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MOM-4, a MAP Kinase Kinase Kinase–Related Protein, Activates WRM-1/LIT-1 Kinase to Transduce Anterior/Posterior Polarity Signals in *C. elegans*

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Summary

In *C. elegans*, a Wnt/WG-like signaling pathway downregulates the TCF/LEF-related protein, POP-1, to specify posterior cell fates. Effectors of this signaling pathway include a β-catenin homolog, WRM-1, and a conserved protein kinase, LIT-1. WRM-1 and LIT-1 form a kinase complex that can directly phosphorylate POP-1, but how signaling activates WRM-1/LIT-1 kinase is not yet known. Here we show that *mom-4*, a genetically defined effector of polarity signaling, encodes a MAP kinase kinase kinase–related protein that stimulates the WRM-1/LIT-1-dependent phosphorylation of POP-1. LIT-1 kinase activity requires a conserved residue analogous to an activating phosphorylation site in other kinases, including MAP kinases. These findings suggest that anterior/posterior polarity signaling in *C. elegans* may involve a MAP kinase–like signaling mechanism.

Introduction

During *C. elegans* embryonic development, many of the cell divisions in all regions of the embryo are oriented along the anterior/posterior (AP) axis, and essentially all of these divisions result in AP daughter cells with different fates (Sulston et al., 1983). Genetic studies of this polarity signaling process have defined a mechanism that involves several proteins with similarities to known Wnt/WG signaling components (Rocheleau et al., 1997; Thorpe et al., 1997). For example, MOM-2 is related to the secreted protein Wnt/WG, and MOM-5 is related to the membrane protein Frizzled, a candidate Wnt/WG receptor. As in other Wnt/WG systems, these upstream factors appear to act through a β-catenin-related protein, WRM-1 (Rocheleau et al., 1997; for review, see Cadigan and Nusse, 1997; Han, 1997).

A key difference between polarity signaling in *C. elegans* and Wnt/WG signaling in vertebrates and *Drosophila* concerns the relationship of WRM-1 and a protein related to vertebrate TCF (T cell factor)/LEF (lymphoid enhancer factor) transcription factors, POP-1 (Lin et al., 1995). In vertebrates and *Drosophila*, the WRM-1-related proteins, β-catenin and Armadillo, are thought to enter the nucleus in response to signaling, where they bind to and activate TCF/LEF-related factors (for review, see Cavallo et al., 1997; Kuhl and Wedlich, 1997; Willert and Nusse, 1998). In *C. elegans*, although WRM-1 is an effector of signaling, WRM-1 appears to have the opposite downstream activity, downregulating rather than activating POP-1 (Rocheleau et al., 1997). In studies on wild-type embryos, POP-1 exhibits a lower level of nuclear immunofluorescence staining in the posterior daughters of many AP divisions than in the anterior daughters (Lin et al., 1995, 1998). Genetic studies have shown that WRM-1 and other signaling components are required for this POP-1 asymmetry between AP sister cells (Rocheleau et al., 1997, 1999; Thorpe et al., 1997; Lin et al., 1998). Thus, in *C. elegans*, Wnt/WG signaling through WRM-1 leads to downregulation of POP-1.

Possible insights into POP-1 regulation by WRM-1 have come from analysis of the gene *lit-1*. Mutations in *lit-1* result in a loss of AP cell fate asymmetries (Kaletta et al., 1997). Phenotypic and genetic analysis of *lit-1* place this gene in both the MOM-2 and LIN-44 Wnt/WG signaling systems in *C. elegans*. In the embryo, LIT-1 appears to function along with WRM-1 in a process that reduces POP-1 levels or activity in posterior daughters of AP divisions (Rocheleau et al., 1999). The LIT-1 protein is related to serine/threonine protein kinases and is most similar to the *Drosophila* tissue polarity protein, Nemo (Choi and Benzer, 1994), and to the mouse protein Nlk (Brott et al., 1998). WRM-1 and LIT-1 appear to form a stable protein complex in vivo in *C. elegans* and in transfected vertebrate cells (Rocheleau et al., 1999). In vertebrate cells, WRM-1 activates the LIT-1 protein kinase leading to phosphorylation of WRM-1, LIT-1, and POP-1. These observations support a model in which signaling activates the WRM-1/LIT-1 kinase complex. This complex then directly phosphorylates POP-1, leading to its downregulation in posterior daughters of AP divisions.

How upstream signaling events lead to activation of the WRM-1/LIT-1 kinase is not understood. LIT-1/Nemo/Nlk kinases make up a small subfamily of protein serine/threonine kinases distinct from, but closely related to, MAP kinases (MAPK) (Choi and Benzer, 1994; Brott et al., 1998; Rocheleau et al., 1999). MAPK signaling pathways, which involve sequential activation of protein kinases called MAPK kinase kinases and MAPK kinases, are highly conserved from yeast to metazoans and regulate many developmental decisions in *C. elegans*, *Drosophila*, and vertebrates (for review, see Ip and Davis, 1998; Madhani and Fink, 1998; Schaeffer and Weber, 1999; Tan and Kim, 1999). In the present study, we report the cloning of the AP polarity gene, *mom-4*. The *mom-4* locus was previously identified by a set of maternal mutations that cause defects in polarity signaling in the early embryo (Rocheleau et al., 1997; Thorpe et al., 1997). We show here that *mom-4* activity is required for POP-1 asymmetries between anterior and posterior

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Table 1. Genetic Analysis of Endoderm Specification in *mom-4*

Embryo Type	Percent of Embryos Lacking Endoderm (n)
mom-4(*ne4*)	9 (214)
mom-4(*ne135*)	11 (252)
mom-4(*ne82*)	17 (382)
mom-4(*ne19*)	43 (1134)
mom-4(*ne19*)/*qDf9*	36 (341)
mom-4(*ne19*);*mom-4*(*RNAi*)	51 (285)
mom-2(*ne141*)	53 (304)
mom-2(*ne141*);*mom-4*(*ne19*)	100 (98)
mom-5(*RNAi*)	2 (511)
mom-5(*RNAi*);*mom-4*(*ne19*)	100 (251)
mom-5(*RNAi*);*mom-4*(*ne135*)	100 (54)
mom-5(*zu193*);*mom-4*(*RNAi*)	56 (304)
lit-1(*RNAi*)	96 (604)
lit-1(*RNAi*);*mom-4*(*ne19*)	95 (193)
apr-1(*RNAi*)	42 (234)
apr-1(*RNAi*);*mom-4*(*ne19*)	100 (329)
pop-1(*RNAi*)	0 (117)
pop-1(*RNAi*);*mom-4*(*ne19*)	0 (116)

daughters of AP divisions. *mom-4* encodes a *C. elegans* homolog of mammalian TAK1 (TGF-β activated kinase), which is thought to function as a MAPK kinase kinase (Yamaguchi et al., 1995; Fanger et al., 1997; Shirakabe et al., 1997; Wang et al., 1997). When expressed in cultured mammalian cells, both MOM-4 and TAK1 are able to stimulate WRM-1/LIT-1 kinase activity leading to the increased phosphorylation of POP-1. Finally, we show that this activation is dependent on the putative kinase activation loop of LIT-1 that serves as a target for activating phosphorylation in related kinases. The structural similarities of LIT-1 to MAPK and of MOM-4 to MAPK kinase kinase raise the possibility that a MAPK-like kinase cascade contributes to AP polarity signaling in *C. elegans*.

Results

mom-4 Interacts Genetically with Wnt/WG Components to Regulate POP-1

In an effort to understand the mechanism of AP polarity signaling in *C. elegans*, we sought to identify additional genes that function in this process. Two previous studies reported the identification of the *mom-4* locus as one of a set of *mom* genes whose maternal activities are required for a decision between mesodermal versus endodermal cell fates for daughters of the four-cell stage blastomere called EMS (Rocheleau et al., 1997; Thorpe et al., 1997). The division of EMS is oriented along the AP axis, and the previously described *mom* genes control cell fate differences between the AP daughters of this division (Rocheleau et al., 1997; Thorpe et al., 1997). As with the other *mom* mutants, some *mom-4* embryos (9%–43%, Table 1) lack endoderm and appear to contain excess pharyngeal mesoderm, a phenotype consistent with a posterior-to-anterior transformation for the EMS daughter, E. To further examine this possibility, we used laser ablation to prevent all blastomeres except E from differentiating in *mom-4(ne19)* mutant embryos. In 13 of 22 such embryos examined, we found that E failed to produce endoderm and instead produced mesodermal

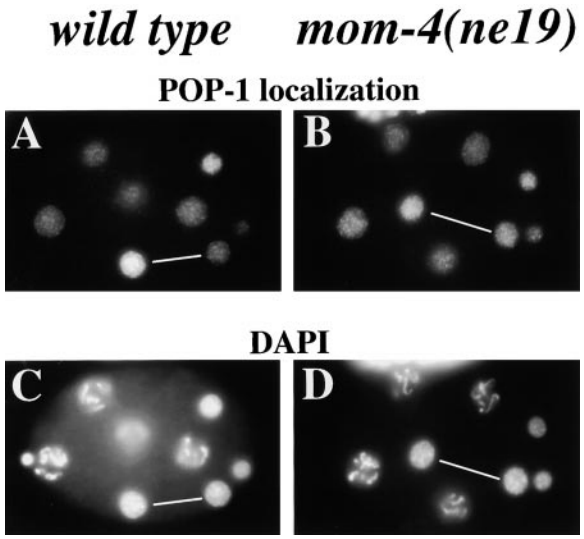


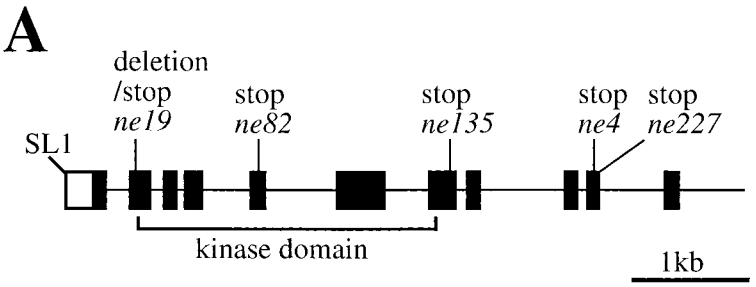
Figure 1. POP-1 Is Localized Symmetrically in *mom-4(ne19)*

Top panels show immunofluorescence staining of POP-1 in either wild-type (A) or *mom-4(ne19)* mutant embryos (B). The anterior of the embryo is to the left, and the posterior to the right. Bars connect nuclei of anterior/posterior sister cells MS and E. In wild-type embryos (A), the anterior cell nucleus shows higher levels of POP-1 immunostaining than the posterior sister. *mom-4(ne19)* embryos (B) show equal staining in the nuclei of both anterior and posterior sisters. The lower panels show corresponding DAPI staining of the nuclei in either wild type (C) or *mom-4(ne19)* (D). Each embryo is approximately 50 microns in length.

tissues similar to those normally produced by its anterior sister, MS (Experimental Procedures and data not shown). Furthermore, in lineaged embryos, this transformation in cell fate was correlated with accelerated division timing in the E lineage (data not shown). In both of these respects, *mom-4* mutant embryos are similar to the previously characterized *mom* mutants (Rocheleau et al., 1997; Thorpe et al., 1997).

It has been reported that *mom-4* mutations strongly enhance EMS polarity defects associated with *mom-2* mutations and that *mom-4* lies genetically upstream of *pop-1* (Thorpe et al., 1997). However, previous studies indicated that not all *mom* mutants synergize with each other, suggesting possible branched pathways that contribute to polarity signaling (Rocheleau et al., 1997). We therefore examined genetic interactions between *mom-4* and the entire set of previously described polarity genes. We found that *mom-4* exhibits strong synergy in genetic tests with *mom-2*, *mom-5*, and *apr-1*, and like these other genes, *mom-4* appears to lie upstream of *pop-1* (Table 1). These genetic findings, together with the previous observation that *mom-4*(+) activity is required in the responding cell for AP polarity signaling (Thorpe et al., 1997), are consistent with a place for *mom-4* either after a convergence between Wnt/WG and other polarity signals or in a pathway parallel to Wnt/WG signaling (see Discussion).

In order to ask if *mom-4* is required for POP-1 asymmetry, we stained *mom-4* mutant embryos with an antibody specific for POP-1. We found that POP-1 staining levels were equal and high in the nuclei of sister cells resulting from AP divisions (Figure 1 and data not shown). Taken together, the genetic relationship of



B

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MOM-4   MDNSSQSKPSSSSSSHSPSPAAITPTQRTTRDSGLCSTIDIPEIQAQCIDNLNSHYLGKG  60
Y105C5  MDR----KPSSSSSTGPPAP----VIQRKTRDSDLCLTSDIPKIPPNCIDELNSHFLGKG  52
TAK1    MSTASAASSSSSSSASEMIEAP-------------SQVLNFEEIDYKEIEVE--EVVGRG  45

MOM-4   TYGLVEKTRYRKTRQDDFRPAAIKYSSQLHMATLIREAKVMWDLRNHPNIIKIYGLYKSP  120
Y105C5  TFGVVEKTKYRKTRDDTYRPAAIKYASATHLSILKREAKHMWNLRNHVNIIKIYGMYESH  112
TAK1    AFGVVCKAKWRAKD------VAIKQIESESERKAFIVELRQLSRVNHPNIVKLYGACLNP  99

MOM-4   RNGQGVVMEYMDCGSMADLLYDRT-HINYTIDHVASWMFQLSSAVDFFHSNS---QVHRD  176
Y105C5  RNGQGLVMEYMDCGCVADLIYDRK-NIEYKMDHVASWMYQLSSAVNFFHSKD---QIHRD  168
TAK1    ---VCLVMEYAEGGSLYNVLHGAEPLPYYTAAHAMSWCLQCSQGVAYLHSMQPKALIHRD  156

MOM-4   LKLQNMLLSDRYRTMKLCDFGTFTSMHQSMTSNRGTPITMAPEVFRCEQYNMKSDIYSIG  236
Y105C5  LKLQNMLLSHHHRTLKICDFGTFTSIHQSMTSNRGSPITMAPEIFRCEPYNMKADIFSIG  228
TAK1    LKPPNLLLVAGGTVLKICDFGTACDIQTHMTNNKGSAAWMAPEVFEGSNYSEKCDVFSWG  216

MOM-4   IIMWQIIARNHPYRRDLSVPGLLYNVATANLRPQELECNP-ILSEFYKKCWNDNADIRPT  295
Y105C5  IIMWQMIARDHPYTMNMPITPFLYNVATRNLRPHEIECNP-ILSNFYKRCWSDNPASRPT  287
TAK1    IILWEVITRRKPFDEIGGPAFRIMWAVHNGTRPPLIKNLPKPIESLMTRCWSKDPSQRPS  276

MOM-4   SSECVEYFTLLKDEYPNGSVPLSDS----------------------STNGPAETPPPHA  333
Y105C5  SADCVEYFRCLRSEYPNGNVPLWDP----------------------ASGASNTVKIPKS  325
TAK1    MEEIVKIMTHLMRYFPGADEPLQYPCQYSDEGQSNSATSTGSFMDIASTNTSNKSDTNME  336

MOM-4   HRPTM-----------------LGTSSGSGIGSNNRTPTASKLLNPQQP-GQGHRRNRSE  375
Y105C5  Q-------------------------------PGGRSPLVCALRILLF-FDAADIFRVL  353
TAK1    QVPATNDTIKRLESKLLKNQAKQQSESGRLSLGASRGSSVESLPPTSEGKRMSADMSEIE  396

MOM-4   TFVVQPDLPY-PTVPGEAGASRI---PKSQSEAKNFRDRAKSEQRQPH--RDARPPPPFE  429
Y105C5  VHTMTRKMS--PLPKTEITPDVK---PKEVAPAKVQETPALLP--------DTLKVPKVE  400
TAK1    ARIVATAGNGQPRRRSIQDLTVTGTEPGQVSSRSSSPSVRMITTSGPTSEKPARSHPWTP  457

MOM-4   HRRDSNDEEKHAVFMNICS-NEETRPIDPDTRDEKSLEIFHQHCDNNKQYADAWVIKKEV  488
Y105C5  RQI-SNEEEKQKIFMTALS-NEDTRPIDPNEGDESSLELYHQHCLVNLNYAEVLLAKKEA  458
TAK1    DDSTDTNGSDNSIPMAYLTLDHQLQPLAPCPNSKESMAVFEQHCKMAQEYMKVQTEIALL  516

MOM-4   MRAKHELIAQWPQHDH-----TVELLERKYYLEQEIARYRDIQD-DNYFSTERM------  536
Y105C5  INEKHKLVQKWAEHER-----TLELLERIACLEQEIAYQECNNAKEKSSFSVRM------  507
TAK1    LQRKQELVAELDQDEKDQQNTSRLVQEHKKLLDENKSLSTYYQQCKKQLEVIRSQQQKRQ  576

MOM-4   ---
Y105C5  ---
TAK1    GTS  579
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Figure 2. MOM-4 Encodes a TAK1 Homolog

(A) Schematic representation of *mom-4* genomic structure. *mom-4* is *trans*-spliced to SL1. Black boxes indicate coding exons from which the amino acid sequence in (B) was deduced. The exons containing the kinase domain are indicated with a bracket. Positions of the *mom-4* lesions *ne4*, *ne19*, *ne82*, *ne135*, and *ne227* are shown (See Experimental Procedures).

(B) Alignment of the MOM-4 amino acid sequence with a *C. elegans* MOM-4 homolog on Y105C5, and vertebrate TAK1. Identical residues are shaded in gray. The MOM-4-related sequence on clone Y105C5 was predicted based on homology with MOM-4 and on nucleotide homology with known splice acceptor and donor sequences.

mom-4 with Wnt/WG signaling and its strong effect on POP-1 asymmetry suggests that *mom-4* is an integral part of the polarity signaling mechanisms that downregulate the activity of POP-1.

mom-4 Encodes a Homolog of Vertebrate TAK1

To clone the *mom-4* gene, we mapped it to a small interval on genetic and physical maps of LGI. We then used RNA interference (RNAi) to determine whether the loss of function of any of the predicted genes within this interval could result in a phenotype similar to that of *mom-4* (Rocheleau et al., 1997; see Experimental Procedures). One of the genes we tested in this RNAi assay (F52F12.3) gave a low frequency of *mom-4*-like dead embryos (data not shown). We further analyzed this gene by conducting the RNAi assay in the genetic background of the *mom-5* mutation and found that the Mom phenotype associated with *mom-5* was strongly enhanced (Table 1). These observations suggested that F52F12.3 may encode the *mom-4* gene. We therefore sequenced the F52F12.3 gene in five *mom-4* mutant alleles and found that each contains a mutation in the predicted exons (Figure 2A). We conclude that F52F12.3 is *mom-4*. The predicted MOM-4 protein shows an overall similarity to vertebrate TAK1 (Figure 2B; Yamaguchi et al., 1995) and resembles MAPK kinase kinases in its predicted kinase domain. MOM-4 and TAK1 share additional similarities outside of the respective kinase domain, including a serine-rich N-terminal region (Figure 2B).

The five *mom-4* mutants appeared to be strong hypomorphs, possibly including null alleles (Figure 2A; see also Experimental Procedures). For example, the *mom-4(ne19)* allele is predicted to cause an early frameshift after codon 46 and a premature translation termination as a consequence. We also found that *mom-4(ne19)* behaves like a deficiency in genetic complementation tests (Table 1). Nevertheless, none of the *mom-4* mutants produced a fully penetrant maternal effect Mom phenotype, suggesting that the *mom-4* function is not absolutely required for AP polarity control in the EMS cell. This could reflect a role for MOM-4 in one of multiple parallel pathways for activating the WRM-1/LIT-1 kinase (see Discussion). One possible source of AP polarity signaling in the absence of *mom-4*(+) activity is a highly similar *mom-4* homolog present in clone Y105C5 in the *C. elegans* genome sequence (Figure 2B). However, we found that RNAi targeting this Y105C5 homolog failed to enhance the phenotype of *mom-4(ne19)* (data not shown). Further genetic tests will be needed to determine if this *mom-4* homolog contributes to AP polarity signaling.

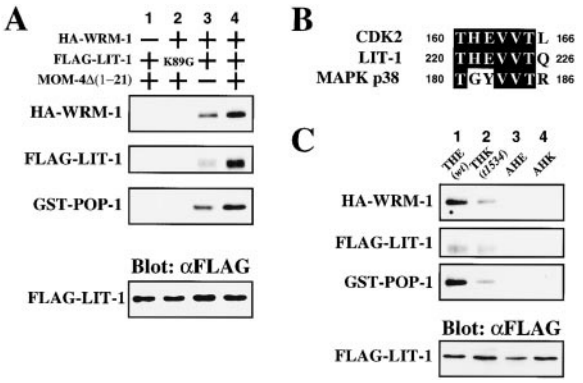

Figure 3. WRM-1/LIT-1 Kinase Is Activated by MOM-4 and Is Dependent on the LIT-1 Activation Loop–like Motif

(A) MOM-4 stimulates phosphorylation of POP-1, LIT-1, and WRM-1 by LIT-1 immunoprecipitation complex. COS-7 cells were transfected with expression plasmids encoding epitope-tagged LIT-1, WRM-1, and/or N-terminally truncated MOM-4 as indicated at the top of the gel. FLAG-LIT-1 was either wild-type or mutant K89G, which is predicted to be defective in the ATP-binding site and therefore to be kinase inactive. In vitro kinase assays were performed on FLAG-LIT-1 immunoprecipitates with bacterially produced GST-POP-1 as a substrate. FLAG-LIT-1 coimmunoprecipitated with, and subsequently phosphorylated, WRM-1. Quantitative comparisons of the radioactive bands reveal that phosphorylation of POP-1, WRM-1, and LIT-1 are increased by 3.5-, 3.3- and 3.8-fold (average of three experiments), respectively, when FLAG-LIT-1 complex was precipitated from MOM-4Δ(1–21) expressing cells (lane 4) compared to FLAG-LIT-1 complex from nonexpressing cells (lane 3). The anti-FLAG immunoblot (lower panel) shows comparable amounts of FLAG-LIT-1 present in each immunoprecipitate.

(B) Alignment of the activation loop regions of human CDK2, LIT-1, and human p38 MAPK. Thr-160 of human CDK2, and Thr-180 and Tyr-182 of human p38 MAPK, are the sites of activating phosphorylation.

(C) The activation loop of LIT-1 is required for its kinase activity. Lysates from COS-7 cells transfected with epitope-tagged LIT-1 and WRM-1 were subjected to an immunoprecipitation/kinase assay. FLAG-LIT-1 was either wild type or contained the following amino acid substitutions within the putative activation loop: Glu222Lys (THK; lane 2), Thr220Ala (AHE; lane 3), and double Glu222Lys Thr220Ala (AHK; lane 4). The genetically identified *lit-1(t1534)* (Kaletta et al., 1997) is predicted to encode a Glu222Lys (THK) mutant protein, with diminished kinase activity (lane 2).

MOM-4 Activates the WRM-1/LIT-1 Kinase

The identification of MOM-4 as a MAPK kinase kinase homolog prompted us to test whether MOM-4 can activate the MAPK-related kinase LIT-1. Previous work on vertebrate TAK1 had suggested that the serine-rich N-terminal region negatively regulates TAK1 and had shown that an N-terminal truncation that removes this region activates TAK1 (Yamaguchi et al., 1995). We therefore constructed an analogous N-terminal truncation of MOM-4, MOM-4Δ(1–21), that removes the first 21 amino acids of MOM-4. As previously reported (Rocheleau et al., 1999), LIT-1, expressed in and immunoprecipitated from cultured mammalian cells, can phosphorylate itself, WRM-1, and POP-1 in a manner strictly dependent on WRM-1 (Figure 3A, lane 3). Coexpression of MOM-4Δ(1–21) resulted in a 3- to 4-fold increase in the phosphorylation of all three substrates by LIT-1 immunoprecipitates (Figure 3A, lane 4). The increased LIT-1, WRM-1, and POP-1 phosphorylation was completely dependent on LIT-1 kinase activity since a point mutation in the predicted ATP-binding site in LIT-1, K89G, abolished all phosphorylation (Figure 3A, lane 2). Full-length MOM-4 expressed at comparable levels to MOM-4Δ(1–21) failed to stimulate LIT-1 kinase activity (data not shown), suggesting that as in TAK1, the N-terminal region of MOM-4 may be a site of negative regulation in mammalian cells. The MOM-4Δ(1–21)-dependent increase in LIT-1 kinase activity required WRM-1 (Figure 3A, lane 1), suggesting that MOM-4 activation does not simply bypass the WRM-1-dependent mechanism for LIT-1 activation. Furthermore, when MOM-4Δ(1–21) was coexpressed, we did not observe an increase in the amount of WRM-1 protein present in cell extracts or in coimmunoprecipitates with LIT-1 (data not shown). This finding suggests that the stimulation in LIT-1 kinase activity does not reflect an increase in WRM-1 protein levels or in WRM-1 affinity for LIT-1.

In the MAPK signaling cascade, MAPK kinase kinase induces the activation of MAPK through the phosphorylation/activation of MAPK kinase. MAPK kinase in turn phosphorylates MAPK on threonine and tyrosine residues in its activation loop (Payne et al., 1991), which correspond to Thr-180 and Tyr-182 in human p38 MAPK (see Figure 3B). LIT-1 contains a putative activation loop within kinase subdomains VII and VIII (Rocheleau et al., 1999), and Thr-220 in LIT-1 is analogous to Thr-180 in p38 MAPK (Figure 3B). LIT-1 does not contain a Tyr-182 equivalent but instead contains a negatively charged Glu-222 at this position. We refer to these residues using the single-letter amino acid designations in Figures 3B and 3C. We examined the potential importance of the putative activation motif in LIT-1 by altering Thr-220 and Glu-222 separately or in combination. The hypomorphic allele, *lit-1(t1534)*, is reported to contain a single Glu-222 to Lys mutation ("THK" instead of "THE" in Figure 3C). The majority of *lit-1(t1534)* mutant embryos are severely defective in morphogenesis and yet are able to form the endoderm (Kaletta et al., 1997; Rocheleau et al., 1999). Consistent with these observations, we found that the THK mutant protein could phosphorylate POP-1, LIT-1, and WRM-1 but only weakly (Figure 3C, compare lanes 1 and 2). The single mutant protein Thr220Ala (AHE, in Figure 3C) and the double mutant protein AHK showed no detectable kinase activity either in the absence (Figure 3C, lanes 3 and 4), or in the presence of MOM-4Δ(1–21) (data not shown). These observations support the view that the activation loop–like motif in LIT-1 is important for LIT-1 kinase activity.

Discussion

Branched Pathways and a Potential Kinase Cascade for Polarity Signaling in *C. elegans*

In *C. elegans*, the relative AP positions of sister cells at birth is coupled to cell fate decisions by a polarity signaling mechanism that involves Wnt/WG-like components (Lin et al., 1995, 1998; Rocheleau et al., 1997; Thorpe et al., 1997). A key target of this signaling pathway is the POP-1 protein, which appears to be downregulated by signaling (Rocheleau et al., 1997; Thorpe et al., 1997; Lin et al., 1998). Two proteins, WRM-1, a β-catenin-related protein, and LIT-1, a conserved serine/threonine protein kinase, appear to be essential for POP-1

downregulation (Rocheleau et al., 1999). In cell culture assays, WRM-1 and LIT-1 form an active kinase complex that can directly phosphorylate the POP-1 protein, suggesting that the WRM-1/LIT-1 kinase may directly downregulate POP-1 in vivo. Genetic studies suggest that the signaling pathway is branched upstream of WRM-1 and LIT-1 and may have polarity inputs from sources other than the MOM-2/Wnt-related protein (Rocheleau et al., 1997). For example, a large percentage of embryos from mutant strains carrying apparent null alleles of both *mom-2*(Wnt) and *mom-5*(Frizzled) nevertheless exhibit proper specification of posterior cell fates, strongly suggesting that alternative polarity signals must be able to activate the WRM-1/LIT-1 kinase.

In the present study, we have described a novel polarity signaling protein, MOM-4, homologous to the vertebrate protein TAK1. Mutations in *mom-4* strongly synergize with mutations in *mom-2* and *mom-5*, raising the possibility that *mom-4*(+) activity is required for Wnt/WG independent polarity signaling. TAK1 is thought to be a MAPK kinase kinase and can phosphorylate and activate MAPK kinases both in vitro and in transfection assays (Yamaguchi et al., 1995; Fanger et al., 1997; Wang et al., 1997; for review, see Ninomiya-Tsuji et al., 1999). The LIT-1/Nemo/Nlk kinases belong to a small subfamily of serine/threonine kinases that are distinct from but closely related to MAPK (Choi and Benzer, 1994; Brott et al., 1998; Rocheleau et al., 1999). Within its kinase domain, LIT-1 is approximately 45% identical (132 out of 292 residues) to human p38 MAP kinase and 43% identical (126 out of 292 residues) to human ERK1, respectively. Furthermore, we have shown here that LIT-1 activation appears to require a conserved motif analogous to a site required for activating phosphorylation by MAPK kinases. Thus, we have shown that MOM-4 is similar to MAPK kinase kinase and that LIT-1, which is similar to MAPK in amino acid sequence, is also similar to MAPK in its activation. These observations together with the genetic synergy between *mom-4* mutants and Wnt/WG pathway mutants suggest that a MAP kinase–like cascade may function in parallel with Wnt/WG signaling to specify AP cell fate differences during *C. elegans* development. In the future, understanding how MOM-4 is activated and how MOM-4 in turn activates LIT-1 is likely to shed light on how Wnt/WG signals interact with other signaling pathways to control cell polarity and cell fate.

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, *dpy-5(e61), unc-13(e1091), hDf9, nDf25, nDf24, nDf29, qDf9, qDf10, mnDf111, lin-11(n566), hT1(I;lV), hT2(I;III)*; LGII, *bli-2(e768)*; LGIII, *unc-32(e189), dpy-18(e364), eT1(III;V), qC1*; LGIV, *unc-5(e53), nT1(IV;V)*; LGV, *dpy-11(e224), him-5(e1409)*; and 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-4(ne135)* to the *unc-13 lin-11* interval on LGI, and deficiency mapping was used to map *mom-4(ne19)* between cloned genes *mec-8* and *mom-5*. Data from these crosses are available from the *C. elegans* database, ACEDB. Self-progeny from *mom-4(ne19)* 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-4 (ne19)* against all other *mom-4* alleles, and *mom-4(ne19)* against the chromosomal deficiency *qDf9*. In all cases, heterozygotes grew to adults that produced dead embryos resembling those produced by *mom-4(ne19)*.

RNAi Reverse Genetics

RNAi was performed for 29 *mom-4* candidate genes in the *mec-8 lin-11* genetic interval (data not shown). If available, cDNA clones from Yuji Kohara's collection were used as template for RNA synthesis; if no cDNA clone was available, template was made by PCR using primers tagged with the T7 promoter (Rocheleau et al., 1997). Synthesis and injection of RNAi were done as described in Fire et al. (1998) and Rocheleau et al. (1997).

Molecular Analysis and Plasmids

Coding sequences in *mom-4* were determined by sequencing RT-PCR products amplified using primers for the SL1 *trans*-splice leader sequence and the gene-specific sequence. The *mom-4* coding sequences we determined show some inconsistencies with the GeneFinder predictions for the corresponding open reading frame F52F12.3; these differences are detailed in the GenBank accession number listed in this paper. Mutant alleles were sequenced using standard protocols from PCR-amplified genomic DNA. *ne19* encodes a single nucleotide deletion in codon 46 that results in a frameshift followed by a subsequent stop codon. *ne82*, *ne4*, and *ne135*, respectively, encode for Trp-156, Trp-482, and Arg-370 to stop. *ne227* changes a splice site at the end of exon 10 to a stop.

For expression in COS-7 cells, we cloned the full-length MOM-4 (2523 nt) and N-terminally truncated MOM-4 (lacking the first 21 amino acids), tagged at the N terminus with the T7 gene 10-protein epitope, into pCDNA3 (Invitrogen). The full-length cDNA for the LIT-1 isoform LIT-1a (1365nt) was tagged at the N terminus with the FLAG epitope and cloned into pCDNA3. We used a PCR-based site-directed mutagenesis method to create amino acid changes in the putative T loop within the LIT-1 kinase domain. The HA-WRM-1 and the GST-POP-1 plasmids were described previously (Rocheleau et al., 1999).

Cell Culture, Transfection, and Immunoprecipitation Kinase Assays

Cell culture and transfection procedures and the immunoprecipitation kinase assays were performed as previously described (Rocheleau et al., 1999). For quantitative analyses of LIT-1 kinase activity, the amounts of FLAG-LIT-1 and HA-WRM-1 were normalized for each immunoprecipitation. Following the kinase reaction, radioactive bands corresponding to FLAG-LIT-1, HA-WRM, and GST-POP-1 were quantitated using a phosphorimager (Molecular Dynamics).

Microscopy

Light and immunofluorescence microscopy and laser microsurgery were as described previously (Bowerman et al., 1992; Rocheleau et al., 1997; Lin et al., 1998). The identities of differentiated cells were 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. (1992) and Mello et al. (1992). The POP-1 mABRL2 antibody and the staining procedure were described previously (Lin et al. 1998).

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