing APX-1 toward the side of P1 that contacts AB. It should be possible to discriminate between these models by analyzing APX-1 localization in isolated P1 or P2 blastomeres or in glp-1 mutants.

The MS signal

At the 4-cell stage, the P2 blastomere functions as a signalling cell but its sister, EMS, does not. However, when EMS divides, one of its daughters, MS, becomes a signalling cell. Since both the P2 and MS signals activate GLP-1 in AB descendants, it seemed possible that the P2 and MS signals were identical or related proteins. Indeed, recent studies with chimeric embryos suggest that P2 can substitute for MS as a signalling cell in the 12-cell stage interaction (C. Shelton and B. Bowerman, personal communication). Although our results strongly suggest that APX-1 is the P2 signal, we find no evidence for APX-1 being the MS signal. Previous work suggested that the MS signal may require an embryonically transcribed component (Mello et al., 1994); we did not detect embryonic transcription of apx-1 in MS, though transcription was detected in other cells later in development. While APX-1 protein is present at high levels in P2 at the 4-cell stage, we have not been able to observe any APX-1 protein in the MS blastomere. Finally, we also have shown that apx-1(ts) mutants have only one temperature-sensitive period, corresponding to the 4-cell stage interaction. This apx-1(ts) mutation does not result in any apparent defects in MS signalling, nor do any of the non-conditional apx-1 mutations isolated to date (Mango et al., 1994; Mello et al., 1994).

The C. elegans genes apx-1, lag-2 and arg-1 can all encode closely related proteins, and appear to be functionally interchangeable (Mello et al., 1994; Henderson et al., 1994; Tax et al., 1994; Fitzgerald and Greenwald, 1995; the Genbank accession number for arg-1 is U50143). LAG-2 has been shown to function as a GLP-1 ligand in some cell-cell interactions in late embryonic and in postembryonic development, and the function of arg-1 has not been established (Lambie and Kimble, 1991; Henderson et al., 1994; Tax et al., 1994; Wilkinson et al., 1994). We have constructed a strain that produces embryos homozygous for chromosomal deficiencies deleting the apx-1, lag-2 and arg-1 genes simultaneously. Such embryos appear to have the normal 12-cell stage interaction, indicating that embryonic expression of these genes is not required for MS signalling (C. C. M., unpublished). It remains possible that maternal lag-2 or arg-1 functions in MS, or that MS signalling occurs through a fourth APX-1-related protein, or through a novel protein.

In summary, the fates of AB descendants are diversified rapidly during the first few cleavages of the C. elegans embryo. We have shown here that the 4-cell stage interaction involves two asymmetries. The first asymmetry is that the receptor GLP-1 and the ligand APX-1 are not expressed in the same blastomeres, preventing the types of lateral interactions observed with the AC/VU cells during larval development in C. elegans. The second asymmetry is that APX-1 is present in only one P1 daughter, in contrast to GLP-1 which is expressed in both AB daughters. This asymmetrical pattern of expression results in only one of the GLP-1-expressing daughters coming

*Fig. 5.* APX-1 is reduced or absent in the P2 blastomere of pie-1(zu154) mutants. Fluorescence micrograph of a 4-cell embryo stained for APX-1 (A) and a Nomarski micrograph of a similar stage to visualize cell membranes (B). APX-1 is not detected in the P2 blastomere of a pie-1(zu154)-4-cell embryo, consistent with the pie-1 phenotype of a loss of ABp-specific fates. (See also Table 2.)
into contact with ligand at the 4-cell stage, thus allowing the AB daughters to adopt different fates.

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REFERENCES


Mengo, S. E., Thorpe, C. J., Martin, P. R., Chamberlain, S. H. and Bowerman, B. (1994). Two maternal genes, apx-1 and pie-1, are required to distinguish the fates of equivalent blastomeres in the early Caenorhabditis elegans embryo. Development 120, 2305-2315.


