

# POP-1 and Anterior–Posterior Fate Decisions in *C. elegans* Embryos

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## Summary

Blastomeres in *C. elegans* embryos execute lineage programs wherein the fate of a cell is correlated reproducibly with the division sequence by which that cell is born. We provide evidence that the *pop-1* gene functions to link anterior–posterior cell divisions with cell fate decisions. Each anterior cell resulting from an anterior–posterior division appears to have a higher level of nuclear POP-1 protein than does its posterior sister. Genes in the *C. elegans* Wnt pathway are required for this inequality in POP-1 levels. We show that loss of *pop-1*(+) activity leads to several types of anterior cells adopting the fates of their posterior sisters. These results suggest a mechanism for the invariance of blastomere lineages.

## Introduction

During the first few cleavages of the *Caenorhabditis elegans* embryo, localized expression of factors that regulate transcription or that mediate cell–cell interactions results in each blastomere acquiring a distinct “identity,” or potential to differentiate (for reviews, see McGhee, 1995; Kemphues and Strome, 1997; Schnabel and Priess, 1997). Each blastomere then executes a unique and nearly invariant lineage, producing numerous cell types through a series of predominantly anterior/posterior (a/p) cleavages. Because blastomere lineages are essentially invariant, this means that patterns of cell division are correlated reproducibly with specific patterns of cell differentiation. For example, in the lineage of a blastomere called MS, the MS descendant born from the division sequence p-a-a-p-p invariably undergoes programmed cell death or apoptosis; none of the other MS descendants born at the same time, but from different division sequences, undergo apoptosis (Sulston et al., 1983).

Within a lineage, how is cell type differentiation reproducibly matched with division sequence? Invariant cleavage patterns could place cells consistently in the same position with respect to determinative environmental signals in the embryo. However, several studies have shown that, after about the 12-cell stage of embryogenesis, blastomeres have remarkable abilities to

execute their normal lineages even after neighboring blastomeres are killed or removed (Sulston et al., 1983; Junkersdorf and Schierenberg, 1992; Mello et al., 1992; Hutter and Schnabel, 1995). For example, the MS descendant born from the division sequence p-a-a-p-p undergoes apoptosis even if every blastomere except for MS is killed (Mello et al., 1992). Thus, in some lineages, cell fates do not appear to be determined by external, environmental cues within the embryo.

Despite the different molecular mechanisms that establish blastomere identities and the very different lineages that blastomeres execute, there is suggestive evidence that cells in all parts of the embryo share a mechanism for recognizing the division sequences by which they were born. Several mutants have been identified that result in the mislocalized expression, or activity, of factors that normally control blastomere identity. For example, in *mex-1* mutants, a transcription factor involved in specifying the MS identity is expressed inappropriately in several blastomeres located in different positions in the embryo; each of these blastomeres now produce mesoderm like a wild-type MS blastomere (Mello et al., 1992; Bowerman et al., 1993; Schnabel et al., 1996). These blastomeres also partly reproduce the MS lineage; cells born from the p-a-a-p-p division sequence can undergo apoptosis, as in the wild-type MS lineage. Thus, cells in several regions of the embryo appear to recognize that they were born from this specific division sequence and, in the presence of the proper transcription factor, respond in a similar manner. Analogous examples of this phenomenon have been described in mutants in which blastomere identities are changed by inappropriate cell–cell interactions (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994; Moskowitz and Rothman, 1996). These observations raise the possibility that within a lineage, cell differentiation may somehow be influenced directly by division sequence.

In the present study, we provide evidence that the *pop-1* gene is part of a general system for transducing information about division sequences into changes in the cell nucleus that affect differentiation. The *pop-1* gene was identified originally because of its role in the development of the MS blastomere (Lin et al., 1995). MS normally produces mesodermal tissues, and its sister E produces only endoderm. In a *pop-1* mutant, MS adopts an E-like fate and produces endoderm.

A signaling pathway similar to the Wnt pathway of vertebrates and *Drosophila melanogaster* has been shown to be required for MS and E to have different fates (Goldstein 1992, 1993; Rocheleau et al., 1997; Thorpe et al., 1997). In models for Wnt signaling, reception of the Wnt signal results in the nuclear localization of a beta-catenin such as the Armadillo protein in *Drosophila*; a *C. elegans* homolog WRM-1 is required for MS and E differences, but the localization of WRM-1 has not yet been determined. Once in the nucleus, beta-catenin is thought to interact with constitutive nuclear proteins such as Tcf-1 in vertebrates or the related dTcf-1 in *Drosophila* to regulate transcription; the POP-1 protein

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in *C. elegans* has sequence similarity to Tcf-1 and acts downstream in the Wnt-like pathway.

A polyclonal antiserum raised against the POP-1 protein shows a slightly lower level of staining in the E nucleus than in the MS nucleus in most, but not all, *C. elegans* embryos (Lin et al., 1995). We describe here a monoclonal antibody specific to the POP-1 protein that shows different levels of nuclear staining in almost all a/p pairs of sister blastomeres in the early embryo, including the MS/E pair. In each of these a/p pairs, we detect a higher level of POP-1 staining in the anterior sister than in the posterior sister. We show that loss of *pop-1(+)* activity causes several anterior cells to adopt fates similar to the fates of their posterior sisters. These studies show that the Wnt-like signaling pathway is required for generating or interpreting a/p polarity throughout the early *C. elegans* embryo and that POP-1 appears to be part of a general mechanism that couples division sequence to different patterns of gene expression in sister cells born from a/p cleavages.

## Results

### POP-1 Asymmetry in A/P Pairs of Sister Cells

An antiserum to POP-1 described previously gave variable immunostaining results and had a reactivity to prophase nuclei that appeared to be nonspecific but could not be removed by affinity purification (Lin et al., 1995). To better define the pattern of POP-1 localization, we generated monoclonal antibodies against the POP-1 protein (Experimental Procedures). Immunolocalization results described in this paper are with mABRL2.

Cell divisions during *C. elegans* embryogenesis are either anterior/posterior (a/p) or transverse (dorsal/ventral or left/right). Because of steric factors in the embryo, such as the eggshell, sister blastomeres resulting from a transverse cleavage may ultimately adopt slightly different a/p positions in the embryo; we thus describe a division as a/p or transverse on the basis of the initial spindle position (Figure 1). To identify POP-1-containing cells in fixed embryos after immunostaining, we compared their patterns of DAPI-stained nuclei with the positions of nuclei in living embryos.

Between the 2-cell and 28-cell stages of embryogenesis, we detect equal levels of POP-1 staining in sister cells born from transverse cleavages. However, we detect different levels of POP-1 staining in almost all pairs of sister cells resulting from an a/p cleavage (Figure 2). For example, in the AB lineage we do not detect POP-1 differences after the first two divisions, which are transverse (data not shown; see Figure 1), but observe differences after the third division, which is a/p (Figures 2A–2D). After the first a/p division in the AB lineage, as well as after a/p divisions in other lineages, we invariably see higher levels of staining in the nucleus of the anterior sister than in the nucleus of its posterior sister; we do not yet know the molecular basis for this staining difference and refer to it here as POP-1 asymmetry. POP-1 asymmetry appears in interphase nuclei, then POP-1 staining diminishes and is not observed in prophase nuclei (for examples, see Figure 2E). The only anterior cell in which we fail to detect POP-1 is the P<sub>4</sub> blastomere (Table 1 and data not shown). A likely explanation for

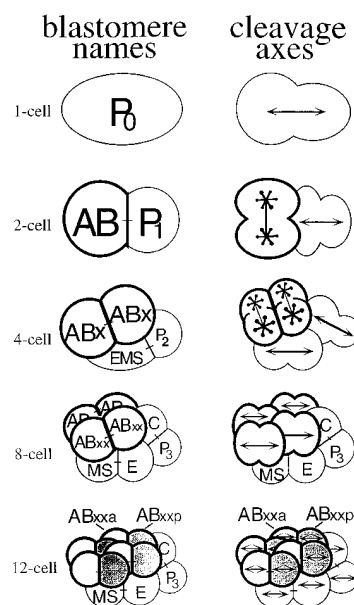


Figure 1. Early Embryonic Cell Divisions

Schematic diagrams of early blastomeres and their cleavage axes. AB and its descendants are outlined in bold. The first two daughters of AB are called ABa and ABp, represented here by the general term ABx. Blastomeres resulting from the second and third divisions in the AB lineage are labeled ABxx, and ABxxa or ABxxp, respectively. The first two divisions in the AB lineage are transverse (lines connected by asterisks). All a/p cleavages are indicated by double-headed arrows; anterior is to the left.

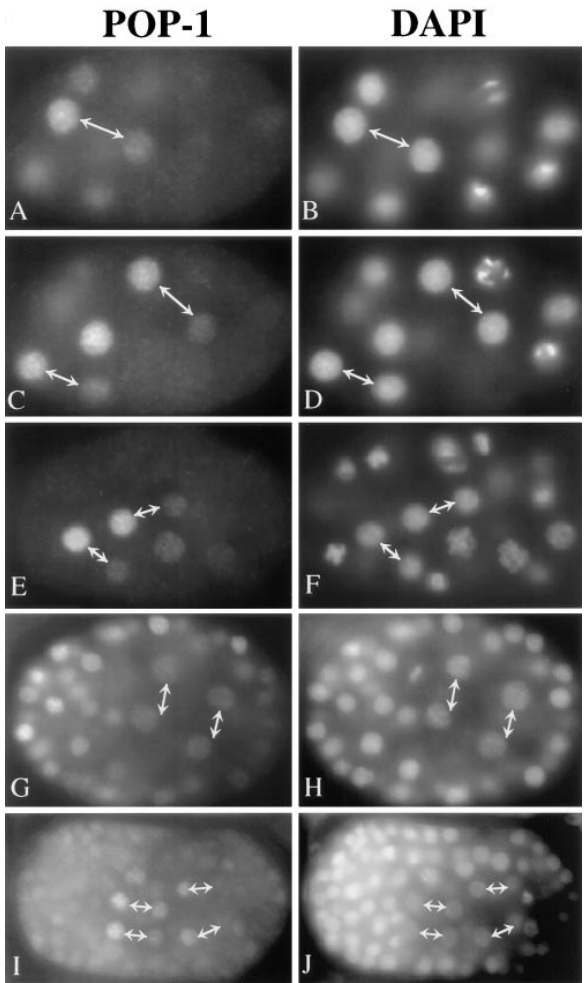
the lack of POP-1 in P<sub>4</sub> is that embryonic transcription is inhibited in this blastomere (Seydoux et al., 1996), and maternally-provided POP-1 appears to be depleted by the time P<sub>4</sub> is born (see below).

Heterogeneity in POP-1 staining is observed between many neighboring nuclei in embryos after the 28-cell stage (data not shown). The descendants of the E lineage can be identified readily in fixed embryos at all stages; we find POP-1 asymmetry after the first, third (Figure 2I), and fourth divisions of the E lineage, which are a/p. The second division of the E lineage is transverse (left/right), and we find symmetrical levels of POP-1 in both pairs of sister blastomeres (Figure 2G; Table 1). In late-stage embryos, POP-1 is prominent in the developing nervous system but absent from some other tissues like the hypodermis (data not shown).

In larvae during postembryonic development, POP-1 is present in the row of hypodermal cells, called seam cells, along the lateral surfaces of the body. POP-1 asymmetry is observed after the seam cells divide a/p; in each pair of sisters, POP-1 appears at higher levels in the anterior sister than in the posterior sister (Figures 3A–3D and 5C). In addition, POP-1 is detected in migratory cells called the Q neuroblasts and in the developing gonad and vulva (Figures 3A and 3B, and data not shown).

### POP-1 Is Involved in Multiple A/P Decisions

The events that make a/p sister blastomeres such as MS and E different can be described as an a/p decision. Loss of *pop-1(+)* activity affects the MS/E decision, causing MS to adopt an E-like fate (Lin et al., 1995); this



**Figure 2. POP-1 Asymmetry in Early Wild-Type Embryos**  
Immunofluorescence micrographs of embryos stained for POP-1 (left column) or DAPI to visualize nuclei (right column). Embryos are oriented with anterior to the left. Sister cells are indicated by double-headed arrows.  
(A–D) Two focal planes of the same 12 cell-stage embryo showing three of the four pairs of ABxxa/ABxyp sisters; these sisters are born in an a/p division. Note that the anterior sister has a higher level of staining in all cases.  
(E and F) A 28 cell-stage embryo showing the two pairs of MS granddaughters; these are born in an a/p division (MSaa/MSap, MSPa/MSPp). Note the several prophase-stage nuclei (visible in F) that do not show POP-1 staining.  
(G and H) An approximately 100 cell-stage embryo showing the two pairs of E granddaughters; these are born from a transverse (left/right) division.  
(I and J) An approximately 200 cell-stage embryo showing the four pairs of E great-granddaughters; these are born from an a/p division.

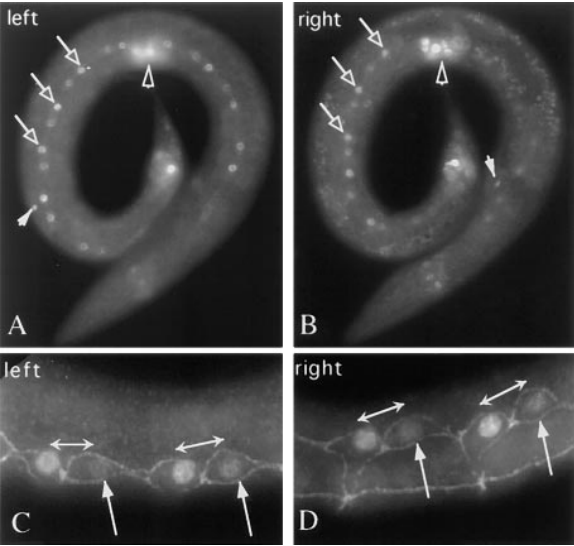
defect can be considered to be an anterior to posterior transformation in fate. Does *pop-1(+)* activity play a role in a/p decisions in the numerous other a/p sisters that exhibit similar POP-1 asymmetry? We addressed this question by examining the AB lineage. In the AB lineage, POP-1 asymmetry appears after the AB descendants undergo their third cleavage, which is a/p (Figures 1, 2A, and 2B). Each of the four a/p pairs of sister cells can be referred to by the general name ABxxa/ABxyp, where each x represents one of the two transverse

Table 1. POP-1 Staining in Early Blastomeres

Stage	Sister Blastomeres	Division Axis <sup>a</sup>	POP-1
2-cell	AB/P <sub>1</sub>	a/p	symmetrical
4-cell	ABa/ABp	d/v	symmetrical
	EMS/P <sub>2</sub>	a/p	asymmetrical
8-cell	ABxl/ABxr <sup>b</sup>	l/r	symmetrical
	MS/E	a/p	asymmetrical
	C/P <sub>3</sub>	a/p	asymmetrical
16-cell	ABxxa/ABxyp	a/p	asymmetrical
	MSa/MSp	a/p	asymmetrical
	Ea/Ep	a/p	asymmetrical
	Ca/Cp	a/p	asymmetrical
24 to 28-cell	ABxxa/ABxyp	a/p	asymmetrical
	MSa/MSp	a/p	asymmetrical
	Cxa/Cxp	a/p	asymmetrical
	P <sub>4</sub> /D	a/p	absent
Later stages	Exl/Exr	l/r	symmetrical
	Exxa/Exxp	a/p	asymmetrical
	Exxa/Exxp	a/p	asymmetrical

<sup>a</sup> d/v, dorsal/ventral, l/r, left/right; a/p, anterior/posterior.  
<sup>b</sup> x indicates both daughters born in a given division in that particular lineage.

cleavages that preceded the third a/p cleavage. In wild-type embryogenesis, different ABxxa and ABxyp blastomeres have distinct fates because of cell-cell interactions mediated by the receptor GLP-1, a protein related to *Drosophila* Notch (for reviews, see McGhee 1995; Schnabel and Priess, 1997). The *glp-1(e2141ts)* mutation



**Figure 3. POP-1 in Postembryonic Development**  
(A and B) Immunofluorescence micrographs of the left and right sides of a young second stage (L2) larva stained for POP-1. The row of nuclei with alternating high levels (arrows) and low levels of POP-1 correspond to seam cells. POP-1 staining also is seen in the developing gonad (open arrowheads) and in left/right asymmetric migrating cells called Q neuroblasts (closed arrowheads). (C and D) High magnification view of the seam cell nuclei in an L1 larva; seam nuclei are one cell division earlier than the nuclei shown in (A). Larvae were stained simultaneously for POP-1 and for an adherens junction component to outline cell boundaries. Sister cells are indicated by double-headed arrows. Note the higher level of POP-1 in the nucleus of the anterior sister than in the posterior (arrow) sister.

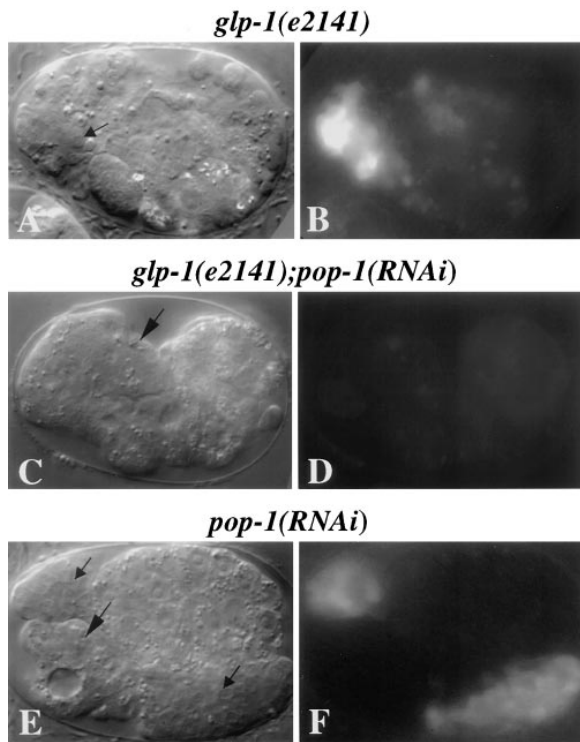


Figure 4. *pop-1(+)* Is Required for ABxxa/ABxyp Differences in *glp-1* Mutants

Nomarski photomicrographs of embryos are shown in the left column; fluorescent micrographs of the same embryos are shown in the right column. Genotypes are listed above panels. All embryos carry the fluorescent neuronal marker H2O-GFP. Cells with neuronal morphologies (small arrows) and hypodermal morphologies (large arrows) are indicated. In (C), the anterior half of the embryo beneath the arrow is comprised of hypodermal cells.

disrupts these interactions, resulting in embryos in which all ABxxa blastomeres produce predominantly neuronal cell types, and all ABxyp blastomeres produce predominantly hypodermal (epithelial) cell types. If *pop-1(+)* activity has a role in the ABxxa/ABxyp decision that is similar to its role in the MS/E decision, *glp-1* mutants lacking *pop-1(+)* activity should overproduce hypodermal cells and lack neuronal cells; an anterior to posterior transformation in fate.

We injected *pop-1* anti-sense or sense RNA into the gonads of homozygous *glp-1(e2141ts)* adults carrying the neuronal marker H2O-GFP (see Experimental Procedures). As shown previously, this RNA technique results in embryos that are accurate phenocopies of mutant embryos; embryos produced by this technique are represented by the gene name followed by the term *RNAi* (for RNA-mediated interference; Guo and Kemphues, 1995; Lin et al., 1995; Hunter and Kenyon, 1996; Mello et al., 1996; Guedes and Priess, 1997; Rocheleau et al., 1997). We found that *glp-1(e2141ts);pop-1(RNAi)* embryos had a much larger amount of hypodermal tissue than did *glp-1(e2141ts)* mutant embryos, but few if any neurons, as indicated by the lack of H2O-GFP expression (Figures 4C and 4D). The development of the first two AB daughters was analyzed individually in *glp-1(e2141ts);pop-1(RNAi)* embryos by killing all other blastomeres

with a laser microbeam (Table 2). Each AB daughter produced hypodermal cells almost exclusively. The lack of neurons in these experimental embryos indicates that *pop-1(+)* activity is required for the ABxxa fate. Moreover, the abundance of hypodermal cells (the ABxyp fate) suggests that *pop-1(+)* activity could be involved in the ABxxa/ABxyp decision.

To examine the fate of ABxxa/ABxyp sisters in a *glp-1(+)* background, we first analyzed AB lineages in a *pop-1(zu189)* mutant embryo. *pop-1(zu189)* is a maternal effect lethal mutation that prevents POP-1 expression in early blastomeres, including ABxxa and ABxyp. Subsequently, however, embryonically expressed POP-1 is present in the descendants of ABxxa and ABxyp in *pop-1(zu189)* mutants (Lin et al., 1995; data not shown). By 4D video microscopy reconstruction (Experimental Procedures), we found that an ABxyp lineage appeared wild-type in the *pop-1(zu189)* mutant, whereas each ABxxa lineage examined was abnormal (Table 3). The blastomeres ABala and ABpla both provide examples of abnormal ABxxa lineages. Several ABala descendants that become neurons in wild-type embryos differentiated as hypodermal cells in *pop-1(zu189)* embryos, while several ABpla descendants that normally become hypodermal cells differentiated as neurons. These reciprocal tissue transformations indicate that *pop-1(+)* activity does not appear to specify a particular tissue type. The abnormal ABxxa lineages matched almost exactly the lineages predicted if ABxxa blastomeres had been transformed into their ABxyp sisters; these similarities include highly patterned cell deaths, unequal divisions, and patterns of cell differentiation (Table 3). Thus, *pop-1(+)* activity appears to play a primary role in making each ABxxa blastomere different from its ABxyp sister, irrespective of the particular fates of those sisters.

ABxxa and ABxyp are born in an a/p division and subsequently undergo several a/p divisions. To determine whether *pop-1(+)* activity was required for a/p differences associated with these later divisions, we examined the lineages of the ABxxa and ABxyp blastomeres in *pop-1(RNAi)* embryos. These embryos lack both maternal and embryonic expression of POP-1 (data not shown) and thus have no detectable POP-1 in ABxxa, ABxyp, or the descendants of these blastomeres. *pop-1(RNAi)* embryos appear to have more severe developmental defects than do *pop-1(zu189)* mutants. For example, morphogenesis occurs in many *pop-1(zu189)* mutants (Lin et al., 1995) but does not occur in *pop-1(RNAi)* embryos (Figure 4E). We observed complex lineage defects in the ABxxa blastomeres of *pop-1(RNAi)* embryos (Table 3). These abnormal lineages do not match with the lineages predicted for simple ABxxa into ABxyp transformations, in contrast to our results with *pop-1(zu189)* mutants. Instead, both ABxxa and ABxyp produced relatively homogenous clones of the predominant tissue type produced by the posterior branches of the wild-type ABxyp lineage (Table 3). These results suggest that *pop-1(+)* functions in the ABxxa/ABxyp decision and in subsequent a/p decisions.

Although we have analyzed only selected lineages, we believe that anterior to posterior transformations in fate are likely to occur in all ABxxa/ABxyp descendants

Table 2. Tissue Differentiation in Partial Embryos

Blastomere Isolated <sup>a</sup>	Embryo Type	Amount of Hypodermal Tissue <sup>b</sup>		
		–	+	+++
ABa	Wild type <i>glp-1(e2141);pop-1(RNAi)</i>		100% (18) <sup>c</sup>	100% (3)
ABp	wild type <i>glp-1(e2141);pop-1(RNAi)</i>		100% (13)	100% (18)
ABa	<i>pop-1(RNAi)</i>		5% (22)	95% (22)
ABp	<i>pop-1(RNAi)</i>	32% (47)	68% (47)	
ABa	<i>mom-2(or9);apr-1(RNAi)</i>	100% (4)		
	<i>mom-2(RNAi);apr-1(RNAi)</i>	100% (17)		
	<i>mom-2(RNAi);apr-1(RNAi);pop-1(RNAi)</i>	5% (40)	32% (40)	63% (40)
	<i>mom-2(or9)</i>		100% (6)	
	<i>mom-2(RNAi)</i>		100% (20)	
	<i>apr-1(RNAi)</i>	5% (19)	5% (19)	

<sup>a</sup> The daughters of the AB blastomere are called ABa and ABp although they are born by a transverse cleavage.

<sup>b</sup> Amounts of hypodermal tissue scored by light microscopy; plus sign corresponds to a partial embryo with 20–40 hypodermal cells; triple plus sign indicates a partial embryo with predominantly hypodermal cells. In cases where no hypodermal cells (–) are produced in partial embryos, a reverse proportional amount of neuronal tissues was observed.

<sup>c</sup> Percentage of operated embryos; total numbers of operated embryos are indicated in parentheses.

of the ABa blastomere in *pop-1(RNAi)* embryos. In most cases, we found that an ABa blastomere isolated by killing all other blastomeres produces the predicted large numbers of hypodermal cells and few neurons (Table 2). However, additional interactions may influence the fates of at least some ABxxa/ABxxp descendants of the ABp blastomere. Based on our current understanding of cell fate determination in the early embryo, an ABp blastomere isolated as described above would be expected to produce no hypodermal cells if all descendants had simple posterior transformations in fate (see Schnabel and Priess, 1997). Instead, more than half of the ABp blastomeres isolated from *pop-1(RNAi)* embryos produce some hypodermal cells (Table 2).

#### Genes Involved in a Wnt-like Pathway Are Required for POP-1 Asymmetry

The MS/E decision requires components of a Wnt-like signaling pathway (see Introduction; Rocheleau et al., 1997; Thorpe et al., 1997). Studies in vertebrates and *Drosophila* have led to a model in which Wnt signaling regulates an interaction between beta-catenin and POP-1-related proteins such as Tcf-1 or Lef-1. Additional studies indicate that the APC (human adenomatous polyposis) protein also can regulate beta-catenin, but it has not been resolved whether APC acts downstream, or in parallel to, Wnt.

In *C. elegans*, the loss of wild-type activity of the *wrm-1* (beta-catenin) gene alone, or the simultaneous loss of *mom-2* (Wnt) and *apr-1* (APC) activities, prevents the MS/E decision and causes MS and E to have similar levels of POP-1 (Rocheleau et al., 1997). Therefore, we wanted to ask whether these genes were required for POP-1 asymmetry in other a/p pairs of sisters. We found that all a/p pairs of sister blastomeres appeared to have equivalent levels of POP-1 in *wrm-1(RNAi)* embryos and in *mom-2(or9);apr-1(RNAi)* embryos (Figure 5). Surprisingly, *mom-2(or9)* single mutants retained POP-1 asymmetry in AB descendants though they lacked POP-1 asymmetry in the MS/E blastomeres (data not shown).

Thus, WRM-1 is essential for all POP-1 asymmetry, while MOM-2 appears to play less of an essential role in ABxxa/ABxxp asymmetry than in MS/E asymmetry (see Discussion).

To ask whether POP-1 asymmetry in postembryonic development is regulated by genes in the Wnt pathway, we examined the male tail seam cells in *lin-17* mutants. A/P sister cells in the wild-type male tail seam show POP-1 asymmetry and have different fates in wild-type development (see Figure 5C; Sulston et al., 1980). The gene *lin-17* encodes a Frizzled-like protein, a putative Wnt receptor, and mutations in *lin-17* cause a loss of polarity in the development of the male tail seam cells (Sternberg and Horvitz, 1988; Sawa et al., 1996). We found that *lin-17(n671)* mutant animals have equivalent levels of POP-1 staining in each pair of a/p sisters in the male tail seam (Figure 5D). These results suggest that POP-1 asymmetry in many, if not all, cells in *C. elegans* depends on Wnt-like signaling pathways.

#### Ectopic Expression of POP-1

If low levels of *pop-1(+)* activity cause anterior cells to adopt the fates of their posterior sisters, high levels of *pop-1(+)* activity could force posterior cells such as ABxxp to adopt the fates of their anterior ABxxa sisters. Because disrupting the Wnt-like signaling pathway results in high levels of POP-1 in the ABxxp blastomeres, we asked if this disruption caused a *pop-1(+)*-dependent defect in the ABxxa/ABxxp decision. For these experiments, we examined the development of the AB daughter called ABa after killing all other blastomeres. An ABa blastomere isolated by this procedure will not be subjected to either of the two *glp-1*-mediated cell interactions that normally occur during the early cleavage stages. Therefore, the *C. elegans* lineage predicts that if the ABxxp descendants of ABa adopt ABxxa fates, these partial embryos should contain neuronal cells and no hypodermal cells. We found that an ABa blastomere from a *mom-2(or9);apr-1(RNAi)* embryo or from a *mom-2(RNAi);apr-1(RNAi)* embryo produced large

Table 3. Cell Lineage of AB Blastomeres in *pop-1* Mutant embryos

Experimental Group	Blastomere <sup>a</sup>	Lineage Source <sup>b</sup>	Cell Lineage <sup>c</sup>
I. ABxxa in <i>pop-1(zu189)</i>	ABpla	wild type	DDHH HHHH DDDD HHHH DDHH <i>HHHD</i> HHHH DDHH
		prediction 1 (ABplp)	DDDD DDDD DDDD DxDD DxDD DDDD DDDx DDDD
		mutant #25	DDDD DDDD DDDD DDDD DxDD DDDD DDDx D---
	ABala	wild type	DDDD DDDD DxDD xDDD DDDD xDDD xDDD DDDD
		prediction 1 (ABarp)	DDDx HHHH DDHH HHHH nHHn HHHH nHHn HHHH
		mutant #35	DDDx HHHH nHUu HHHH <H-> HHHH
II. ABxxp in <i>pop-1(zu189)</i>	ABpra	wild type	DDHH <i>HHHD</i> HHHH DDHH
		prediction 1 (ABprp)	DxDD DDDD DDDx DDDD
		mutant #25	Dx-D DDDD DDDx ----
	ABarp	wild type	nHHn HHHH nHHn HHHH
		mutant #25	uHHu HHHH uHHu HUHH
III. ABxxa in <i>pop-1(RNAi)</i>	ABala	wild type	DDDD DDDD DxDD xDDD DDDD xDDD xDDD DDDD
		prediction 1 (ABarp)	DDDx HHHH DDHH HHHH nHHn HHHH nHHn HHHH
		prediction 2	HHHH HHHH HHHH HHHH HHHH HHHH HHHH HHHH
		mutant #20	HHHH HHHH HHHH --HH ---- HHHH HHHH
		mutant #14	-HHH -HHH H-HH H-HH
		mutant #21	--DD DDDD ---- DDDD DDDD DDDD -DD DDDD
	ABpla	wild type	DDHH HHHH DDDD HHHH DDHH <i>HHHD</i> HHHH DDHH
		prediction 1 (ABplp)	DDDD DDDD DDDD DxDD DxDD DDDD DDDx DDDD
		prediction 2	DDDD DDDD DDDD DDDD DDDD DDDD DDDD DDDD
		mutant #14	DDDD DDDD DDDD DDDD DDDD DDDD DDDD DDDD
		mutant #20	DDDD DDDD HHHH DDDD HHHH HHHH HHHH HHHH
		mutant #21	--DD DDDD ---- DDDD DDDD DDDD -DD DDDD
IV. ABxxp in <i>pop-1(RNAi)</i>	ABpra	wild type	DDDD DDDD DDDD HHHH DDHH <i>HHHD</i> HHHH DDHH
		prediction 1 (ABprp)	DDDD DDDD DDDD DDDD DxDD DDDD DDDx DDDD
		prediction 2	DDDD DDDD DDDD DDDD DDDD DDDD DDDD DDDD
		mutant #14	DDDD DDDD DDDD DDDD DDD- DDDD DDDD DDD-
		mutant #20	DDDx DDDD DDDD DDDD
IV. ABxxp in <i>pop-1(RNAi)</i>	ABarp	wild type	DDDx HHHH DDHH HHHH nHHn HHHH nHHn HHHH
		prediction 2	HHHH HHHH HHHH HHHH HHHH HHHH HHHH HHHH
		mutant #14	HHHH HHHH HHHH HHHH HHHH HHHH HHHH HHHH
		mutant #20	HHUH HHHH HHHH HHHH

Cell lineages were followed through the eighth round of cleavage from AB. At this time of development, fates can be assigned on the basis of tissue-type differentiation or cell division behavior (code shown below). Sister cells are indicated in the lineage by adjacent letters, starting from the most anterior (left). In addition to the data shown, selected ABxxa lineage branches were observed in two additional *pop-1(zu189)* mutants; one had abnormalities identical to those shown for mutant #25, and the other was wild-type.

<sup>a</sup> Cell interactions mediated by GLP-1 divide the AB great-granddaughters into three groups that have a distinct anterior and posterior fate: ABala and ABarp, ABp(l/r)a and ABp(l/r)p, and ABara and ABalp. The fates of ABara and ABalp are not easily distinguished by lineage analysis and were therefore not analyzed.

<sup>b</sup> The source of the lineage to the left is indicated as follows: wild type, the cell lineage of the blastomere in wild type (Sulston, 1983); prediction 1, the cell lineage predicted if an ABxxa blastomere adopts the fate of the corresponding ABxxp blastomere; prediction 2, the predicted lineage if anterior-posterior differences are lost in divisions after the birth of ABxxa/ABxxp (in the case of ABxxa blastomeres, this is in addition to the initial ABxxa to ABxxp transformation); mutant #, cell lineage is from the given mutant embryo. Because of the GLP-1 mediated interactions, ABala would not be predicted to transform into ABalp, but rather ABarp (see Schnabel and Priess, 1997).

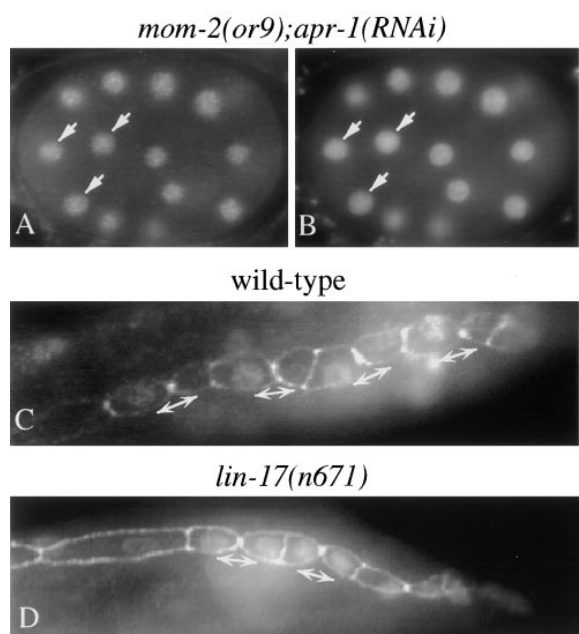
<sup>c</sup> Cell lineage. Each letter indicates the fate of one of the 32 descendants of the given ABxxa/ABxxp blastomere, tabulated anterior to posterior (from ABxxxxaaaa to ABxxxxpppp). D, cell division; H, hypodermis; N, neuron; X, cell death; U, undivided, tissue type not scorable; lowercase indicates cell is smaller than its sister; (-), fate of cell could not be determined; (<-), cell smaller than posterior sister, cell type not scorable; (>-), cell smaller than anterior sister, cell type not scorable; italics indicates that cell divides in late wild-type embryogenesis and division and is not observable by 4D analysis.

numbers of neurons but no hypodermal cells. In contrast, most ABa blastomeres from *mom-2(RNAi);apr-1(RNAi);pop-1(RNAi)* embryos produced few neurons and large numbers of hypodermal cells. Thus, inappropriate *pop-1(+)* activity appears to result in the ABxxp descendants of ABa adopting ABxxa characteristics; a posterior to anterior transformation in fate reciprocal to the anterior to posterior transformations observed in embryos lacking *pop-1(+)* activity.

### P<sub>2</sub> Signaling and POP-1 Asymmetry

The P<sub>2</sub> blastomere appears to provide the Wnt signal involved in the MS/E decision (see Introduction). To test

whether P<sub>2</sub> is required for POP-1 asymmetry in the MS/E pair of sister blastomeres, we removed the P<sub>2</sub> blastomere early in the 4-cell stage to prevent signaling, allowed the parent of MS and E to divide, and then stained the resulting embryo for POP-1. We found that MS and E had equivalent levels of POP-1 in 8 of 9 embryos (Figure 6E). To test whether P<sub>2</sub> is required for POP-1 asymmetry in the ABxxa/ABxxp pair of sisters, we repeated the above experiment but allowed the partial embryos to develop until ABxxa and ABxxp were born. POP-1 asymmetry was apparent in 5 of 6 embryos (Figure 6A), and staining was too faint to evaluate in the sixth. We conclude that POP-1 asymmetry in the ABxxa/



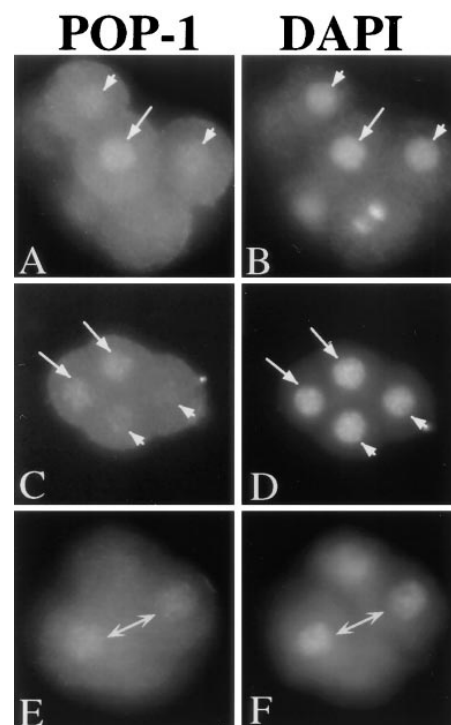
**Figure 5. The Wnt Pathway and POP-1 Asymmetry**  
Immunofluorescence micrographs of embryos (A) or larvae (C and D); genotypes are listed above panels. (A) An embryo is stained for POP-1. All AB descendants (three descendants are indicated by arrows) have similar levels of staining. (B) Nuclei in the embryo shown in (A) stained with DAPI. (C and D) Seam cells in the tail of a male larva stained simultaneously for POP-1 and for adherens junctions to visualize cell boundaries. Sister cells indicated by double-headed arrows.

ABxxp pairs of blastomeres does not appear to require  $P_2$  signaling (see Discussion).

POP-1 asymmetry is apparent in the a/p descendants of MS (see Figure 2E) and the descendants of E (see Figure 2I). Because POP-1 asymmetry in MS/E requires  $P_2$  signaling, we wanted to know whether  $P_2$  or  $P_2$  descendants were required to maintain POP-1 asymmetry in later a/p divisions. We allowed  $P_2$  to contact EMS for a time period sufficient for  $P_2$ -EMS signaling (see Experimental Procedures), then isolated EMS by removing the  $P_2$  blastomere and the AB daughters. The isolated EMS blastomere was allowed to divide into MS and E, then both MS and E were allowed to divide once more before fixation and immunostaining. In each of two partial embryos examined, POP-1 asymmetry was apparent in the resulting blastomeres (Figure 6C). Thus, if  $P_2$ -EMS signaling is allowed to occur, POP-1 asymmetry can persist in descendants of MS or E without the continued presence of  $P_2$  or AB descendants.

## Discussion

The remarkable invariance of the cell lineage of the *C. elegans* embryo means that there is a reproducible relationship between the division sequence by which a cell is born and the specific differentiated fate of that cell. How do different patterns of division sequence result in different patterns of gene expression? We have shown that POP-1 asymmetry is observed after almost all a/p



**Figure 6. POP-1 in Partial Embryos**  
Immunofluorescence micrographs of partial embryos stained for POP-1 (left column) and stained with DAPI (right column) to visualize nuclei.  
(A and B) A partial embryo in which  $P_2$  was removed before  $P_2$ -EMS signaling. The embryo was fixed after the birth of the ABxxa and ABxxp sisters. One of four blastomeres with high POP-1 is visible (arrow), and two of four blastomeres with low POP-1 are visible (arrowheads) on this focal plane. The dividing blastomere (lower right in each panel) is MS or E.  
(C and D) A partial embryo derived from an EMS blastomere isolated after  $P_2$ -EMS signaling. EMS was allowed to divide twice, then fixed and stained; POP-1 is high in two EMS descendants (arrows) and low in the other two (arrowheads).  
(E and F) A partial embryo in which  $P_2$  was removed before  $P_2$ -EMS signaling. The partial embryo was allowed to develop until EMS divided, then fixed and stained. The daughters of EMS (arrows) have similar levels of POP-1.

divisions in the early embryo; each anterior sister appears to have a higher level of POP-1 than its posterior sister. Thus, a cell that is born from a division sequence such as a-p-a is unique in experiencing changes in POP-1 that vary in the order high-low-high. Although the mechanism underlying POP-1 asymmetry is not known, these results provide clear evidence for a link between division sequence and changes in the cell nucleus.

## POP-1 Asymmetry

The sister blastomeres resulting from the first two transverse divisions in the AB lineage have a high level of POP-1. After the third a/p division, the anterior sisters have a high level of POP-1, but the posterior sisters have a low level. Thus, a posterior cell somehow recognizes that it was born by an a/p cleavage, and in response to this information reduces its apparent level of nuclear POP-1. For example, the mechanism that lowers

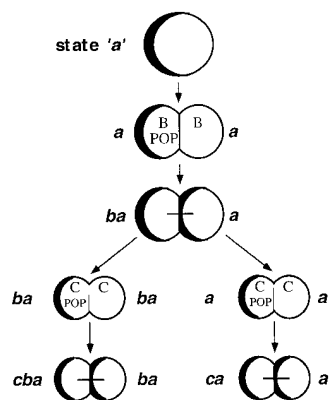


Figure 7. POP-1 and Lineage Patterns

In this model, a cell has an a/p polarity because of the anterior (left) localization of factors that maintain POP-1 at high levels (represented by the black crescent); these factors are partitioned into the anterior (left) daughter at division. Transcription factors B and C are expressed sequentially in the lineage, and in the presence of POP-1 activity, they can change the state of a cell (lowercase, italicized letters). Note that a/p polarity is regenerated at each division in posterior cells independent of POP-1 activity (see Discussion).

POP-1 levels could be inhibited by a factor(s) localized to the anterior cortex of the parental cell (Figure 7); only the anterior daughter of such a cell would retain a high level of POP-1 during the next cell cycle.

We have shown that POP-1 asymmetry is present at each of several sequential a/p divisions. This result means that each parental cell must produce an anterior daughter with a high level of POP-1 and a posterior daughter with a low level of POP-1, irrespective of the level in the parental cell. How is POP-1 asymmetry maintained within a lineage? In the MS/E blastomeres, POP-1 asymmetry depends on a signal from a third blastomere called  $P_2$ . Thus, POP-1 asymmetry in later MS or E divisions could depend on continued signaling by  $P_2$  descendants. We have shown that this is not the case, because POP-1 asymmetry is present in the descendants of an EMS blastomere isolated after  $P_2$  signaling. In an alternative model,  $P_2$  signaling could cause one or more of the EMS descendants to become a new source of signaling. Our experiments do not rule out this possibility; however, we consider it very unlikely. First, a/p asymmetries in the MS lineage, such as the first programmed cell death, clearly persist after killing the E blastomere (see Introduction). Second, if a lineage contained special signaling cells, we might expect those cells to affect POP-1 localization in neighboring cells from other lineages. Instead, the POP-1 asymmetries we observe in immunostained embryos correlate precisely with the lineage histories of cells rather than their position. For these reasons, we favor the hypothesis that once POP-1 asymmetry is initiated, it can be propagated without further signaling. In the simple model shown in Figure 7, a posterior cell would need to be able to recognize its anterior pole and regenerate the inhibitory factor at that site. For example, the midbody resulting from every a/p cell division would invariably be at the anterior pole of a posterior cell and thus could provide a cell-intrinsic marker of the anterior.

### ***pop-1(+)* Activity Is Required for Many Anterior Fates**

Our analysis of tissue differentiation in *glp-1(e2141); pop-1(RNAi)* embryos and in *pop-1(zu189)* mutant embryos demonstrates that *pop-1(+)* function has a role in AB development in addition to its role in MS development described previously. In MS development, loss of *pop-1(+)* activity causes a mesoderm to endoderm transformation. We have shown examples in this paper of neuronal to hypodermal, as well as hypodermal to neuronal, transformations. Thus, *pop-1(+)* activity is not limited to specifying one type of tissue.

POP-1 reappears at each of successive a/p divisions within the early embryo, raising the issue of whether POP-1 functions in each of the sequential a/p decisions. An ideal test of this model would be to compare the effect of removing *pop-1(+)* activity from just one a/p division with the effect of removing *pop-1(+)* activity from two a/p divisions in the same lineage. We have analyzed AB lineages in *pop-1(zu189)* embryos, which lack detectable POP-1 in the ABxxa/ABxxp blastomeres but express POP-1 in subsequent descendants. In these embryos, we find clear evidence that the ABxxa blastomeres are adopting the fates predicted if they were transformed into their ABxxp sisters; subsequent a/p asymmetries appear normal in these lineages. For comparison, we have analyzed AB lineages in *pop-1(RNAi)* embryos, which lack POP-1 in the ABxxa/ABxxp blastomeres and in the descendants of these blastomeres. In these embryos, we find lineage defects that can be interpreted as failures to specify anterior fates in multiple, sequential a/p decisions.

For the reciprocal test, we would like to know whether forced expression of POP-1 in posterior sisters, where POP-1 normally appears at low levels, would lead to these cells adopting the fates of their anterior sisters. The results from our analysis of ABa development in embryos defective in the Wnt-like signaling pathway are consistent with this hypothesis. We therefore propose that *pop-1(+)* activity is part of a general mechanism that can be used to distinguish the fates of otherwise equivalent a/p sisters in some blastomere lineages.

It is likely that POP-1 interacts with multiple transcription factors in determining a/p differences. POP-1 and the transcription factor SKN-1 are both required for the MS fate; SKN-1 is present at equal levels in MS and E, while POP-1 is asymmetric between MS and E (Bowerman et al., 1992, 1993; Lin et al., 1995). Therefore, POP-1 could interact with SKN-1 to specify MS but not function in E. SKN-1 is not expressed in ABxxa or ABxxp, so POP-1 could have a similar mode of action in these blastomeres, but with a different transcription factor. If this basic idea is correct, an intriguing possibility is that POP-1 interacts with a succession of temporally, but not spatially, regulated transcription factors within a lineage. In the model shown in Figure 7, we imagine that a transcription factor "B" is expressed in two sisters, but only functions in the anterior sister with high POP-1; the activities of B plus POP-1 change the state of the anterior cell from "a" to "ba"; the state of the posterior sister remains unchanged until the next division, when a second transcription factor "C" is expressed in all cells. Thus, POP-1 asymmetry coupled with the sequential expression of two different transcription factors could



result in an invariant lineage that produces four distinct cell types (cba, ba, ca, and a).

### P<sub>1</sub> versus P<sub>2</sub> Signaling

Although POP-1 asymmetry demonstrates that there is a common component to a/p asymmetry in all of the blastomere lineages, it is less clear how a/p asymmetry is initiated in each of those lineages. There is good evidence that the P<sub>2</sub> blastomere is the source of the initial a/p difference between MS and E, and is required for POP-1 asymmetry between MS and E. However, the ABxxa/ABxyp blastomeres have POP-1 asymmetry (our present results) and a/p differences in fate (Gendreau et al., 1994; Hutter and Schnabel, 1995) even after the P<sub>2</sub> blastomere is removed or killed. Thus, P<sub>2</sub> cannot be the sole source of a/p differences in the ABxxa/ABxyp blastomeres.

Hutter and Schnabel (1995) proposed that polarity in the AB lineage was induced by the P<sub>1</sub> blastomere, which is the parent of P<sub>2</sub>. They found that killing the P<sub>1</sub> blastomere caused defects in the ABxxa/ABxyp decision; in about 50% of the blastomeres examined, the ABxyp blastomere adopted an ABxxa-like fate. However, Gendreau et al. (1994) observed ABxxa/ABxyp differences even after the P<sub>1</sub> blastomere was removed early in the 2-cell stage. The basis for these different results has not yet been determined.

Although we favor the simplicity of the P<sub>1</sub> signaling model of Hutter and Schnabel (1995), we note that their experimental results do not match precisely with the results predicted from our analysis of *pop-1* function. If the source of POP-1 asymmetry was destroyed, all blastomeres should have high levels of POP-1. We would expect such embryos to have fate transformations that are the reciprocal of the lineage transformations we observe in *pop-1(RNAi)* embryos, where POP-1 expression is not detected in any AB descendants. Specifically, we would expect ABxyp blastomeres to be transformed into ABxxa-like blastomeres, with additional defects in later a/p decisions for both blastomeres. However, in the experiments of Hutter and Schnabel (1995), ABxyp blastomeres appear to adopt ABxxa lineages without defects in later a/p decisions. This result is exactly the reciprocal of the lineage transformation we observe in *pop-1(zu189)* mutants, where the expression of POP-1 is delayed but not abolished.

### Wnt Signaling and POP-1

The recent discovery that P<sub>2</sub> signaling involves a Wnt-like pathway (Rocheleau et al., 1997; Thorpe et al., 1997) suggests the interesting possibility that all a/p polarity in the early *C. elegans* embryo could be controlled by Wnt-like signals. For example, each of the P blastomeres (P<sub>1</sub>, P<sub>2</sub>, etc.) could sequentially express a Wnt-like signal that polarizes their respective sister (AB, EMS, etc.) and thus, in a stepwise fashion, provide a/p polarity to all other embryonic blastomeres.

In models for Wnt signaling pathways, Wnt signaling is believed to cause beta-catenins (such as the WRM-1 protein) to enter the nucleus and interact with proteins related to POP-1 and thus regulate transcription (see Nusse and Varmus, 1992; Peifer, 1996; Moon et al.,

1997). We have shown here that *wrm-1(+)* activity is required for POP-1 asymmetry in all lineages. The localization of WRM-1 in *C. elegans* has not yet been determined, and there are several possible roles for WRM-1 that are compatible with the existing data. If WRM-1 and POP-1 function together in every a/p division, it is possible that WRM-1 binding to POP-1 affects the immunostaining properties of POP-1 and is the source of the POP-1 asymmetry described in this report. Alternatively, it is possible that WRM-1 functions only to initiate POP-1 asymmetry.

The requirement for MOM-2 in POP-1 asymmetry appears to be more complex than the requirement for WRM-1. MOM-2 is the presumptive Wnt signal expressed by P<sub>2</sub>, and MOM-2 is required for POP-1 asymmetry in the MS/E blastomeres (Rocheleau et al., 1997; Thorpe et al., 1997). Nevertheless, we have found that the majority of *mom-2* mutant embryos have POP-1 asymmetry in AB descendants. Thus, if P<sub>1</sub> is source of a/p polarity for AB descendants, it may express a Wnt other than MOM-2. The predicted APR-1 protein is related to vertebrate APC; APC activity appears to regulate beta-catenin levels, but it is not clear whether this regulation is in direct response to Wnt signaling (Rocheleau et al., 1997; for review, see Moon and Miller, 1997). Previous studies have shown that the loss of *apr-1(+)* activity strongly enhances MS/E phenotypes caused by mutations in *mom-2*, and we have shown here that POP-1 asymmetry is abolished in embryos that lack *apr-1(+)* and *mom-2(+)* activities simultaneously. Thus, a/p asymmetries in the MS/E and ABxxa/ABxyp blastomeres both involve POP-1, but there are differences in how POP-1 asymmetry is initiated in these blastomeres, including the source of the signal, and the role of MOM-2 in that signaling event.

If Wnt signaling initiates a/p polarity, does it do so through POP-1? Our results suggest that POP-1 asymmetry is a response to an a/p polarity determined independently of *pop-1(+)* activity itself. *pop-1(zu189)* mutant embryos lack detectable POP-1 during all of the early cleavage stages, when P<sub>2</sub> signaling, and possibly P<sub>1</sub> signaling, occur. However, POP-1 asymmetry is apparent in these embryos after embryonic expression of POP-1 is initiated, and our lineage analysis shows the correct a/p asymmetry during these later cleavage stages. Thus, we propose that a/p polarity is initiated in these embryos and propagated for the first few divisions in the absence of *pop-1(+)* activity. Wnt signaling might thus define a/p polarity independently of *pop-1*-dependent transcription; this apparent split in the Wnt pathway is consistent with previous results showing a role of MOM-2 in mitotic spindle orientation that does not involve either POP-1 or WRM-1 (Rocheleau et al., 1997).

In conclusion, we propose that POP-1 is part of a general a/p coordinate system that operates throughout the early embryo. We believe that the existence of this system provides an explanation for the several observations described previously on lineage autonomy in wild-type and mutant *C. elegans* embryos. Because POP-1 is required for several different types of cell differentiation, an important task for the future will be in understanding how POP-1 interacts with other transcription

factors to control cell fates. Finally, it will be of interest to determine at the molecular level how polarity is initiated, and propagated, in these lineages.

## Experimental Procedures

### Strains and Alleles

The Bristol strain N2 was used as the standard wild-type strain. The genetic markers used in this paper are listed by chromosomes as follows: linkage group I (LGI), *dpy5(e61)*, *pop-1(zu189)*, *lin-17(n671)*, *goa-1(n1134)*, and *hT1(I;V)*; LGIII, *dpy-20(e1282)* and *glp-1(e2141ts)*; LGV, *dpy-11(e224)*, *mom-2(or9)*, and *nT1(IV;V)*. *mom-2(or9)* was kindly provided by B. Bowerman; all other mutants were obtained from the *C. elegans* Genetic Stock Center. Basic methods for worm culture and genetics were performed as described by Brenner (1974).

### Generation and Characterization of POP-1 Monoclonal Antibody

RBF/Dn mice (The Jackson Laboratory) were injected subcutaneously with 100  $\mu$ g of His-tagged POP-1 fusion protein (Lin et al., 1995) and boosted at 2 week intervals. Sera from injected mice were assayed by ELISA and immunofluorescence. Monoclonal antibodies were generated according to published protocols (Wayner and Carter, 1987) in the Hybridoma Production Facility at the Fred Hutchinson Cancer Research Center. Two hybridoma lines were isolated that recognize the POP-1 protein in *C. elegans* embryos: mABRL1 (P1A11) and mABRL2(P4G4); both monoclonal antibodies are IgG1.

### Immunofluorescence

Embryos and larvae were processed for immunostaining as described previously (Lin et al., 1995), except that the fixation time was reduced to 5–10 min, and after fixation embryos were immersed in 100% dimethyl formamide at  $-20^{\circ}\text{C}$  for 5 min instead of in methanol. Embryos were incubated with 10% goat serum (GIBCO) in Tris-Tween at room temperature for 15 min and incubated at  $4^{\circ}\text{C}$  for 12 hr with either mABRL1 or mABRL2 at a 1/50 dilution in Tris-Tween.

### Analysis of Embryos

Blastomere isolations and culture medium were as described previously (Thorpe et al., 1997), except  $\text{MgCl}_2$  was added to 5 mM in the culture medium. Light microscopy was performed with a Zeiss Axioplan microscope equipped with epifluorescence, polarizing, and differential interference contrast (DIC) optics. Photographs were taken using a Dage MTI VE1000 video camera.

Neurons were distinguished from hypodermal cells either by expression of the H2O-GFP transgene or by morphological criteria; a hypodermal cell has a large nucleus with a relatively clear nucleoplasm and a prominent nucleolus, while neurons have small, punctate nuclei.

The AB lineage was determined by observing the sequence of cell divisions in 4D movies recorded by videomicroscopy. The 4D imaging system used was developed by Charles Thomas and John White (Thomas et al., 1996). Most of the major hypodermal cells produced by AB are born at the 8th round of AB cleavage and do not divide again; most neuroblasts and pharyngeal precursors undergo at least one additional round of cleavage within 90 min of their birth. Cells born at the eighth round were followed until they divided or until their pattern of differentiation could be determined by morphological criteria. To confirm that cells were nonmitotic, cells were followed for 2 hr past the time corresponding to the 9th round of cleavage. For the lineages analyzed, cell division at the 9th round is usually a predictor for a neuronal fate.

### RNAi

Antisense and/or sense RNA from the *pop-1*, *wrm-1*, *mom-2*, and *apr-1* genes were generated and injected into adult *C. elegans* as described previously (Lin et al., 1995; Rocheleau et al., 1997). All RNAi experiments, except those using *glp-1(e2141)* or *mom-2(or9)*, were done in a *goa-1(n1134)* strain that lays embryos at an early

stage of embryogenesis. Epistasis between *pop-1(RNAi)* and *apr-1(RNAi);mom-2(RNAi)* was done by sequential injection of the RNAs as described previously (Rocheleau et al., 1997).

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