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Wnt Signaling and an APC-Related Gene Specify Endoderm in Early C. elegans Embryos

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Summary

In a 4-cell stage C. elegans embryo, signaling by the P_2 blastomere induces anterior–posterior polarity in the adjacent EMS blastomere, leading to endoderm formation. We have taken genetic and reverse genetic approaches toward understanding the molecular basis for this induction. These studies have identified a set of genes with sequence similarity to genes that have been shown to be, or are implicated in, Wnt/Wingless signaling pathways in other systems. The C. elegans genes described here are related to *wnt/wingless*, *porcupine*, *frizzled*, *β-catenin/armadillo*, and the human adenomatous polyposis coli gene, APC. We present evidence that there may be partially redundant inputs into endoderm specification and that a subset of these genes appear also to function in determining cytoskeletal polarity in certain early blastomeres.

Introduction

In C. elegans embyogenesis, many of the divisions of early cells, called blastomeres, result in anterior and posterior sisters with different fates. Part of the anterior–posterior (a–p) polarity of the early embryo appears to be determined by the site of sperm entry (Goldstein and Hird, 1996). A second source of a–p polarity requires a cell–cell interaction at the 4-cell stage of embryogenesis between blastomeres called EMS and P_2 (Goldstein, 1992, 1993). In normal development, the EMS blastomere divides into an anterior daughter, called MS, that produces mesoderm and a posterior daughter, called E, that produces only endoderm. The ability of EMS to produce daughters with different fates appears to require signaling by the P_2 blastomere. If P_2 is prevented from contacting EMS, neither EMS daughter produces endoderm, and both daughters adopt MS-like fates. P_2 normally contacts the posterior surface of EMS; however, if P_2 is repositioned to contact the anterior surface, the fates of the EMS daughters are interchanged. Thus, P_2 induces both endoderm formation and the a–p polarity of the EMS blastomeres; we refer to this induction as P_2-EMS signaling.

Previous studies suggested that P_2-EMS signaling may induce the E fate by lowering the amount or activity of POP-1 protein in the E blastomere (Lin et al., 1995). In a wild-type embryo, POP-1 appears to be present at a high level in the MS nucleus and at a lower level in the E nucleus. In a mutant lacking detectable POP-1 in both MS and E, both blastomeres adopt E-like fates and produce endoderm. POP-1 is an HMG-domain protein similar to the vertebrate Tcf-1 and Lef-1 proteins and to the recently described Pangolin/dTCF protein of Drosophila melanogaster (Brunner et al., 1997; van de Wetering et al., 1997). Genetic and/or biochemical studies suggest that Pangolin/dTCF and TCF/LEF family members can function as downstream components in a conserved Wnt/Wingless (Wnt/WG) signaling pathway (see Nusse and Varmus, 1992; Klingensmith and Nusse, 1994; and Moon et al., 1997, for reviews). Thus, P_2-EMS signaling might involve POP-1 and a Wnt/WG-like pathway in Caenorhabditis elegans. However, loss of WG signaling and mutations in *pangolin* cause similar phenotypes (Brunner et al., 1997), while loss of P_2-EMS signaling and mutations in *pop-1* cause opposite phenotypes (Lin et al., 1995).

To determine the molecular basis for P_2-EMS signaling, we performed genetic screens to look for mutants that lacked endoderm (the E fate) and that overproduced mesoderm (the MS fate). As a second approach, we searched the C. elegans genome sequence database for homologs of genes implicated in Wnt/WG signaling and tested by a reverse genetic assay whether these genes were required for P_2-EMS signaling. In this report, we show that three new genes identified in our genetic screens encode products that are similar to components of the Wnt/WG pathway. Two additional genes identified by our reverse genetic assay also appear to be required for P_2-EMS signaling: one can encode a protein related to β-catenin, and the other is similar to the human adenomatous polyposis coli (APC) gene (Groden et al., 1991). Our results provide strong evidence that P_2-EMS signaling in C. elegans involves a Wnt/WG-like pathway, although downstream components in this pathway may have novel roles. Finally, our studies provide evidence for partial redundancy within this pathway involving the APC-related gene. These observations indicate that the E blastomere, which has one of the simplest developmental patterns of any cell in C. elegans, may be specified by a surprisingly complex mechanism.

Results

Molecular Identification of Genes Required for Endoderm Formation

Previous genetic screens by several laboratories have identified genes required for endoderm development. For example, mutations in the *skn-1* gene cause a high percentage of embryos to lack endoderm (Bowerman

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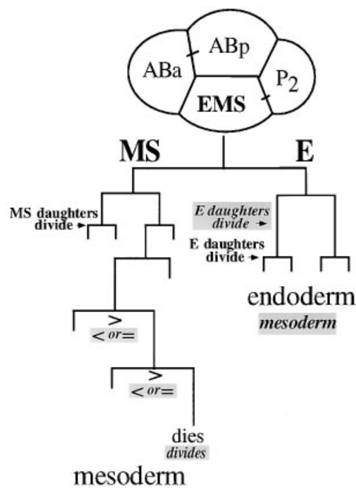

Figure 1. MS and E Development

A schematic diagram of a 4-cell stage embryo is shown with abbreviated lineages of the sister blastomeres MS and E. These lineages show the different division times of the MS and E daughters in wild-type embryogenesis and the unequal divisions (>) and cell death that normally occur in one branch of the MS lineage. The shaded boxes refer to abnormalities observed in mutants described in the text.

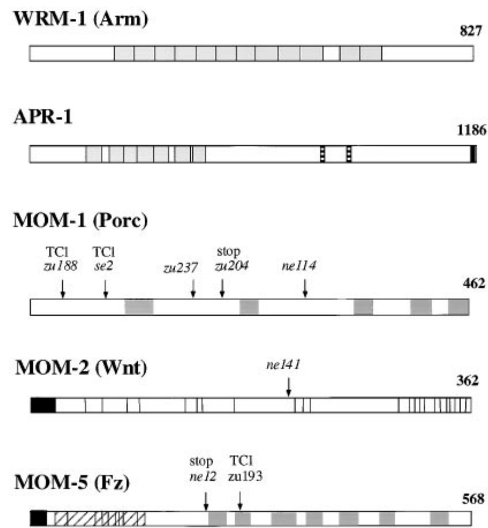

Figure 2. Schematic Representations of the Predicted WRM-1, APR-1, MOM-1, MOM-2, and MOM-5 Proteins

The predicted proteins shown are based on cDNA sequences and/or genomic sequences available from the C. elegans Genome Sequencing Project; molecular lesions in some of the *mom* mutants are indicated. Proteins are not drawn to scale; the predicted sizes in amino acids are indicated at the C termini (right). Potential signal peptides and transmembrane domains are indicated by black and gray boxes, respectively. WRM-1 has 12 arm motifs (stippled boxes) that are 23% identical to human β-catenin (Hülsken et al., 1994) and 22% identical to Drosophila Armadillo (Riggleman, 1989); no significant homologies are observed outside of these domains. APR-1 is much smaller than the 2843-amino-acid APC protein in humans (Groden et al., 1991); however, both proteins contain seven arm motifs, and APR-1 and APC are 31% identical in these regions. APC and APR-1 contain three and two copies, respectively, of the sequence [I/L-L-X-E/R-C/S-I-X-S-A/E-M-P-T/K]; in APC this motif occurs at residues 1574, 1723, and 2038 within a region believed to be important in interactions with β-catenin. MOM-1 has 29% identity to Drosophila Porcupine (Kadowaki et al., 1996), including five similarly positioned possible transmembrane domains. MOM-2 has 34% identity to Drosophila Wingless (Rijsewijk et al., 1987) and 35% identity to human Wnt2 (Wainwright et al., 1988). Conserved cysteine residues found in all Wnt proteins are indicated by vertical lines. MOM-5 has 37% identity to Drosophila Frizzled (Adler et al., 1990) and 33% identity to Frizzled2 (Bhanot et al., 1996). The hatched box at the N terminus corresponds to the CRD (cysteine-rich extracellular domain) thought to mediate interactions between Frizzled2 and WG (Bhanot et al., 1996); conserved cysteine residues are indicated by vertical lines.

et al., 1992a). However, none of the mutations reported thus far cause the specific transformations predicted from a defect in P_2-EMS signaling. When P_2-EMS signaling is prevented in wild-type embryos, the E blastomere develops like an MS blastomere (Goldstein, 1992, 1993). First, E does not produce endoderm and instead produces pharyngeal tissue and body wall muscles, which are mesodermal tissues normally produced by MS. Second, the E blastomere adopts an accelerated cleavage rate similar to MS; for example, the E daughters divide prematurely at about the same time as the MS daughters (Figure 1).

We used a reverse genetic assay to test whether 12 genes in the C. elegans database that have homology to known or potential components of the Wnt/WG pathway function in P_2-EMS signaling (see Experimental Procedures). For this assay, RNA from the relevant cDNA or PCR-amplified gene fragment was injected into the gonads of wild-type adult hermaphrodites (see Experimental Procedures). We and others have found that this RNA procedure precisely reproduces phenotypes that are known to result from strong or null mutations in almost all of the maternally expressed genes tested (Guo and Kemphues, 1995; Lin et al., 1995; Guo and Kemphues, 1996; Mello et al., 1996; Powell-Coffman et al., 1996; Guedes and Priess, 1997). For each of the genes examined thus far, this procedure has been shown to result in a lack of protein expression (Lin et al., 1995; Powell-Coffman et al., 1996; C. C. M., unpublished data). The mechanism underlying the RNA-induced defect appears to be distinct from that of conventional antisense RNA because both the sense and antisense RNA strands cause similar defects (see above references; S. Driver and C. C. M., unpublished data). Because the mechanism is not known, we will refer to this technique as RNAi, for RNA-mediated interference; embryos thus treated will be indicated by listing the gene name followed by *RNAi*.

We identified a gene related to Drosophila *armadillo* and vertebrate β-catenin and call this gene *wrm-1* (for worm arm motif gene; Figure 2). The predicted WRM-1 protein contains the same number of the repeated "arm" motif that is found in Armadillo/β-catenin (Riggleman, 1989) but is considerably diverged in overall amino acid sequence identity (Figure 2 and data not shown). We found that *wrm-1(RNAi)* embryos have phenotypic defects that are very similar to those caused by defects in P_2-EMS signaling: all of these embryos fail to produce endoderm and instead produce abnormally large quantities of pharyngeal tissue (Figure 3 and Table 1). We examined the fate of the E blastomere in *wrm-1(RNAi)*

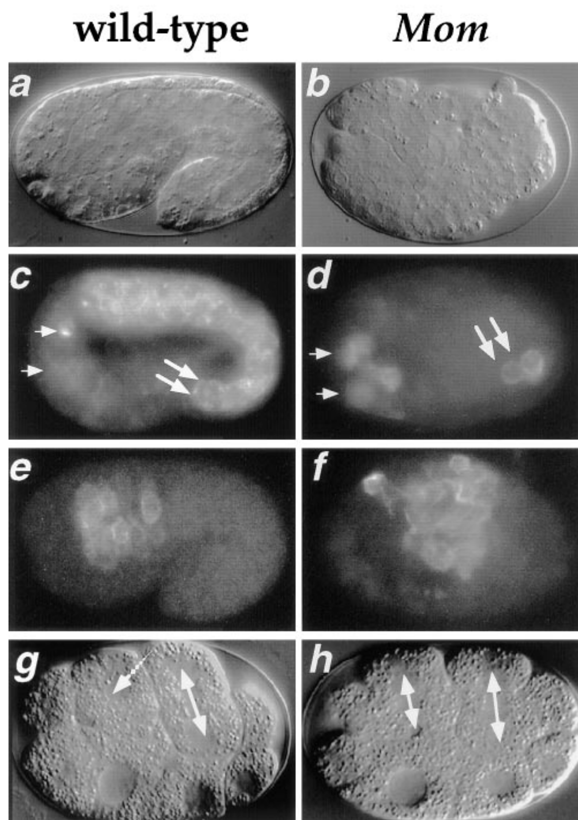

Figure 3. The *mom* Phenotype

The E blastomere in *wrm-1(RNAi)* embryos, *apr-1(RNAi)* embryos, and *mom* mutant embryos can produce mesoderm instead of endoderm. This defect results in the phenotype illustrated by the *mom-1(se2)* mutant shown in the right column; the ABar cleavage defect unique to the *mom* mutants is shown in (h). (a and b) Light micrographs of living embryos. The wild-type embryo has initiated morphogenesis and will ultimately elongate 4-fold. The terminal stage *mom* mutant embryo has not elongated. (c and d) Immunofluorescence micrographs of terminal stage embryos stained with mAB-ICB4: this antibody recognizes the intestine (endoderm), nonendodermal valve cells (pair of long arrows) (Bowerman et al., 1992b) and a few neurons (pair of short arrows). (e and f) Immunofluorescence micrographs of embryos after staining with mAB3NB12: this antibody stains a subset of muscles in the pharynx, many of which are derived from the MS blastomere. (g and h) Early cell divisions. In the wild-type embryo, the ABar spindle (left arrow) is orthogonal to other AB descendants such as ABpr (right double arrow). In the *mom* mutant, ABar (left double arrow) and ABpr (right double arrow) have parallel spindles.

embryos by killing all other blastomeres with a laser microbeam after the period that P_2-EMS signaling normally occurs in wild-type embryogenesis (see Experimental Procedures). After E was allowed to develop for several hours, the resulting partial embryos were examined then fixed and stained with tissue-type specific antibodies. The E blastomere failed to produce endoderm and instead produced pharyngeal tissue in 8/8 laser-operated embryos. We followed the early cleavage patterns in 11 *wrm-1(RNAi)* embryos and found that these patterns are normal except for the E daughters, which divide prematurely (Figure 1 and Table 1). The close similarity between these defects and those caused by defects in P_2-EMS signaling suggests that the *wrm-1* gene plays an essential role in P_2-EMS signaling in wild-type embryogenesis.

To test if *wrm-1* was required for the different apparent levels of POP-1 protein observed in the MS and E nuclei of wild-type embryos (Figure 4a), we stained 8-cell stage *wrm-1(RNAi)* embryos with an antibody that recognizes POP-1. We found that the E nucleus appears to contain an abnormally high level of POP-1 that is similar to the level in the MS nucleus (Figure 4b). To determine whether *pop-1(+)* activity prevents E from producing endoderm in the *wrm-1(RNAi)* embryos, we constructed and analyzed *pop-1(zu189);wrm-1(RNAi)* embryos. In contrast to *wrm-1(RNAi)* embryos, which produce no endoderm (Table 1), all of the *pop-1(zu189);wrm-1(RNAi)* embryos contain endoderm, as do *pop-1(RNAi);wrm-1(RNAi)* embryos (Table 2; see also Experimental Procedures). To determine if a loss of *pop-1(+)* activity allows E to produce endoderm irrespective of P_2 signaling, we removed the P_2 blastomere from 4-cell stage *pop-1(zu189)* embryos following the procedures of Goldstein (1992) and found that both daughters of EMS produced endoderm in 7/7 experiments (data not shown). These results together are consistent with a model that P_2-EMS signaling decreases or inhibits *pop-1(+)* activity in E (Lin et al., 1995) and suggest that *wrm-1(+)* activity plays a role in this process.

Several recent studies have implicated the human colon cancer–associated gene, APC, as a possible regulator of β-catenin (Korinek et al., 1997; Morin et al., 1997; Rubinfeld et al., 1997). We identified from the database a gene related to APC that we call *apr-1*, for *APC-related* gene (Figure 2). We found that ∼26% of *apr-1(RNAi)* embryos lack endoderm, overproduce pharyngeal tissue, and have a premature division of the E daughters (Table 1); these embryos have partial body morphogenesis, in contrast to *wrm-1(RNAi)* embryos that have no body morphogenesis (Figure 3 and data not shown). Laser ablation experiments similar to those described above confirmed that the E blastomere in 2/9 *apr-1(RNAi)* embryos adopts an MS-like fate: E produces pharyngeal tissue and the E daughters divide prematurely. Thus, *apr-1* and *wrm-1* both appear to play a role in endoderm specification, while *wrm-1* may have additional functions required for proper morphogenesis.

We tested 11 other genes that showed sequence similarity to components of the Wnt/WG-signaling pathway for their effects on endoderm development (see Experimental Procedures). None of our experiments on two *disheveled*-related genes resulted in endoderm defects. However, experiments on one of five *wingless*-related genes and one of three *frizzled*-related genes caused endoderm defects. Because these same two genes were identified independently in our genetic screens, they are described below.

The *mom* Genes and P_2-EMS Signaling

In genetic screens, we identified 13 independent mutants that produce embryos that lack endoderm and that have abnormally large amounts of pharyngeal tissue; we call these *mom* mutants, for more mesoderm. These mutants identified five genes: *mom-1* (five alleles),

Table 1. Endoderm Specification

Embryo Type	% Embryos Lacking Endoderm (n)	MS/E Cleavage Timing[a]		
		0–5 min	6–15 min	16–25 min
Wild-Type	0	0	0	5
pop-1(zu189)	0 (350)	ND	ND	ND
(RNAi)	0 (200)	7[b]	0	0
wrm-1(RNAi)	100 (179)	14	0	0
apr-1(RNAi)	26 (383)	5	5	3
mom-1(se2)	62 (144)	2	2	0
(ne117)	62 (100)	ND	ND	ND
(zu188)	56 (296)	ND	ND	ND
(zu204)	41 (397)	ND	ND	ND
(zu237)	41 (100)	ND	ND	ND
mom-2(ne141)	39 (879)	4	3	2
(RNAi)	14 (269)	0	2	8
(ne141)/mDf3	40 (322)	ND	ND	ND
(ne141)/(RNAi)	67 (196)	ND	ND	ND
mom-5(ne12)	5 (63)	0	2	2
(zu193)	5 (126)	0	2	10
(RNAi)	2 (186)	0	0	7
(zu193)/(RNAi)	8 (229)	ND	ND	ND

Development of the E Blastomere.
[a]Wild-type E daughters divide 20 to 25 min after MS daughters (see Figure 1). The time between MS and E divisions has been broken into three intervals. The number of embryos that exhibited E division within each interval is indicated. For example, in all 14 *wrm-1(RNAi)* lineages examined, the E daughters divided within a 5 min interval following the division of the MS daughters.
[b]In *pop-1(RNAi)* embryos, MS daughters adopt an E-like cleavage and divide later than normal.
ND, not determined.

mom-2 (one allele), *mom-3* (one allele), *mom-4* (four alleles), and *mom-5* (two alleles). In this paper, we describe the *mom-1*, *mom-2*, and *mom-5* genes, which we have cloned. All of the mutations in the *mom* genes cause maternal effect lethality (see Experimental Procedures). One hundred percent of the embryos produced by homozygous *mom* mutant mothers fail to hatch and exhibit severe abnormalities in morphogenesis (Figure 3).

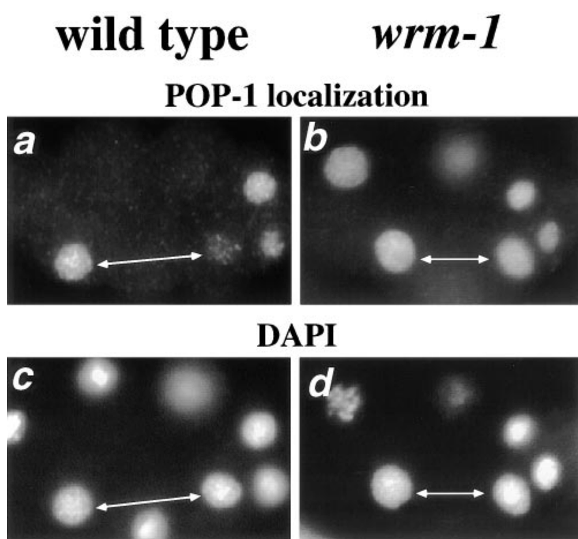

Figure 4. POP-1 Localization

Immunofluorescence micrographs of a wild-type and a *wrm-1(RNAi)* embryo at the 8-cell stage stained with mABRL2, an antibody that recognizes the POP-1 protein (a and b) and with DAPI to visualize nuclei (c and d). Each double arrow points to an MS nucleus (left end) and an E nucleus (right end).

The percentage of embryos lacking endoderm varies in the different *mom* mutants and also varies among broods from individuals in each strain. For example, a total of 39% of the embryos from *mom-2(ne141)* mutants lacks endoderm (Table 1; see also Figure 5a), but this percentage varies between 14% to 76% in individual broods; embryos lacking endoderm invariably have large amounts of pharyngeal tissue. Laser ablation experiments as described above showed that in *mom-1*

Table 2. Genetic Analysis of Endoderm Development

Embryo Type	% Embryos Lacking Endoderm (n)
pop-1(zu189);wrm-1(RNAi)	0 (86)
pop-1(zu189);apr-1(RNAi)	0 (173)
pop-1(RNAi);wrm-1(RNAi)	0 (69)
pop-1(RNAi);apr-1(RNAi)	0 (121)
pop-1(RNAi);mom-2(ne141)	0 (88)
pop-1(RNAi);mom-5(zu193)	0 (47)
wrm-1(RNAi);apr-1(RNAi)	100 (134)
wrm-1(RNAi);mom-2(ne141)	98 (256)
wrm-1(RNAi);mom-5(RNAi)	100 (79)
mom-2(ne141)	39 (879)
mom-5(zu193)	5 (126)
mom-2(ne141);mom-5(zu193)	8 (200)
mom-2(ne141);mom-5(RNAi)	8 (607)
mom-2(RNAi);mom-5(RNAi)	7 (259)
apr-1(RNAi);	26 (383)
apr-1(RNAi);mom-2(ne141)	99 (145)
apr-1(RNAi);mom-2(RNAi)	98 (326)
apr-1(RNAi);mom-5(zu193)	100 (98)
apr-1(RNAi);mom-5(RNAi)	99 (237)
apr-1(RNAi);mom-2(ne141);pop-1(RNAi)	0 (58)
apr-1(RNAi);mom-2(ne141);mom-5(RNAi)	100 (160)

Endoderm Formation.

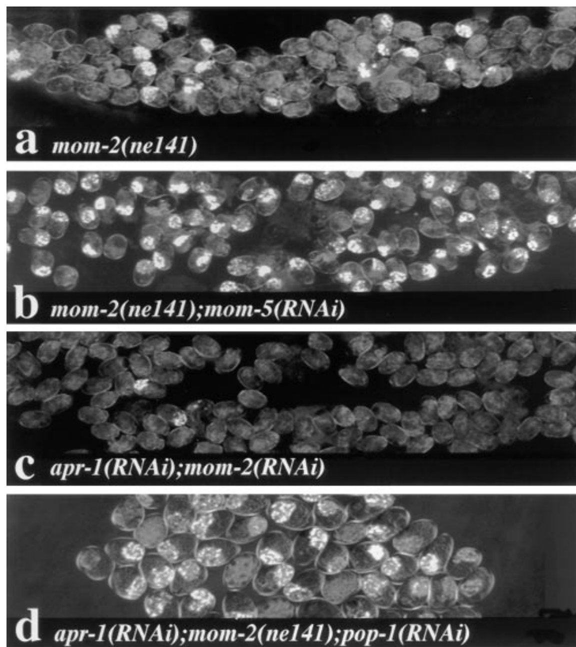

Figure 5. Endoderm Differentiation

(a)–(d) each show a field of embryos viewed with polarization microscopy; endoderm-specific granules are birefrigent and appear white.

and *mom-2* embryos the MS blastomere produces pharyngeal tissue as in wild-type embryos; however, the E blastomere also adopts an MS-like fate: E produces pharyngeal cells, and the E daughters divide prematurely. The percentage of isolated E blastomeres with this pattern of development correlated with the expected frequencies of embryos lacking endoderm: E produced pharyngeal cells instead of endoderm in 10/16 *mom-1(se2)* mutants, 4/10 *mom-2(ne141)* mutants, and 2/15 *mom-5(zu193)* mutants. We stained 8-cell stage *mom-1(se2)* mutants for POP-1 and found that about 65% (n = 30) had higher than normal levels of staining in the E nucleus (data not shown). These results suggest that the *mom* genes are involved in endoderm specification and that they are likely to have a role in P_2-EMS signaling.

In contrast to the variable penetrance of the endoderm defect, all of the *mom-1*, *mom-2*, and *mom-5* embryos examined had defects in the cleavage of one of the early blastomeres, a defect that was not observed in *wrm-1(RNAi)* or *apr-1(RNAi)* embryos, or in *pop-1* mutants or *pop-1(RNAi)* embryos. In a wild-type, 8-cell stage embryo, there are four descendants of the AB blastomere. Three of these descendants have mitotic spindles that are oriented approximately in parallel; however, the spindle of the fourth descendant, ABar, is oriented perpendicularly to the others (Figure 3g). We found that the orientation of the ABar spindle is abnormal in *mom* mutants, such that it is parallel to that of the other AB descendants (Figure 3h). The ABar spindle was also abnormal in 4/4 *mom-2(ne141);pop-1(RNAi)* embryos. Because *pop-1* mutants and *pop-1(RNAi)* embryos have normal ABar spindles, these observations suggest that *mom-2(+)* acts through a *pop-1*-independent mechanism to control spindle orientation (see Discussion).

To determine if mutations in the *mom* genes cause cleavage defects in other lineages, we examined the development of the MS blastomere in *mom-1(se2)* mutants. We found that certain MS descendants that have unequal cleavages in wild-type embryos instead have equal or reversed cleavages in *mom-1* mutants: defects were observed in 5/5 embryos examined, although the nature of the polarity defect was variable (Figure 1). One of these polarity defects correlated with a defect in cell differentiation. In wild-type embryos, an MS descendant called MSpaapp is born in an unequal cleavage and soon afterward undergoes programmed cell death. In lineaged *mom-1* mutant embryos, we found that MSpaapp was born in a cleavage that was either equal or reversed and did not undergo programmed cell death (Figure 1). Thus, in addition to their role in the P_2-EMS interaction, the *mom* genes have a role in determining the orientation of the ABar spindle, and at least *mom-1* has a role in determining the proper polarities of later MS divisions.

Molecular Analysis of *mom-1*, *mom-2*, and *mom-5*

Mutations in *mom-1* and *mom-5* were identified in screens using a genetic background in which the transposon Tc1 is mobilized (Mello et al., 1994). We found novel Tc1 insertions that mapped to the physical–genetic locations of the *mom-1* and *mom-5* mutations and used standard techniques to recover the flanking sequences and clone the genes (see Experimental Procedures). We found that *mom-1* encodes a gene related to the Drosophila gene *porcupine* (Kadowaki et al., 1996) and that *mom-5* encodes a member of the *frizzled* gene family (Vinson et al., 1989).

The *mom-2* gene was identified by correlating the genetic position of *mom-2(ne141)* to cloned genes present on the C. elegans genome sequence. Within this interval, we found a gene with homology to *wnt/wg* that gave a *mom-2*-like phenotype when tested by the RNAi assay. We confirmed that this gene was *mom-2* by showing that the *mom-2(ne141)* mutation is a lesion in this gene (Figure 2). Thus, the predicted MOM-2 protein is homologous to WG, and the predicted MOM-1 and MOM-5 proteins are homologous to proteins implicated in the secretion and reception of the Wnt/WG signal, respectively.

Because our mutations in the *mom* genes cause incomplete penetrance of the endoderm defect, we initially expected to find that these mutations would create only minor changes in the predicted protein products. However, many of the mutations in the *mom-1* gene would be predicted to create a severely truncated protein (Figure 2). The predicted MOM-1 protein is 442 amino acids in length with five potential transmembrane domains; the *mom-1(zu188)* mutation would be expected to truncate the MOM-1 protein before the first transmembrane domain at only 47 amino acids. The *mom-2(ne141)* mutation is expected to replace a glycine that is highly conserved in Wnt/WG proteins with an arginine; a glycine to aspartic acid mutation at this same site in the Drosophila *wglN67* mutation appears to block WG secretion and results in a strong, embryonic lethal phenotype resembling the presumptive null phenotype

(van den Heuvel et al., 1993). Similarly, the *mom-5(ne12)* mutation would be expected to truncate the MOM-5 protein before the first transmembrane domain.

Genetic Analysis of Endoderm Specification

To address further whether the variability in the endoderm defect of the *mom* mutants could be attributed to partial activity of the *mom* gene products, we compared the phenotypes of *mom-2* and *mom-5* mutants with *mom-2(RNAi)* and *mom-5(RNAi)* embryos. We found that *mom-2(RNAi)* and *mom-5(RNAi)* embryos lack endoderm at frequencies that are comparable to the *mom-2* or *mom-5* mutants (Table 1). In another test, we constructed and examined *mom-2(ne141);mom-5(zu193)* double mutants. We expected to find the endoderm defect in this double mutant to be at least as severe as in the *mom-2(ne141)* mutant. However, only 8% of these double-mutant embryos lack endoderm compared to 39% of the *mom-2(ne141)* single mutants (Table 2). Essentially identical results were observed in *mom-2(ne141);mom-5(RNAi)* embryos (Table 2 and Figure 5b) and in *mom-2(RNAi);mom-5(RNAi)* embryos (Table 2). Finally, we tested the *mom-2(or42)* allele, which contains a partial deletion of the gene (see accompanying paper, Thorpe et al., 1997 [this issue of *Cell*]), in combination with *mom-5(RNAi)* and found that only ~9% of the resulting embryos lack endoderm (data not shown). The observation that >90% of the embryos with mutations in both *mom-2* and *mom-5* are nevertheless able to produce endoderm suggests that neither of these genes is essential for endoderm development (see Discussion).

Because 26% of the *apr-1(RNAi)* embryos lack endoderm, we asked whether mutations in the *mom* genes would suppress or enhance this defect. We found very strong enhancement in all combinations tested (Table 2). For example, 100% of *apr-1(RNAi);mom-5(zu193)* embryos lack endoderm and produce excess mesoderm, as do 99% of both *apr-1(RNAi);mom-2(ne141)* embryos and *apr-1(RNAi);mom-2(RNAi)* embryos (Table 2 and Figure 5c). Furthermore, we found that 22/22 of the latter embryos showed premature division of the E daughters. To determine if *pop-1(+)* activity is responsible for the lack of endoderm in *apr-1(RNAi);mom-2(ne141)* embryos, we constructed *apr-1(RNAi);mom-2(ne141);pop-1(RNAi)* embryos and found that all such embryos have endoderm (Table 2 and Figure 5d).

Autonomous Endoderm in *pie-1* Mutants May Involve Autocrine Signaling

In addition to signaling the EMS blastomere to produce endoderm, the P_2 blastomere appears to have a latent ability to produce endoderm itself. In wild-type embryos, P_2 is prevented from producing endoderm by the maternal gene *pie-1* (Mello et al., 1992, 1996), which appears to function in a general repression of transcription in P_2 (Seydoux et al., 1996). Although an isolated wild-type or *pie-1* mutant EMS blastomere cannot produce endoderm without P_2 signaling, an isolated *pie-1* mutant P_2 blastomere is able to produce endoderm (Goldstein, 1995). Therefore, we were interested in determining whether the genes described in this paper also are required for the "autonomous" endoderm from P_2 in *pie-1* mutants. We found that in all combinations tested (Table 3), the endoderm produced by the P_2 and EMS blastomeres in *pie-1* mutant embryos shows the same dependence on these genes as does the endoderm produced by the EMS blastomere in *pie-1(+)* embryos. For example, many *pie-1(zu127);mom-5(RNAi)* and *pie-1(zu127);apr-1(RNAi)* embryos have endoderm, while almost no *pie-1(zu127);apr-1(RNAi);mom-5(RNAi)* embryos have endoderm. The observation that the P_2 blastomere requires *wrm-1*, *apr-1*, and the *mom* genes in order to produce endoderm suggests that P_2 may undergo autocrine signaling in *pie-1* mutant embryos.

Table 3. Endoderm Development in *pie-1* Mutant Embryos

Embryo Type	% Embryos Lacking Endoderm (n)
pie-1(zu127)	0 (400)
pie-1(zu127);wrm-1(RNAi)	99 (443)
pie-1(zu127);wrm-1(RNAi);pop-1(RNAi)	0 (173)
pie-1(zu127);apr-1(RNAi)	54 (147)
pie-1(zu127);mom-2(ne141)	9 (200)
pie-1(zu127);apr-1(RNAi);mom-2(RNAi)	93 (659)
pie-1(zu127);apr-1(RNAi);mom-2(RNAi);pop-1(RNAi)	0 (48)
pie-1(zu127);mom-5(zu193)	3 (104)
pie-1(zu127);apr-1(RNAi);mom-5(RNAi)	99 (673)
pie-1(zu127);apr-1(RNAi);mom-5(RNAi);pop-1(RNAi)	0 (91)

Endoderm Formation in *pie-1* Mutants.

Discussion

The *mom* Genes

In an effort to understand how interactions between blastomeres contribute to the numerous a–p differences observed in the early C. elegans embryo, we have begun a genetic analysis of P_2-EMS signaling. In this report, we described the results of genetics screens for *mom* mutants that lack endoderm and that have abnormally large quantities of mesoderm, the terminal phenotype predicted for a mutant defective in P_2-EMS signaling. Our analysis suggests that when the E blastomere fails to produce endoderm in the *mom* mutants, it produces pharyngeal tissues that normally are made by MS. Thus, the *mom* genes appear to play a role in the P_2-EMS signaling pathway as defined by the blastomere isolation and recombination experiments of Goldstein (1992, 1993). Our molecular cloning of the *mom* genes showed that each could encode a protein similar to one of the known components of the Wnt/WG pathway: MOM-2 is related to the signaling protein Wnt/WG and MOM-1 is related to PORC, a protein involved in WG secretion. These molecular results are consistent with the results of Thorpe et al. (1997), showing that in chimeric embryos *mom-2(+)* and *mom-1(+)* functions are required in the signaling blastomere, P_2.

The predicted MOM-5 protein has nearly equal similarity to the Frizzled2 (FZ2) and Frizzled (FZ) proteins of Drosophila. FZ2 and FZ are closely related, serpentine receptor-like transmembrane proteins. FZ2 has been

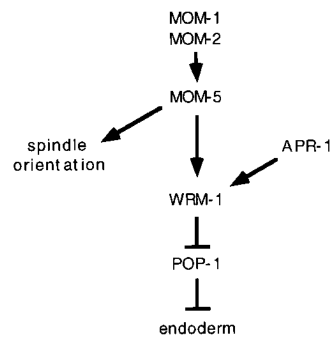

Figure 6. Genetic Model for the Role of the *mom* Genes in Spindle Orientation and in Endoderm Development

wrm-1, *pop-1*, and *apr-1* appear to have no role in determining the early spindle orientations and so are shown on a separate branch leading to endoderm formation. In the absence of signaling (as in the *mom-1* and *mom-2* mutants), negative interactions may exist between *mom-5* and *apr-1* (not shown).

proposed to function as a receptor in WG-mediated signaling events such as cell fate determination (for reviews, see Klingensmith and Nusse, 1994; Orsulic and Peifer, 1996; Perrimon, 1996). FZ appears to function in a signaling pathway that coordinates the cytoskeletal polarities of epidermal cells (for review, see Adler, 1992). Relatively little is known about the molecular components of the FZ pathway; however, the only component known to be shared with the WG pathway is Disheveled (Gubb, 1993; Theisen et al., 1994). A recent study has implicated a RhoA homolog in the FZ pathway and suggested possible similarities with G protein–mediated pheromone signaling in yeast (Strutt et al., 1997). Thus, FZ and FZ2 may function in distinct pathways, either or both of which may be relevant to understanding *mom-5* function in C. elegans. Because the *mom* mutants are defective in both cytoskeletal polarity and cell fate determination, we discuss these defects separately below.

The Role of the *mom* Genes in Spindle Orientation

We have shown that *mom-1*, *mom-2*, and *mom-5* mutants have fully penetrant defects in the mitotic spindle orientation of an early AB descendant called ABar, and lineage analysis of *mom-1* embryos revealed additional, later defects in the cleavage polarities of other blastomeres. Although the effect of P_2 removal on the ABar spindle has not been reported, laser ablation of the P_2 blastomere markedly alters the development of ABar while having no, or minor, effects on the development of the other early AB descendants (Hutter and Schnabel, 1995; Schnabel, 1995). There appear to be complex and distinct mechanisms that control the normally invariant spindle orientations of early blastomeres in C. elegans (see Goldstein et al., 1993, for review). G protein signaling is implicated in these controls: mutations in *gbp-1*, a gene encoding the G β subunit, cause the early mitotic spindles to be randomly oriented, and the GBP-1 protein localizes to the spindle asters of dividing blastomeres (Zwaal et al., 1996). Thus, the *mom* genes play a role in spindle orientation (Figure 6), and it is possible that this role involves G proteins.

As discussed below, *pop-1* and *wrm-1* may function downstream of the *mom* genes to regulate transcriptional events required for endoderm specification; however, we have not detected a function for these genes in controlling spindle orientations. Thus, the role of the *mom* genes in spindle orientation may either not involve transcription or may involve transcriptional regulators other than *pop-1*. Although most models for Wnt/WG signaling emphasize transcriptional or chromatin targets of this pathway, early Xenopus laevis embryos that are transcriptionally silent have been shown to respond to Wnt-1 class members by increasing gap junctional permeability (Olson et al., 1991). These and similar results have suggested that chromatin cannot be the sole target of Wnt signaling (for review, see Moon et al., 1997). In experiments to address which features of early C. elegans development require transcription, Powell-Coffman et al. (1996) inhibited transcription by injecting antisense RNA from the *ama-1* gene into the gonads of wild-type adults and analyzed the resulting embryos; the *ama-1* gene encodes the large subunit of RNA polymerase. They found that all blastomeres had normal cleavages until the 26-cell stage of embryogenesis, consistent with earlier observations that alpha-amanatin-treated embryos appeared to have normal early cleavages (Edgar et al., 1994). We have repeated similar *ama-1(RNAi)* experiments to examine the orientation of the ABar spindle specifically and found that it was normal (unpublished data). The observation that spindle orientations in early wild-type blastomeres do not appear to be determined by transcriptional events suggests that *mom* mutants disrupt spindle orientations by nontranscriptional effects or through inappropriate transcription.

The Role of *wrm-1*, *apr-1*, and the *mom* Genes in Determining the E Fate

In wild-type embryogenesis, one of the first visible consequences of P_2-EMS signaling is the delayed division of the E daughters relative to the division of the MS daughters. This delay does not occur in transcription-inhibited embryos (Powell-Coffman et al., 1996), in *wrm-1(RNAi)* embryos, *apr-1(RNAi)* embryos, or in *mom* mutant embryos. Thus, we propose that each of these genes, at some level, is involved in an early transcription-mediated event that makes E different from MS and ultimately leads to E producing endoderm rather than mesoderm. We have shown that *wrm-1* can encode a protein related to β-catenins such as the Armadillo protein. In Drosophila, Armadillo has been shown to have functions in cell signaling through the WG pathway, in addition to functioning in cell adhesion (see Peifer, 1995, for review). We see no evidence of defects in cell adhesion among the early blastomeres in *wrm-1(RNAi)* embryos, and recent studies have shown that a second β-catenin-like protein, HMP-2, is expressed in these blastomeres: *hmp-2* mutants and *hmp-2(RNAi)* embryos show no defects in endoderm development (M. Costa and J. R. P., unpublished data). Thus, the early C. elegans embryo contains at least two β-catenin-like proteins that may have different functions. Mutations in the maternal gene *pop-1*, and in *pop-1(RNAi)*, allow endoderm to form in *wrm-1(RNAi)* embryos and allow the E blastomere to produce endoderm independent of

P_2-EMS signaling (Lin et al., 1995; this paper). Thus, we propose that the E fate in normal development requires low or no *pop-1(+)* activity and that the *wrm-1* gene product is required to repress *pop-1(+)* activity (Figure 6). In wild-type embryos, an antiserum against the POP-1 protein shows a relatively low level of staining in the E nucleus compared to the MS nucleus; we have shown that MS and E in *wrm-1(RNAi)* have comparable, high levels of POP-1 staining. Thus, it is possible that *wrm-1(+)* activity decreases the absolute level of POP-1 protein in the E nucleus or that the mechanism by which *wrm-1(+)* represses *pop-1(+)* activity alters the ability of POP-1 to be recognized in immunostaining experiments. POP-1 is an HMG domain protein related to the vertebrate transcription factors Lef-1 or Tcf-1 and Drosophila PAN. Therefore, we propose that *wrm-1* plays a role in transcriptional regulation through *pop-1*. In current models of Wnt/WG-signaling pathways, β-catenin has been proposed to form a complex with a LEF-1-related protein to activate transcription. Therefore, mutations in the genes that encode LEF-1 and β-catenin would be predicted to result in similar phenotypes, while our results show that *pop-1* mutants and *wrm-1(RNAi)* embryos have opposite phenotypes. Thus, *pop-1* and/or *wrm-1* may have novel roles in the Wnt/WG-like pathway in C. elegans.

The APC protein has been shown to interact with β-catenin and glycogen synthase kinase 3 (GSK-3), both of which are components of the Wnt/WG-signaling pathway (for review, see Peifer, 1996). Recent studies have shown that APC and β-catenin appear to have similar properties in axis-inducing assays in Xenopus (Vleminckx et al., 1997). We have analyzed the C. elegans APC-related gene *apr-1* and have shown that *apr-1(RNAi)* embryos and *wrm-1(RNAi)* embryos have similar defects in the development of the E blastomere and do not affect the early spindle orientations, suggesting that *apr-1* might function with *wrm-1* in the P_2-EMS-signaling pathway leading to E specification.

E Specification May Involve Multiple or Branched Pathways

In contrast to *wrm-1(RNAi)* embryos, all of which lack endoderm, the *mom* mutants produce many embryos that contain endoderm. For example, endoderm is present in about 40% and 90% of the *mom-2* and *mom-5* mutant embryos, respectively. The incomplete penetrance of the endoderm defect contrasts with the completely penetrant defects in spindle orientation caused by every *mom-1*, *mom-2*, and *mom-5* allele we identified. Incompletely penetrant defects could simply result from non-null alleles. However, our molecular analysis suggests that many of the mutations in the *mom* genes should result in truncated or severely defective proteins. For example, the *mom-5(ne12)* mutation is a stop codon before the first of the seven transmembrane spanning domains, making it difficult to imagine how this mutant protein would retain partial function in signal transduction. We also do not consider it likely that the *mom-5(ne12)* mutation creates a partial protein with novel patterns of interaction with other components of the signaling pathway, because *mom-5(RNAi)* embryos exhibit phenotypes identical to our mutant alleles.

How is endoderm specified in the absence of wild-type *mom* gene products? We have shown that although only 26% of *apr-1(RNAi)* embryos lack endoderm when *mom-2(+)* and *mom-5(+)* activities are both present, *apr-1(+)* function becomes essential for endoderm formation when either *mom-2(+)* or *mom-5(+)* activities are not present. A simple interpretation of these results is that there are partially redundant inputs into the specification of the E fate (and thus endoderm formation). The first input is the product of a linear pathway containing the *mom-1, mom-2,* and *mom-5* genes and the second input involves *apr-1*. Both inputs might then converge to alter *wrm-1(+)* activity and consequently *pop-1(+)* activity (Figure 6).

If the *mom* genes provide only a single, linear input into endoderm formation, null alleles in all *mom* genes might be expected to cause similar percentages of embryos with endoderm. However, we have shown that 90% of *mom-5* mutants have endoderm, compared to much lower percentages in *mom-1* or *mom-2* embryos. Because removing *mom-5(+)* activity in a *mom-2* mutant background (as in the *mom-2;mom-5* double mutant) greatly increases the number of embryos that have endoderm, this result suggests that *mom-5(+)* activity has a negative influence on endoderm formation when *mom-2(+)* activity is not present. Several models are consistent with this result; for example, as an extension of the model presented above, it is possible that when the *mom-2* signal is not present, *mom-5(+)* activity has a negative effect on the *apr-1* input to endoderm formation. Genetic interactions between WG and FZ2 have not yet been analyzed in Drosophila; however, complex genetic interactions analogous to those reported here have also been observed in postembryonic development in C. elegans between a second *Wnt/wg*-like gene, *lin-44,* and a *fz*-like gene, *lin-17* (Sawa et al., 1996).

In summary, our results show that the MOM proteins are related to components of the Wnt/WG-signaling pathway and are required for proper cytoskeletal polarity as well as cell fate determination in the early C. elegans embryo. However, the *mom* pathway appears to involve some complexities compared to models for the Wnt/WG pathway. First, our results suggest that the effect of the *mom* genes on endoderm formation is mediated by *wrm-1* and *pop-1* but that the effect of the *mom* genes on spindle orientation is not mediated by either *wrm-1* or *pop-1*. Second, WRM-1 and/or POP-1 may have different roles than Drosophila ARM and PAN/dTCF, respectively, since *pop-1* mutants and *wrm-1(RNAi)* embryos have opposite phenotypes. Finally, our results provide evidence that the specification of the E fate may involve parallel inputs provided by the *mom* genes and *apr-1*. The ability to isolate and recombine the blastomeres involved in these interactions plus the ability to test rapidly by RNAi experiments whether specific genes are involved suggest that a detailed molecular understanding of these interactions in the early C. elegans embryo should be possible.

Experimental Procedures

Strains and Alleles

The Bristol strain N2 was used as the standard wild-type strain. The marker mutations, deficiencies, and balancer chromosomes used

are listed by chromosome as follows: LGI: *pop-1(zu189), dpy-5(e61), unc-13(e1091);nDf9, nDf24, qDf6, mnDf111, lin-11(n566), hT1(I;IV), hT2(I;III)*; LGII: *bli-2(e768)*; LGIII: *unc-32(e189), dpy-18(e364), eT1(III;V)*; LGIV: *unc-5(e53), DnT1(IV;V)*; LGV: *dpy-11(e224), unc-42(e270), sDf35, mDf3, him-5(e1409)*; LGX: *lin-2(e1309), lon-2(e678)*. C. elegans culture, mutagenesis, and genetics were as described in Brenner (1974).

Genetic Analysis

Mutant alleles were isolated as described previously (Mello et al., 1992, 1994). Standard genetic crosses were used to map *mom-1(se2)* to the *dpy-8*, *unc-6* interval on LGX, *mom-2(ne141)* to the *dpy-11*, *unc-42* interval of LGV, and *mom-5(zu193)* and *mom-5(ne12)* to the *unc-13*, *lin-11* interval on LGI. Data from these crosses are available from the C. elegans data base, ACEDB. Self progeny from *mom-1(se2)*, *mom-2(ne141)*, or *mom-5(zu193)* adult hermaphrodites appeared identical in all respects to cross progeny by wild-type males, indicating that these gene activities are required maternally.

Complementation tests were performed as follows: *mom-1(se2)* against all other *mom-1* alleles, *mom-2(ne141)* against *mom-2(or42)*, *mom-2(ne141)* against the chromosomal deficiency *mDf3*, *mom-5(zu193)* against *mom-5(ne12)*. In all cases, heterozygotes grew to adults that produced dead embryos resembling those produced by the test strain (first listed). All *mom-1* mutant strains have a noncomplementing, incompletely penetrant vulva defect as adults.

RNAi Reverse Genetics

Templates for RNA synthesis were produced from PCR directly on cDNA phage lysates using T7 and T3 primers. For genomic clones, PCR primers were chosen to span exons and generate fragments between 0.5 and 2 kb in size. A nested pair of PCR primers, one containing the T7 promoter (5′TAATACGACTCACTATAGGGAGACC AC3′), was used for a second round of PCR. RNAs were synthesized using T7 polymerase and commercially available synthesis kits. Unmodified RNA was resuspended for injection at 1 to 5 mg/ml in DEPC-treated water. Microinjection into both arms of hermaphrodite gonads was performed as described in Mello et al. (1991). Preliminary studies indicate that cosuppression of different but related genes requires at least 70% to 80% nucleotide sequence identity (C. C. M., unpublished data).

Coinjection of two RNAs appears to result in the phenotype equivalent to that observed in doubles constructed with actual mutant alleles for the corresponding genes (C. C. M., unpublished data; this study). Because RNA injections might fail for technical reasons, additional controls were performed where epistasis was expected. For example, *wrm-1(RNAi);pop-1(RNAi)* coinjections yield a phenotype identical to *pop-1(RNAi)* singles, as do injections of *wrm-1(RNAi)* into *pop-1(zu189)* mutant mothers. To ensure that *wrm-1* activity was blocked, we injected wild-type animals with *wrm-1(RNAi)* first, followed 12 hr later by a second injection of *pop-1(RNAi)*. These sequential assays allowed us first to confirm conversion to the *wrm-1* no-endoderm phenotype, followed by conversion to the *pop-1(RNAi)* extra-endoderm phenotype. Performing the injections in the opposite order resulted in the same final result, embryos with extra endoderm.

RNAs from the following clones or genes were tested but failed to induce embryonic phenotypes. Wnt-related: *lin-44*(yk120c7), *cwn-1, cwn-2,* and W08D2.1. Frizzled-related: Y34D9.00718/(yk107h2) and F27E11/(yk117b4). Disheveled-related: C34F11.9a/(yk10b11), yk46b11. We do not know whether these genes have no function in the early embryo or whether the RNA procedure did not inhibit the function of these genes.

Molecular Analysis

The genes described in this study correspond to completely sequenced genomic clones. Coding sequences were determined by sequencing cDNA clones or by sequencing RT-PCR products amplified using primers predicted from genomic sequence. Our results for *mom-5*/T23D8.1 and *apr-1*/K04G2.8 were consistent with Genefinder predictions. Our results for *mom-1*/T07H6.2, *mom-2*/F38E1.7, and *wrm-1*/B0336.1 did not agree with Genefinder predictions; these differences are detailed in the GenBank accessions listed in this paper. Mutant alleles were sequenced using standard protocols from PCR-amplified genomic DNA.

Microscopy

Light and immunofluorescence microscopy and laser microsurgery were as described in Bowerman et al. (1992a, 1993). The identity of differentiated cells was assigned based on morphological criteria in the light microscope, followed in most cases by fixation and staining with tissue-specific probes. Criteria for assigning cell fates and antibodies were as described in Bowerman et al. (1992a) and Mello et al. (1992); the mABRL2 antibody has staining properties similar to the 94I antiserum (Lin et al., 1995) and will be described elsewhere.

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References

Adler, P.N. (1992). The genetic control of tissue polarity in Drosophila. Bioessays *14*, 735–741.

Adler, P.N., Vinson, C., Park, W.J., Conover, S., and Klein, L. (1990). Molecular structure of frizzled, a Drosophila tissue polarity gene. Genetics *126*, 401–416.

Bhanot, P., Brink, M., Samos, C.H., Hsieh, J.-C., Wang, Y., Macke, J.P., Andrew, D., Nathans, J., and Nusse, R. (1996). A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature *382*, 225–230.

Bowerman, B., Eaton, B.A., and Priess, J.R. (1992a). *skn-1*, a maternally expressed gene required to specify the fate of ventral blastomeres in the early C. elegans embryo. Cell *68*, 1061–1075.

Bowerman, B., Tax, F.E., Thomas, J.H., and Priess, J.R. (1992b). Cell interactions involved in development of the bilaterally symmetrical intestinal valve cells during embryogenesis in *Caenorhabditis elegans*. Development *116*, 1113–1122.

Bowerman, B., Draper, B.W., Mello, C.C., and Priess, J.R. (1993). The maternal gene *skn-1* encodes a protein that is distributed unequally in early C. elegans embryos. Cell *74*, 443–452.

Brenner, E. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71–94.

Brunner, E., Peter, O., Schweizer, L., and Basler, K. (1997). pangolin encodes a Lef-1 homologue that acts downstream of Armadillo to transduce the Wingless signal in Drosophila. Nature *385*, 829–833.

Edgar, L.G., Wolf, N., and Wood, W.B. (1994). Early transcription in Caenorhabditis elegans embryos. Development *120*, 443–451.

Goldstein, B. (1992). Induction of gut in Caenorhabditis elegans embryos. Nature *357*, 255–257.

Goldstein, B. (1993). Establishment of gut fate in the E lineage of C. elegans: the roles of lineage-dependent mechanisms and cell interactions. Development *118*, 1267–1277.

Goldstein, B. (1995). An analysis of the response to gut induction in the C. elegans embryo. Development *121* 1227–1236.

Goldstein, B., and Hird, S.N. (1996). Specification of the anteroposterior axis in Caenorhabditis elegans. Development *122*, 1467–1474.

Goldstein, B., Hird, S.N., and White, J.G. (1993). Cell polarity in early C. elegans development. Development *Supplement*, 279–287.

Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gerlbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., et al. (1991). Identification and characterization of the familial adenomatous polyposis coli gene. Cell *66*, 589–600.

Gubb, D. (1993). Genes controlling cellular polarity in Drosophila. Development *Supplement*, 269–277.

Guedes, S., and Priess, J.R. (1997). The C. elegans MEX-1 protein is present in germline blastomeres and is a P granule component. Development *124*, 731–739.

Guo, S., and Kemphues, K.J. (1995). *par-1*, a gene required for establishing polarity in C. elegans embryos, encodes a putative Ser/Thr kinase that is asymmetrically distributed. Cell *81*, 611–620.

Guo, S., and Kemphues, K.J. (1996). Molecular genetics of asymmetric cleavage in the early Caenorhabditis elegans embryo. Curr. Opin. Genet. Dev. *6*, 408–415.

Hülsken, J., Behrens, J., and Birchmeier, W. (1994). Tumor-suppressor gene products in cell contacts: the cadherin-APC-armadillo connection. Curr. Opin. Cell Biol. *6*, 711–716.

Hutter, H., and Schnabel, R. (1995). Specification of anterior-posterior differences within the AB lineage in the C. elegans embryo: a polarizing induction. Development *121*, 1559–1568.

Kadowaki, T., Wilder, E., Klingensmith, J., Zachary, K., and Perrimon, N. (1996). The segment polarity gene *porcupine* encodes a putative multitransmembrane protein involved in Wingless processing. Genes Dev. *10*, 3116–3128.

Kemphues, K.J., and Strome, S. (1996). Fertilization and establishment of polarity in the embryo. In The Nematode C. elegans, T.B.D. Riddle, B.J. Meyer, and J. Priess, eds. (New York: Cold Spring Harbor Laboratory), pp. 335–359.

Klingensmith, J., and Nusse, R. (1994). Signaling by wingless in Drosophila. Dev. Biol. *166*, 396–414.

Korinek, V., Barker, N., Morin, P.J., van Wichen, D., de Weger, R., Kinzler, K.W., Vogelstein, B., and Clevers, H. (1997). Constitutive transcriptional activation by a β-catenin-Tcf complex in APC-/- colon carcinoma. Science *275*, 1784–1787.

Lin, R., Thompson, S., and Priess, J.R. (1995). *pop-1* encodes an HMG box protein required for the specification of a mesoderm precursor in early C. elegans embryos. Cell *83*, 599–609.

Mello, C.C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in C. elegans: extrachromosomal maintenance and integration of transforming sequences. EMBO J. *10*, 3959–3970.

Mello, C.C., Draper, B.W., Krause, M., Weintraub, H., and Priess, J.R. (1992). The *pie-1* and *mex-1* genes and maternal control of blastomere identity in early C. elegans embryos. Cell *70*, 163–176.

Mello, C.C., Draper, B.W., and Priess, J.R. (1994). The maternal genes *apx-1* and *glp-1* and establishment of dorsal–ventral polarity in the early C. elegans embryo. Cell *77*, 95–106.

Mello, C.C., Schubert, C., Draper, B., Zhang, W., Lobel, R., and Priess, J.R. (1996). The PIE-1 protein and germline specification in C. elegans embryos. Nature *382*, 710–712.

Moon, R.T., Brown, J.D., and Torres, M. (1997). Wnts modulate cell fate and behavior during vertebrate development. Trends Genet. *13*, 157–162.

Morin, P.J., Sparks, A.B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K.W. (1997). Activation of beta-Catenin-Tcf signaling in colon cancer by mutations in beta-Catenin or APC. Science *275*, 1787–1790.

Nusse, R., and Varmus, H.E. (1992). Wnt genes. Cell *69*, 1073–1087.

Olson, D.J., Christian, J.L., and Moon, R.T. (1991). Effect of Wnt-1 and related proteins on gap junctional communication in Xenopus embryos. Science *252*, 1173–1176.

Orsulic, S., and Peifer, M. (1996). Cell-cell signaling: Wingless lands at last. Curr. Biol. *6*, 1363–1367.

Peifer, M. (1995). Cell adhesion and signal transduction: the Armadillo connection. Trends Cell Biol. *5*, 224–229.

Peifer, M. (1996). Regulating cell proliferation: as easy as APC. Science *272*, 974–975.

Perrimon, N. (1996). Serpentine proteins slither into the Wingless and Hedgehog fields. Cell *86*, 513–516.

Powell-Coffman, J.A., Knight, J., and Wood, W.B. (1996). Onset of C. elegans gastrulation is blocked by inhibition of embryonic transcription with an RNA polymerase antisense RNA. Dev. Biol. *178*, 472–483.

Riggleman, R. (1989). Molecular analysis of the armadillo locus; uniformly distributed transcripts and a protein with novel internal repeats are associated with a Drosophila segment polarity gene. Genes Dev. *3*, 96–113.

Rijsewijk, F., Schuermann, M., Wagenaar, E., Parren, P., Weigel, D., and Nusse, R. (1987). The Drosophila homolog of the mouse mammary oncogene *int-1* is identical to the segment polarity gene *wingless*. Cell *50*, 649–657.

Rubinfeld, B., Robbins, P., El-Gamil, M., Albert, I., Porfiri, E., and Polakis, P. (1997). Stabilization of β-catenin by genetic defects in melanoma cell lines. Science *275*, 1790–1792.

Sawa, H., Lobel, L., and Horvitz, H.R. (1996). The Caenorhabditis elegans gene lin-17, which is required for certain asymmetric cell divisions, encodes a putative seven-transmembrane protein similar to the Drosophila frizzled protein. Genes Dev. *10*, 2189–2197.

Schnabel, R. (1995). Duels without obvious sense: counteracting inductions involved in body wall muscle development in the Caenorhabditis elegans embryo. Development *121*, 2219–2232.

Seydoux, G., Mello, C.C., Pettitt, J., Wood, W.B., Priess, J.R., and Fire, A. (1996). Repression of gene expression in the embryonic germ lineage of C. elegans. Nature *382*, 713–716.

Strutt, D.I., Weber, U., and Mlodzik, M. (1997). The role of RhoA in tissue polarity and Frizzled signaling. Nature *387*, 292–295.

Theisen, H., Purcell, J., Bennett, M., Kansagara, D., Syed, A., and Marsh, J.L. (1994). *dishevelled* is required during wingless signaling to establish both cell polarity and cell identity. Development *120*, 347–360.

Thorpe, C.J., Schlesinger, A., Carter, J.C., and Bowerman, B. (1997). Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm. Cell, this issue, *90*, 695–705.

van de Wetering, M., Cavallo, R., Dooijes, D., van Beest, M., van Es, J., Loureiro, J., Ypma, A., Hursh, D., Jones, T., Bejsovec, A., Peifer, M. et al. (1997). Armadillo coactivates transcription driven by the product of the Drosophila segment polarity gene dTCF. Cell *88*, 789–799.

van den Heuvel, M., Harryman-Samos, C., Klingensmith, J., Perrimon, N., and Nusse, R. (1993). Mutations in the segment polarity genes wingless and porcupine impair secretion of the wingless protein. EMBO J. *12*, 5293–5302.

Vinson, C.R., Conover, S., and Adler, P.N. (1989). A Drosophila tissue polarity locus encodes a protein containing seven potential transmembrane domains. Nature *338*, 263–264.

Vleminckx, K., Wong, E., Guber, K., Rubinfeld, B., Polakis, P., and Gumbiner, B.M. (1997). Adenomatous polyposis coli tumor suppressor protein has signaling activity in Xenopus laevis embryos resulting in the induction of an ectopic dorsoanterior axis. J. Cell Biol. *136*, 411–420.

Wainwright, B.J., Scambler, P.J., Stanier, P., Watson, E.K., Bell, G., Wicking, C., Estivill, X., Courtney, M., Boue, A., Pedersen, P.S., et al. (1988). Isolation of a human gene with protein sequence similarity to human and murine int-1 and the Drosophila segment polarity mutant wingless. EMBO J. *7*, 1743–1748.

Zwaal., R.R., Ahringer, J., van Luenen, H.G.A.M., Rushforth, A., Anderson, P., and Plasterk, R.H.A. (1996). G proteins are required for spatial orientation of early cell cleavages in C. elegans embryos. Cell *86*, 619–629.

GenBank Accession Number

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