Establishment of POP-1 asymmetry in early *C. elegans* embryos

Frederick D. Park and James R. Priess

Division of Basic Sciences, Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Accepted 24 April 2003

SUMMARY

In *Caenorhabditis elegans* embryos, the nuclei of sister cells that are born from anterior/posterior divisions show an invariant high/low asymmetry, respectively, in their level of the transcription factor POP-1. Previous studies have shown that POP-1 asymmetry between the daughters of an embryonic cell called EMS results in part from a Wnt-like signal provided by a neighboring cell, called P2. We identify here additional signaling cells that play a role in POP-1 asymmetry for other early embryonic cells. Some of these cells have signaling properties similar to P2, whereas other cells use apparently distinct signaling pathways. Although cell signaling plays a critical role in POP-1 asymmetry during the first few cell divisions, later embryonic cells have an ability to generate POP-1 asymmetry that appears to be independent of prior Wnt signaling.

Key words: *C. elegans*, Frizzled, polarity, POP-1, Wnt

INTRODUCTION

The pattern of cell cleavage and differentiation in *C. elegans* is largely invariant (Sulston et al., 1983; Schnabel et al., 1997). The spindles of most cells are oriented such that mitosis generates an anterior daughter and a posterior daughter, and most of the early anterior/posterior (a/p) cell divisions result in sister cells with different fates. Although these fates are determined through diverse pathways, several studies have suggested that cells throughout the embryo recognize a common a/p coordinate system in choosing their fates (Mello et al., 1992; Hutter and Schnabel, 1994; Mango et al., 1994; Moskowitz et al., 1994; Way et al., 1994). Recent studies have provided considerable evidence for this hypothesis, and have begun to identify molecular components of the a/p system (Lin et al., 1995; Lin et al., 1998; Kaletta et al., 1997; Meneghini et al., 1999; Rocheleau et al., 1997; Rocheleau et al., 1997; Thorpe et al., 1997; Maduro et al., 2002). The transcription factor POP-1 is present in the nuclei of all early embryonic, and several postembryonic, cells. After an embryonic cell divides, its anterior daughter invariably shows a higher level of nuclear POP-1 than its posterior daughter. Thus each a/p pair of sister cells exhibits POP-1 asymmetry with a reproducible high/low polarity. Conditions that result in high POP-1 in the posterior sister cause an anterior transformation in fate, whereas the absence of POP-1 in the anterior sister causes a posterior transformation in fate. POP-1 functions in a variety of cell-type decisions, including mesodermal/endodermal choices and epidermal/neuronal choices. These observations suggest that POP-1 asymmetry provides the a/p coordinate system that collaborates with more widely expressed transcription factors to diversify sister cells.

POP-1 is related to TCF/Pangolin, a transcriptional effector of the canonical Wnt signaling pathway, and POP-1 has been shown to function in canonical Wnt signaling during larval development (Lin et al., 1995; Herman, 2001). However, POP-1 asymmetry in the early embryo is regulated by a non-canonical Wnt pathway, with parallel input from a mitogen-activated protein kinase (MAPK) pathway (for a review, see Korswagen, 2002). Components of these pathways include MOM-2/Wnt, MOM-5/Frizzled, WRM-1/beta-catenin, MOM-4/MAPKKK/TAK1 and LIT-1/Nemo. Sister cells show POP-1 asymmetry because they differ in their nucleo/cytoplasmic distributions of POP-1 (Maduro et al., 2002). Studies with cultured vertebrate cells suggest that WRM-1/beta-catenin can activate LIT-1/Nemo, resulting in phosphorylated POP-1 that accumulates in the cytoplasm (Rocheleau et al., 1999).

How is the a/p polarity system established? The most detailed experimental studies to date have focused on the development of the EMS cell (Goldstein, 1992; Goldstein, 1993). EMS divides into an anterior, mesodermal precursor and a posterior, endodermal precursor. This a/p polarity is induced during the 4-cell stage of embryogenesis by a neighboring cell called P2. For example, removing P2 causes both EMS daughters to have anterior fates (high POP-1) and repositioning P2 on the opposite surface of EMS reverses the polarity of the division.

Relatively little is known about the cellular events that establish a/p polarity in other embryonic cells. At the 2-cell stage, the posterior cell is called P1 (the parent of P2 and EMS) and the anterior cell is called AB (see Fig. 1). Within the AB lineage, POP-1 asymmetry is first detectable after the third division of AB, when there are four a/p sister pairs of AB descendants (Lin et al., 1998); for convenience we refer to this stage as the AB8 stage. POP-1 function is essential for a/p differences in cell fate within each of the four sister pairs of AB8 cells (Lin et al., 1998). Previous studies on how a/p differences are generated in the AB lineage have reached contradictory conclusions. In one set of experiments, AB was
separated from P1 and allowed to develop to the AB16 stage (Wittmann et al., 1997). As many as eight of the AB16 cells expressed a transgenic marker that normally is expressed in the eight posterior AB16 cells, suggesting that AB has an inherent a/p polarity. In a different study, videomicroscopy was used to follow AB development after killing P1 or P1 descendants (Hutter and Schnabel, 1995). Several AB6 cells showed posterior to anterior transformations in fate after killing P1, but not P1 descendants, suggesting that P1 induces an a/p polarity in AB that is maintained in a latent form until the AB8 stage.

To further analyze the cellular basis for a/p polarity in the AB lineage, we analyzed POP-1 levels directly by immunostaining isolated and cultured embryonic cells. Our results indicate that POP-1 asymmetry at the AB8 stage results from interactions with specific P1 descendants, rather than with P1. These interactions are mediated in part by MOM-2/Wnt signaling. Surprisingly, by the AB16 stage embryonic cells have acquired an ability to generate POP-1 asymmetry that appears to be independent of MOM-2/Wnt signaling or prior interactions with other cells, but that requires MOM-5/FRizzled.

Fig. 1. Early cleavages and POP-1 asymmetry. Schematic diagram of normal and devitellinized embryos from first cleavage to the AB8 stage; anterior is left in all drawings. Descendants of AB are shown in blue and descendants of EMS are shown in yellow. Sister cells are connected by short black lines. For some cells, the axis of the subsequent division is shown with either a double-headed arrow (division within the plane) or an asterisk (divisions into the plane). At the AB8 stage, levels of POP-1 are indicated as high (white [1:2500 mouse mABRL2 (P4G4) (Lin et al., 1998)]; midbody staining following dilutions of antibodies/antisera were used: anti POP-1 of intact embryos was as described previously (Lin et al., 1998). The following dilutions of antibodies/antisera were used: anti POP-1 [1:50 rabbit anti-PKL-2 (kindly provided by M. Land)]; P-granules [1:17000 rabbit anti-PGL-1 (Kawasaki et al., 1998)]. Secondary antibodies were conjugated to either Cy-3 (Jackson ImmunoResearch Laboratories) or FITC (Tago). Cells were stained with DAPI (4,6-diamidino-2-phenylindole) at 60 ng/ml for five minutes. POP-1 was scored only when the PKL-2 staining pattern provided an unambiguous indication of sister pairs, thus some results are scored by the number of sister pairs rather than by the number of experiments.

dsRNA-mediated interference (RNAi)
Standard techniques were used to synthesize double-stranded RNA (dsRNA) from T7 promoter-tagged, PCR-amplified genomic DNA for mom-2, mom-5, goa-1, and gpa-16. PCR primers were chosen to span exons and generate fragments between 0.5 and 2 kb in size. L4 or young adult hermaphrodites were soaked overnight with dsRNA (Nance and Priess, 2002).
1 asymmetry in all cells up to the 28-cell stage, and POP-1 asymmetry has been observed in E descendants and a few MS descendants in later embryos (Lin et al., 1998; Maduro et al., 2002). To identify sister cells in older embryos and in cell culture experiments, we used a POP-1 antibody in conjunction with an antiserum against the kinase PKL-2. This antiserum stains the midbody, or cell-division remnant, between sister pairs. For each of the sister pairs indicated in D (small arrows), the anterior sister has low POP-1. POP-1 staining is cell cycle-dependent and not detected in prophase or anaphase nuclei (large arrows in B,D) (see also Lin et al., 1995). In all panels anterior is left; in B,D and F ventral is down (arrowhead indicates the P1 cell).

**P1 descendants are required for POP-1 asymmetry at the AB8 stage**

In normal embryogenesis, POP-1 asymmetry is first evident in AB8 stage embryos. (A,B) Wild-type. (C,D) mom-2(or42) mutant. (E,F) mom-2(or42);mom-5(RNAi) embryo. Left panels show embryos stained for POP-1; chevrons indicate examples of sister cells. Right panels show DAPI and PKL-2 images superimposed; small arrows indicate examples of midbodies between sister pairs. For each of the sister pairs indicated in D (small arrows), the anterior sister has low POP-1. POP-1 staining is cell cycle-dependent and not detected in prophase or anaphase nuclei (large arrows in B,D) (see also Lin et al., 1995). In all panels anterior is left; in B,D and F ventral is down (arrowhead indicates the P1 cell).

AB descendants at the AB8 stage (Lin et al., 1998). To examine POP-1 in cultured cells, we removed the eggshell from 2-cell stage embryos and allowed the devitellinized embryos to develop to the AB8 stage (see Fig. 1 for a comparison of cell positions in normal and devitellinized embryos). POP-1 asymmetry was observed between most sister pairs of AB8 cells in devitellinized but otherwise intact embryos (78%, 32 sister pairs scored) and in embryos in which AB and P1 were separated then immediately recombined (78%, 32 sister pairs). POP-1 asymmetry was not detectable between any sister pairs of AB8 cells when AB was kept separate from P1 (0%, 56 sister pairs scored; Fig. 3A,B) or when AB was allowed to contact P1 only through the first cell cycle (0%, 44 sister pairs). These results indicate that signaling from P1 is not sufficient for POP-1 asymmetry between sister pairs of AB8 cells, and instead suggest that P1 descendants influence POP-1 asymmetry.

**AB cleavage orientation and P2 signaling**

In *C. elegans* and other animals, Wnt proteins are secreted and can signal non-adjacent target cells (Whangbo et al., 2000; Zecca et al., 1996; Neumann and Cohen, 1997). Because P2, a daughter of P1, expresses MOM-2/Wnt and signals EMS, in principle P2 might signal the AB descendants. However, a previous study showed that P2 is not essential for POP-1
asymmetry in the AB2 cells (Lin et al., 1998). We have confirmed this result using our modified conditions of cell isolation and culture; most sister pairs of AB2 cells show POP-1 asymmetry after P2 is removed from a 4-cell embryo (85%, 48 sister pairs). Moreover, P2 is not sufficient for POP-1 asymmetry; although P2 normally contacts one of the AB2 cells, called ABp, the daughters of ABp do not exhibit POP-1 asymmetry.

Why does P2 signaling fail to alter POP-1 levels in the ABp daughters? Because P2 has been shown to interact with ABp, but not EMS, through a separate, Notch-related signaling pathway, we wondered whether this pathway precluded POP-1 asymmetry. However, embryos that were depleted of the receptor GLP-1/Notch by glp-1(RNAi) did not show POP-1 asymmetry at the ABp division, similar to wild-type embryos (0/5). A second possibility is that the transverse divisions of the AB2 cells, perpendicular to P2, prevent POP-1 asymmetry. A previous study noted that EMS may fail to respond to induction if it divides perpendicular to, rather than in line with, P2, although this analysis is complicated by the fact that signaling from P2 normally orients the EMS division (Goldstein, 1995). To examine whether division orientation influenced POP-1 asymmetry, we used RNAi to inhibit the functions of the G alpha proteins encoded by goa-1 and gpa-16 (see Materials and Methods); such embryos appear to have random spindle orientations (Gotta and Ahringer, 2001; Zwaal et al., 1996). We observed that a/p, but not transverse, divisions of EMS resulted in POP-1 asymmetry (6/6 and 0/5, respectively; Fig. 4A). Similarly, a/p, but not transverse, divisions of the AB2 cells resulted in POP-1 asymmetry (4/4 and 0/4, respectively; Fig. 4A). When the functions of goa-1 and gpa-16 were inhibited in mom-2(or42) mutant embryos, POP-1 asymmetry was not detectable after either an a/p, or transverse, division of the AB2 daughters (0/12 and 0/6, respectively). As a second method for altering the AB2 division axis, a laser microbeam was used to fuse the AB2 daughters together immediately after their birth. As a fused AB2 cell enters mitosis, it often develops two largely separate spindles that orient a/p. After the tetrapolar division, the posterior daughters usually showed lower levels of nuclear POP-1 than the anterior daughters (16/18 sister pairs; Fig. 4C).

We used devitellinized embryos to further examine interactions between P2 and the AB2 cells. In devitellinized embryos, as in normal embryos, the division axes of the AB2 cells are oriented transversely with respect to the position of EMS (asterisks in Fig. 1). We found that the AB2 divisions remained approximately transverse after P2 was removed (32/32 embryos), or after a graft P2 was placed on various surfaces of one or both AB2 cells (22/22 embryos). We conclude that the presence or absence of P2 does not significantly alter the transverse division axis of an AB2 cell. When a graft P2 was positioned perpendicular to the predicted AB2 division axis (short red arrow in Fig. 1), POP-1 asymmetry was not observed between either pair of AB2 daughters (0/8 embryos). In the next set of experiments the graft P2 was placed in line with the predicted division axis of one of the AB2 cells (long red arrow in Fig. 1); we refer to this experimental paradigm as the AB2 polarization assay. In each of these experiments, the AB2 cell in contact with the graft P2 divided into daughters with POP-1 asymmetry (6/6; Fig. 4E,F).

The daughter distal to P2 had high POP-1 and the proximal daughter had low POP-1, similar to the high/low POP-1 polarity that P2 normally induces in the distal/proximal daughters of EMS. The AB2 cell that was not in contact with the graft P2 divided into daughters lacking POP-1 asymmetry (6/6). In a reciprocal experiment, a pair of AB2 cells was isolated and grafted onto the P2 cell of an intact, devitellinized host embryo (position indicated by blue arrow in Fig. 1). In this configuration, the P2 cell of the host embryo is in contact simultaneously with the host EMS and one of the graft AB2 cells, analogous to the pattern of cell contacts that P2 makes in normal 4-cell embryos. In each case in which the graft AB2 cell divided in line with P2, the daughters of that AB2 cell showed high/low POP-1 polarity with respect to P2 (5/5). Taken together, these results suggest that ABp in normal embryogenesis has the potential to respond to signaling from P2, and that interactions between P2 and EMS do not preclude P2 from simultaneously signaling ABp. Instead, the transverse
division of ABp appears to prevent it from responding to P2, such that POP-1 remains high in both ABp daughters.

**P1 descendants induce high/low POP-1 polarity**

The AB2 polarization assay described above (long red arrow in Fig. 1) was used to examine the signaling properties of several cells in the early embryo. We found that graft AB2, AB4, AB8, P1 and EMS cells could not induce POP-1 asymmetry in this assay (Table 1). In contrast, the P1 descendants E, C and P2 appeared equivalent to P2 in their ability to induce POP-1 asymmetry with high/low polarity (Table 1). The P1 descendant MS usually induced POP-1 asymmetry, however the asymmetry was markedly less than that observed with the other P1 descendants (data not shown).

Previous experiments by others have demonstrated that a signal from P2 aligns the EMS division axis and induces the proximal EMS daughter (E) to lengthen its cell cycle and to undergo endodermal differentiation (Goldstein, 1992; Goldstein, 1993). To further compare the signaling properties of MS, E and C with P2, each of these cells was grafted onto an isolated EMS cell. Similar to P2, both E and C were able to align the EMS division axis and to induce the proximal EMS daughter to lengthen its cell cycle and to undergo endodermal differentiation (6/7, 8/8 and 8/8 experiments, respectively; Fig. 5E,F). MS was unable to align the EMS spindle and did not induce endodermal-specific differentiation (0/9 experiments). We conclude that E, C and P2, and to a lesser extent MS, can induce POP-1 polarity, and that the signaling properties of E and C appear identical to those reported previously for P2.

MS, E and C are born during the AB4 stage and make extensive cell contacts with AB descendants (see below). Thus interactions at the AB4 stage might generate POP-1 asymmetry at the AB8 stage. We found that the daughters of an isolated AB4 cell did not show POP-1 asymmetry (Table 1). However, when an MS, E or C cell was grafted onto an isolated AB4 cell, the AB4 daughters showed high/low POP-1 polarity in almost all cases (Fig. 5G,H; Table 1). Surprisingly, in each experiment the spindle of the AB4 cell appeared to align with the signaling cell, in contrast to the spindles of AB2 cells. Grafting an AB4 cell onto a second AB4 cell never resulted in POP-1 asymmetry (Table 1), and these cells showed only infrequent, and probably random, alignment of their spindles (7/38 had aligned spindles). Thus, MS, E and C have the ability to align the AB4

![Fig. 5. Signaling by P1 descendants. (A-D) Nomarski photomicrographs showing the division of ABpr in (A) wild-type and (B) mom-2(or42) embryos, and the division of ABpl in (C) wild-type and (D) mom-2(or42) embryos; the asterisk indicates a division into the focal plane. (E) GFP expression of an endoderm-specific transgene after combining isolated EMS and C cells; DAPI staining of the same cells is shown in F. At the time of fixation the EMS daughter distal to C had divided twice, whereas the proximal EMS daughter divided only once. (G) POP-1 expression after the division of combined AB4 and MS cells; DAPI staining is shown in H. (I) POP-1 expression after the division of the AB4 cells in a devitellinized embryo. Prior to cell division, a graft E cell was placed on one of the AB4 cells. Note that AB4 daughter proximal to E has low POP-1. J shows superimposed DAPI and PKL-2 images with arrows indicating midbodies.

<table>
<thead>
<tr>
<th>Candidate</th>
<th>Assay*</th>
<th>Daughters with POP-1 asymmetry/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>AB2</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>AB4</td>
<td>0/7</td>
</tr>
<tr>
<td></td>
<td>AB8</td>
<td>0/13</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>EMS</td>
<td>0/11</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>4/4</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>5/6 (weak)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>3/3</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8/8</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0/12</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>3/6 (weak)</td>
</tr>
<tr>
<td>mom-2</td>
<td>AB4</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>6/7</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>12/13</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>15/15</td>
</tr>
<tr>
<td></td>
<td>(6 strong, 9 weak)</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>C</td>
<td>1/14</td>
</tr>
<tr>
<td>mom-2</td>
<td>AB4</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>9/9</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>6/7</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>12/13</td>
</tr>
<tr>
<td></td>
<td>MS</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>15/15</td>
</tr>
<tr>
<td></td>
<td>(6 strong, 9 weak)</td>
<td></td>
</tr>
</tbody>
</table>

*Assays using AB2 cells were on devitellinized wild-type embryos (AB2 polarization assay). All other assays were on individual wild-type cells as listed.
spindles and to induce high/low POP-1 polarity in the AB^4 daughters. The ability of AB descendants to respond to signaling persists until at least the AB^16 stage; isolated AB^16 cells that were combined with C divided into daughters with high/low POP-1 polarity relative to C (Table 1).

Videomicroscopy was used to examine the cell contacts and spindle orientations of the four AB^4 cells in live embryos (n=12); these cells are named ABal, ABar, ABpl, and ABpr. ABal contacts only MS, and its spindle normally orients toward MS. ABpr and ABpl contact MS, E and C simultaneously, however their spindles orient toward E and often move slightly toward E before cell division (Fig. 5A,C). Finally, ABar contacts C and MS, and its spindle is oriented approximately between C and MS. The observation that three of the AB^4 cells contact multiple signaling cells simultaneously suggests that the AB^4 cells distinguish, or integrate, the various signals. We used devitellinized 8-cell embryos to test whether an AB^4 cell could distinguish between signals from MS and E. In devitellinized embryos, the spindles of the AB^4 cells usually orient toward MS (see Fig. 1), and the AB^4 daughters that show POP-1 asymmetry invariably have high/low POP-1 polarity with respect to the position of MS. We therefore grafted an E cell onto one of the AB^4 cells of a host embryo at a site either opposite, or orthogonal, to the site of contact with MS (yellow arrow in Fig. 1). In each of six experiments, the spindle of the AB^4 cell aligned toward the graft E, rather than the host MS, and divided with high/low POP-1 polarity relative to the graft E (Fig. 5J). We conclude that signaling from E predominates over signaling from MS. Similarly, we infer that signaling from C may predominate over MS signaling in normal development because POP-1 polarity in ABar is high/low relative to C, rather than MS.

**MOM-2/Wnt and POP-1 asymmetry at the AB^8 stage**

Consistent with previous results by others, we found that mom-2 mutants at the AB^8 stage had a partial loss of POP-1 asymmetry between sister pairs of AB^8 cells (Table 2) (see also Lin et al., 1998; Meneghini et al., 1999). To examine the role of MOM-2/Wnt in signaling, we isolated individual cells from mom-2(or42) mutant embryos to use as graft cells in the AB^2 polarization assay with a wild-type host embryo. Graft P^2 or C cells from a mom-2(or42) mutant were unable to induce POP-1 asymmetry in this assay, whereas the MS cell sometimes induced weak POP-1 asymmetry (Table 1; the E cell adopts an MS-like fate in mom-2 mutants, and was not tested separately). We next grafted either a C or MS cell from a mom-2(or42) mutant onto an isolated, wild-type AB^4 cell. Similar to the above results, C did not align the AB^4 spindle (0/6) and did not induce POP-1 asymmetry between the AB^4 daughters (Table 1). In contrast, MS aligned the AB^4 spindle (15/15) and induced high/low POP-1 polarity with either a wild-type or reduced level of POP-1 asymmetry (Fig. 5G and Table 1). We conclude that MOM-2/Wnt is essential for signaling from C, but not MS. As described above, in wild-type development one AB^4 cell (ABar) contacts MS and C simultaneously, and the ABar daughters show high/low POP-1 polarity with respect to C. In mom-2(or42) mutant embryos, POP-1 asymmetry often was not detectable between the daughters of ABar (7/13 embryos). However, when present, the POP-1 polarity was reversed and oriented high/low with respect to MS (6/6). Thus, MS, rather than C, may orient the polarity of the ABar division in mom-2 mutant embryos.

Previous studies have described defects in the orientation of the ABar spindle in mom-2 mutant embryos (Rocheleau et al., 1997; Thorpe et al., 1997). We found that all of the AB^4 cells have variable defects in spindle orientation (Fig. 5B,D). For example, the ABpl spindle often aligned toward MS or midway between MS and E (Fig. 5D), whereas a wild-type ABpl spindle aligned toward E (Fig. 5C). When an MS, E or C cell from a mom-2(or42) embryo was grafted onto an isolated AB^4 cell from a second mom-2(or42) embryo, in nearly all cases the AB^4 spindle aligned toward MS or E (7/7 and 6/7, respectively) but only rarely aligned toward C (2/13). Thus, the AB^4 spindle defects in mom-2 mutant embryos appear to result from the absence of a signal from C, and from an inability to discriminate between competing signals from MS and E.

**Table 2. POP-1 asymmetry in mom mutant embryos**

<table>
<thead>
<tr>
<th>Sister pairs [% (n)]</th>
<th>mom-2(or42)</th>
<th>mom-5(RNAi)</th>
<th>mom-2(or42); mom-5(RNAi)</th>
<th>mom-4(or39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB^8</td>
<td>54 (63)</td>
<td>13 (16)</td>
<td>0 (24)</td>
<td>0 (32)</td>
</tr>
<tr>
<td>AB^16</td>
<td>95 (52)</td>
<td>65 (23)</td>
<td>0 (54)</td>
<td>0 (104)</td>
</tr>
<tr>
<td>AB^32</td>
<td>100 (101)</td>
<td>84 (55)</td>
<td>0 (24)</td>
<td>0 (144)</td>
</tr>
<tr>
<td>EMS^2</td>
<td>53 (15)</td>
<td>100 (7)</td>
<td>0 (7)</td>
<td>63 (19)</td>
</tr>
<tr>
<td>EMS^4</td>
<td>53 (40)</td>
<td>71 (35)</td>
<td>0 (13)</td>
<td>0 (30)</td>
</tr>
<tr>
<td>EMS^8</td>
<td>100 (42)</td>
<td>88 (8)</td>
<td>0 (30)</td>
<td>0 (104)</td>
</tr>
</tbody>
</table>

n, number of sister pairs examined.

**POP-1 asymmetry beyond the AB^8 stage**

The above results provide evidence that P_1 descendants play an important role in generating POP-1 asymmetry between the AB^8 sister pairs, a stage when POP-1 plays a critical role in specifying a/p differences in cell fate. Surprisingly, we found that if an isolated AB cell was allowed to develop to the AB^16 or AB^32 stages, most of the resulting sister pairs showed POP-1 asymmetry (80%, 45 sister pairs and 97%, 107 sister pairs, respectively; Fig. 3C,D). Although POP-1 asymmetry was never observed between the daughters of an isolated EMS cell (0%, 8 sister pairs), we often observed POP-1 asymmetry between the granddaughters and great-granddaughters (56%, 18 sister pairs and 95%, 40 sister pairs, respectively). Therefore AB and EMS descendants after the AB^8 stage have an ability to generate POP-1 asymmetry that appears to be independent of prior exposure to the signaling cells P^2, MS, E, P^3 or C.

We wanted to determine whether POP-1 asymmetry in sister pairs of AB^16 cells required signaling between AB descendants. For these experiments we separated AB from P^1, then separated each of the successive descendants of AB immediately after each cell division. The sequentially isolated cell was allowed to divide one additional time before staining the resulting sister pair for POP-1. As expected, POP-1 asymmetry was not observed between sequentially isolated sister pairs of AB^4 or AB^8 cells (0/11 and 0/10, respectively). In contrast, POP-1 asymmetry was usually present between sequentially isolated sister pairs of AB^16 and AB^32 cells (18/35 and 29/29, respectively; Fig. 3E,F).
AB8 cells usually showed robust POP-1 asymmetry (Table 3). The daughters and granddaughters of the sequentially isolated (Kadowaki et al., 1996; Rocheleau et al., 1997). We found that for secretion of MOM-2 and other Wnt family members *C. elegans* protein related to Prc, and should thus be required for POP-1 asymmetry, and some a/p sister pairs had low/high polarity. We sequentially isolated AB8 cells from *mom-2*(or42) embryos and from *mom-1*(or10); *mom-2*(RNAi) double mutant embryos, then allowed these cells to divide once or twice in culture before immunostaining for POP-1. The *mom-1* gene encodes the only *C. elegans* protein related to Prc, and should thus be required for secretion of MOM-2 and other Wnt family members (Kadowaki et al., 1996; Rocheleau et al., 1997). We found that the daughters and granddaughters of the sequentially isolated AB8 cells usually showed robust POP-1 asymmetry (Table 3). Thus, MOM-2/Wnt is not essential for POP-1 asymmetry after the AB8 stage, but has a role in POP-1 polarity.

We found that neither the daughters nor granddaughters of a sequentially isolated AB8 cell from a *mom-5*(or57) mutant embryo showed POP-1 asymmetry (Table 3). Similarly, an AB cell that was isolated from a *mom-5*(or57) mutant and allowed to divide to the AB16 or AB32 stages did not show POP-1 asymmetry between any sister pairs (0/8 and 0/4 experiments, respectively). These results suggest that MOM-5/Frizzled has a role in POP-1 asymmetry that is independent of MOM-2/Wnt signaling. Because sister pairs of AB16 or AB32 cells usually show POP-1 asymmetry in intact embryos that have been depleted of MOM-5/Frizzled by RNAi or by mutation, we examined intact embryos that were depleted of both MOM-2/Wnt and MOM-5/Frizzled. These latter embryos lacked POP-1 asymmetry in both AB and EMS descendants at the AB16 and AB32 stages (Fig. 2E,F; Table 2).

**Interactions resulting in low/high POP-1 polarity**

A wild-type, sequentially isolated AB16 cell can divide into a pair of sister cells with POP-1 asymmetry. However, when two adjacent AB16 cells were allowed to divide, their spindles almost invariably aligned to generate a line of two sister pairs with low/high-high/low POP-1 polarity (Fig. 3G). This was the case irrespective of whether two sequentially isolated AB16 cells were combined and allowed to divide once (19/21 experiments), or a sequentially isolated AB8 cell was allowed to divide twice (30/32). Identical results were obtained for the sequentially isolated daughters of the EMS cell (5/5). These results suggest that each parental cell aligned the spindle of the other, with a reciprocal induction of low/high POP-1 polarity. Thus, these cells induce low/high POP-1 in the distal/proximal daughters of the responding cell, in contrast to the high/low POP-1 induced by MOM-2/Wnt signaling.

We observed two additional examples in which low/high POP-1 polarity appeared to be induced in the distal/proximal daughters of a responding cell. First, experiments combining an AB4 cell with an MS, E or C cell, or combining an AB16 cell with a C cell, resulted in a high/low-high/low pattern of POP-1 polarity (Fig. 5G,H). This pattern is consistent with the hypothesis that the AB4 cell induced low/high POP-1 polarity in the MS daughters, whereas MS simultaneously induced high/low POP-1 polarity in the AB4 daughters. The second example involved POP-1 polarity in C and P3, the daughters of the P2 cell (see legend to Fig. 6 for details). In wild-type or *mom-2*(or42) mutant embryos, C is born proximal to ABp and has high POP-1, whereas P3 is distal and has low POP-1 (Fig. 6A, and see Fig. 1). In a devitellinized embryo, in which P2 does not contact ABp, we found that POP-1 polarity was reversed in the P2 daughters (Fig. 6C). We observed the same polarity reversal when isolated EMS and P2 cells were combined and allowed to divide (Fig. 6E). However, the normal pattern of POP-1 polarity was restored after ABp and ABa cells were grafted onto the P2 cell of a devitellinized wild-type host embryo (position of blue arrow in Fig. 1). This was the case irrespective of whether the graft ABp and ABa cells originated from wild-type embryos (10/10), or from *mom-2*(or42) mutants (5/5; Fig. 6G). Thus, AB2 cells can induce low/high POP-1 polarity in the P2 daughters.

We examined whether MOM-2/Wnt, MOM-1/Prc, MOM-5/Frizzled or MOM-4/MAPKKK are required for the polarized division of adjacent AB16 cells (the granddaughters of a sequentially isolated AB8 cell; Table 3). The *mom-2* and *mom-1* mutant cells usually had aligned spindles and low/high-high/low POP-1 polarity. In contrast, the cells from *mom-5* or *mom-4* mutants did not show POP-1 asymmetry. The *mom-4* mutant cells usually aligned their spindles, however the *mom-5* mutant cells did not (Table 3). We conclude that MOM-5/Frizzled and MOM-4/MAPKKK, but not MOM-2/Wnt, play a role in low/high POP-1 polarity.

**DISCUSSION**

The a/p sister cells in early *C. elegans* embryos have an
invariant high/low POP-1 polarity that is propagated in successive cell divisions. The establishment of this simple pattern appears to involve a complex series of cell interactions. As the 4-cell embryo divides, the EMS spindle is aligned with P2, and P2 signaling results in low levels of nuclear POP-1 in the daughter of EMS proximal to P2. The AB2 cells, called ABa and ABp, divide perpendicular to P2 and do not respond to P2 signaling. We have shown that an AB2 cell can respond to signaling if the AB2 spindle and P2 are aligned, either by altering the position of P2 or by altering the orientation of the AB2 spindle. In normal development the perpendicular, left/right division of ABp produces daughters with very similar fates, and the parallel differentiation of these left/right daughters is a major source of bilateral symmetry in C. elegans (Sulston et al., 1983). If the ABp spindle were to align with P2, the a/p division would result in daughters with different levels of POP-1 and presumably different fates.

At the next round of cell division, each AB4 cell divides into daughters with POP-1 asymmetry, as do the subsequent AB8 and AB16 cells. Previous studies reached contradictory conclusions about whether asymmetry in the AB lineage was determined intrinsically or through cell interactions (see Introduction). Our present study provides a resolution of this paradox. We observed POP-1 asymmetry in sister pairs of AB16 cells, but not AB8 cells, which were derived from an isolated AB. These results are consistent with those from a previous study that killing P1 caused posterior to anterior fate transformations at the AB8 stage; POP-1 remains high in all of the AB8 cells after P1 is removed, so all cells should adopt anterior fates (Hutter and Schnabel, 1995). Our results also support the study of Wittmann et al., which suggested AB descendants had an intrinsic asymmetry independent of P1; their study used a transgenic marker that is expressed at the AB16 stage (Wittmann et al., 1997). Thus, cell interactions are essential for POP-1 asymmetry at, but not after, the AB8 stage. Reported examples of AB8 cells that correctly adopted posterior fates after killing or removing P1 may represent cells in transition between the two modes of generating POP-1 asymmetry (Gendreau et al., 1994; Hutter and Schnabel, 1995).

**POP-1 asymmetry at the AB8 stage**

Although we propose that cell interactions determine POP-1 asymmetry in the early AB lineage, our results argue against a previous model that the primary interaction is between AB and P1 (latent polarity model), rather than between AB descendants and P1 descendants (Hutter and Schnabel, 1995). We have shown that exposing AB to P1 is not sufficient to generate POP-1 asymmetry at the AB8 stage, and that P1 does not provide signaling activity in our assays. In the previous study, laser irradiation of the P1 descendants did not prevent a/p polarity at the AB8 stage, suggesting that the P1 descendants were not essential for polarization (Hutter and Schnabel, 1995). However, laser-irradiation does not effectively prevent P2 from signaling EMS, nor does it prevent P2 from signaling ABp through a separate, Notch-related signaling pathway (Mello et al., 1994). All of the known genes that are involved in POP-1 asymmetry in the early embryo are expressed maternally, as are the components of the Notch pathway. Thus, it may be difficult to eliminate translation of maternally provided mRNAs by laser-irradiating the early embryonic cells.

The AB4 cells contact one or more of the P1 descendants MS, E, C and P3. We have shown that each of these P1 descendants can induce high/low POP-1 polarity in AB descendants, similar to the ability of P2 to induce high/low POP-1 polarity in the daughters of EMS. Indeed, at least two of the P1 descendants, E and C, appear to be fully equivalent to P2 in their ability to polarize EMS. C and P2 require MOM-2/Wnt for signaling, however the MS cell appears to provide Notch-related signaling pathway (Mello et al., 1994). All of the known genes that are involved in POP-1 asymmetry in the early embryo are expressed maternally, as are the components of the Notch pathway. Thus, it may be difficult to eliminate translation of maternally provided mRNAs by laser-irradiating the early embryonic cells.
the signaling cells P2, MS, E or C in cell culture experiments. This difference might result from the synthesis of new regulators in the AB4 cells, or from the degradation of inhibitors present in the AB2 cells. In normal development each AB4 division results in an anterior/posterior pair of daughters. However, all of these divisions are oblique, and some are nearly transverse, with respect to the a/p axis of the egg (see Fig. 5A,C). The alignment of the AB4 spindles with posterior-localized signaling cells such as E provides a partial explanation for the a/p pattern of high/low POP-1 polarity at the AB8 stage. In addition, the ability of MS to align the ABal spindle may be crucial for the normal pattern of Notch-mediated interactions. MS can interact with the ABal daughters through a Notch-like signaling pathway, and the alignment of the ABal spindle by MS ensures that only one daughter undergoes this interaction (Mello et al., 1994; Hutter and Schnabel, 1994).

Detailed cell-killing experiments on early C. elegans embryos have revealed unexpected and surprisingly complex networks of positive and negative interactions that are involved in the specification of muscle fates and that are presumably integrated for proper development (Schnabel, 1994; Schnabel, 1995). Although the molecular basis of muscle specification is not yet understood, our present study shows that the embryo contains multiple cells with the potential to influence POP-1 polarity. In our cell culture experiments, signaling from E predominated over signaling from MS when an AB4 cell simultaneously contacted both E and MS. Similarly, we propose that signaling from C predominates over signaling from MS in polarizing the division of ABar. The polarity of POP-1 at the ABar division is reversed in mom-2 mutants, presumably because of the absence of a signal from C and the persistence of a non-MOM-2/Wnt signal from MS. An analogous example was found in a previous analysis of the C. elegans Wnt family member, EGL-20, which functions in the asymmetric division of a larval epidermal cell called V5. In the absence of EGL-20/Wnt, the normal asymmetry of the V5 division is reversed by signaling from a neighboring cell (Whangbo et al., 2000).

**POP-1 asymmetry after the AB8 stage**

By the AB16 stage, the descendants of both AB and EMS have an ability to generate POP-1 asymmetry that appears to be independent of prior contact with signaling cells that express MOM-2/Wnt. This conclusion is based on experiments in which cells were sequentially isolated in culture, but is supported by the observation that intact mom-2;mom-5 mutant embryos show robust POP-1 asymmetry between sister pairs at the AB16 and later stages. Although sequentially isolated AB16 cells can divide with POP-1 asymmetry, contact with other AB descendants can determine POP-1 polarity. These interactions induce low/high POP-1 polarity, in contrast to the high/low polarity induced by MOM-2/Wnt signals. Because adjacent AB16 cells divide with mirror image POP-1 polarity, we assume that each cell can both signal and respond to signaling. Thus, a single, isolated AB16 cell may generate POP-1 asymmetry through self-signaling.

MOM-5/Frizzled is essential for POP-1 asymmetry in isolated cells that have not been exposed to MOM-2/Wnt signaling. Therefore MOM-5/Frizzled may be a component of the signaling pathway that generates low/high POP-1 polarity independent of MOM-2/Wnt. Drosophila Frizzled is an essential component of the planar cell polarity pathway, however the role of Wnt proteins has not been determined (Lawrence et al., 2002). It will be of interest to determine whether other genes involved in Drosophila planar cell polarity have functions in low/high signaling in C. elegans. MOM-4/MAPKKK and proteins such as LIT-1/Nemo and WRM-1/Beta-catenin are essential for POP-1 asymmetry in AB descendants, and thus appear to be core components of the asymmetry-generating machinery (this study) (Kaleta et al., 1997; Lin et al., 1998; Meneghini et al., 1999; Rocheleau et al., 1997; Rocheleau et al., 1999).

The observation that POP-1 asymmetry is present in intact mom-5 mutant embryos, but not in mom-2;mom-5 double mutant embryos, suggests that MOM-2/Wnt signaling can induce POP-1 asymmetry independent of MOM-5/Frizzled. These results support the view from previous genetic studies that MOM-2/Wnt and MOM-5/Frizzled have overlapping, but distinct roles in the early embryo (Rocheleau et al., 1997; Schlesinger et al., 1999). A survey of gene expression patterns in C. elegans embryos has detected mRNAs corresponding to at least two additional Frizzled-related proteins that are candidate receptors for MOM-2/Wnt (Y. Kohara, personal communication; http://nematode.lab.nig.ac.jp/). However, our present study indicates that these Frizzleds cannot be functionally redundant with MOM-5 for the POP-1 asymmetry shown by isolated, cultured cells.

When two adjacent AB8 cells divide in culture they generate a line of two sister pairs with low/high-low/low POP-1 polarity. In an intact, normal embryo, similarly oriented divisions of adjacent AB8 cells would be expected to produce sister pairs with high/low-high/low POP-1 polarity. Thus, the behavior of the isolated cells does not reproduce the normal pattern of POP-1 polarity. Among the cell culture experiments described here, the only condition that resulted in high/low-high/low POP-1 polarity involved combining a low/high signaling cell (AB4) with a high/low signaling cell such as MS, E or C. Thus, it is possible that the normal pattern of POP-1 involves two distinct signaling pathways. We have shown that isolated AB descendants remain responsive to MOM-2/Wnt signaling until at least the AB16 stage, however we do not yet know whether Wnt signals persist in normal embryos at the AB16 and later stages.

We are indebted to Marianne Land and Charles S. Rubin for providing the PLK-2 antiserum prior to publication. We thank Rafal Ciosk and Jeremy Nance for a critical reading of the manuscript, Russell Hill for stimulating interest in this project, and current members of the Priess laboratory for advice and assistance. Some of the nematode strains used in this study were provided by the Caenorhabditis Genetics Center, which is funded by the NIH National Center for Research Resources (NCRRR). F. Park is supported by the Pontzin Scholarship Fund, and J. Priess is supported by the Howard Hughes Medical Institute.

**REFERENCES**


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


