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 P2 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, 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 embryogenesis, 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 Hir, 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 P2 (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 P2 blastomere. If P2 is prevented from contacting EMS, neither EMS daughter produces endoderm, and both daughters adopt MS-like fates. P2 normally contacts the posterior surface of EMS; however, if P2 is repositioned to contact the anterior surface, the fates of the EMS daughters are interchanged. Thus, P2 induces both endoderm formation and the a-p polarity of the EMS blastomeres; we refer to this induction as P2-EMS signaling.

Previous studies suggested that P2-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 Watering 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 VarMS, 1992; Klingensmith and Nusse, 1994; and Moon et al., 1997, for reviews). Thus, P2-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 P2-EMS signaling and mutations in pop-1 cause opposite phenotypes (Lin et al., 1995).

To determine the molecular basis for P2-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 P2-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 P2-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 P2-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...
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.

et al., 1992a). However, none of the mutations reported thus far cause the specific transformations predicted from a defect in P2-EMS signaling. When P2-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 P2-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 b-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/b-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 P2-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)
embryos by killing all other blastomeres with a laser microbeam after the period that P2-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-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 P2-EMS signaling suggests that the wrm-1 gene plays an essential role in P2-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 P2 signaling, we removed the P2 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 P2-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 P2-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),
Development of the E Blastomere.

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.

ND, not determined.

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

<table>
<thead>
<tr>
<th>Embryo Type</th>
<th>% Embryos Lacking Endoderm (n)</th>
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<tr>
<td>pop-1(zu189);wrm-1(RNAi)</td>
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<tr>
<td>pop-1(zu189);apr-1(RNAi)</td>
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<tr>
<td>pop-1(RNAi);apr-1(RNAi)</td>
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<tr>
<td>pop-1(RNAi);mom-2(ne141)</td>
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<td>pop-1(RNAi);mom-5(zu193)</td>
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<tr>
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<td>wrm-1(RNAi);mom-2(ne141)</td>
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<td>wrm-1(RNAi);mom-5(RNAi)</td>
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<tr>
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<td>mom-5(zu193)</td>
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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).
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 P2-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 AB bar 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 P2-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 wgIN67 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 P2 blastomere appears to have a latent ability to produce endoderm itself. In wild-type embryos, P2 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 P2 (Seydoux et al., 1996). Although an isolated wild-type or pie-1 mutant EMS blastomere cannot produce endoderm without P2 signaling, an isolated pie-1 mutant P2 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 P2 in pie-1 mutants. We found that in all combinations tested (Table 3), the endoderm produced by the P2 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 P2 blastomere requires wrm-1, apr-1, and the mom genes in order to produce endoderm suggests that P2 may undergo autocrine signaling in pie-1 mutant embryos.

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 P2-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 P2-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 P2-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, P2.

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

Table 3. Endoderm Development in pie-1 Mutant Embryos

<table>
<thead>
<tr>
<th>Embryo Type</th>
<th>% Embryos Lacking Endoderm (n)</th>
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<td>pie-1(zu27)</td>
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<tr>
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<tr>
<td>pie-1(zu27);apr-1(RNAi)</td>
<td>54 (147)</td>
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<td>pie-1(zu27);mom-2(ne141)</td>
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<td>pie-1(zu27);apr-1(RNAi);mom-5(RNAi);pop-1(RNAi)</td>
<td>0 (91)</td>
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Endoderm Formation in pie-1 Mutants.
proposed to function as a receptor in WG-mediated signaling events such as cell fate determination (for reviews, see Klingensmith and Nusse, 1994; Ornulic 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 and apr-1 functions 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 P₂ removal on the ABar spindle has not been reported, laser ablation of the P₂ 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 Gβ 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-amanitin-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.
P~T-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, ~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 ~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 ~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 ~catenin appear to have similar properties in axis-inducing assays in Xenopus (Vlieghe and Clark, 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~T-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(ne189), dpy-5(e61), unc-13(e1091), ndpD42, qd26, mnD111, lin-11(566), hti-19/4, hti-29/1. LGII: bli-2(e768). LGIII: unc-32(e189), dpy-18(e364), et111(yj2). LGIV: unc-5(e53), DntT1(yj7), LGV: dpy-11(e224), unc-42(e270), dF35, mD3, him-5(e1409), LGX: lin-2(e1309), lon-6(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(e20) 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(ne2), mom-2(ne141), mom-2(zu193) and mom-5(ne12) 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(ne2) against all other mom-1 alleles, mom-2(ne141) against mom-2(or24), mom-2(ne141) against the chromosomal deficiency mD3, 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'TAACAGCTAATAAGGGAGACC 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 hemaphroditic 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 injection might fail for technical reasons, additional controls were performed where epistasis was expected. For example, wtm-1(RNAi); pop-1(RNAi) coinjections yield a phenotype identical to pop-1(RNAi) single, as do injections of wtm-1(RNAi) into pop-1(ne189) mutant mothers. To ensure that wtm-1 activity was blocked, we injected wild-type animals with wtm-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 wtm-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(ky1202c7), cwn-1, cwn-2, and W08D2.1. Frizzled-related: Y34D9.0718(yj107h2) and F27E11(yk1117b4). Disheveled-related: C34F11.9a(yj10b11), 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/T238D.1 and apr-1/K042G.2 were consistent with Genefinder predictions. Our results for mom-1/T07H6.2, mom-2/F138E1.17, and wtm-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., 1992) and will be described elsewhere.

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References


**GenBank Accession Number**

The accession numbers for the sequences described in this paper are as follows: mom-1, AF013489; apr-1, AF013950; wrm-1, AF013951; mom-2, AF013952; and mom-5, AF013953.