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C. elegans MOM-5/Frizzled Functions in MOM-2/Wnt-Independent Cell Polarity and Is Localized Asymmetrically prior to Cell Division

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Summary

C. elegans **embryonic cells have a common anterior/posterior (a/p) polarity that is apparent in the localization of the transcription factor POP-1 [1, 2]. The level of nuclear POP-1 remains high in the anterior daughters of dividing cells but is lowered in the posterior daughters [2, 3]. To generate POP-1 asymmetry, most early embryonic cells require contact with signaling cells that express the ligand MOM-2/Wnt [4, 5]; the point of cell contact specifies the daughter with low nuclear POP-1 [6, 7]. In contrast, slightly older embryonic cells that have no apparent prior exposure to Wnt signaling can generate POP-1 asymmetry, provided these cells express MOM-5/Frizzled [7]. We show here that MOM-5::GFP is enriched at the posterior pole of cells prior to division and that a similar asymmetry is observed in cultured cells with no apparent prior exposure to Wnt signaling. While depleting these latter cells of MOM-5/Frizzled causes both daughter cells to have high levels of POP-1 [7], we show that both daughter cells have low levels of POP-1 in embryos with atypically high levels of MOM-5::GFP. These results suggest that MOM-5/Frizzled asymmetry leads to POP-1 asymmetry. In later embryogenesis, we find that MOM-5::GFP localizes to the leading edges of epidermal cells during ventral enclosure. These localization patterns suggest a parallel between MOM-5/Frizzled and the roles of *Drosophila* Frizzled in planar polarity and dorsal enclosure.**

Results and Discussion

Previously, we showed that isolated embryonic cells that appeared to have no prior exposure to Wnt signaling were, nevertheless, able to generate POP-1 asymmetry after a few rounds of division [7]. To examine how these cells generate POP-1 asymmetry, we asked whether MOM-5/Frizzled might be localized asymmetrically. A reporter was constructed that encodes a full-length MOM-5 protein fused at its C terminus to Green Fluorescent Protein (GFP). For these studies we used a heterologous promoter and 3′UTR that provide strong maternal and embryonic expression (see Experimental Procedures). The transgene encoding MOM-5::GFP was introduced into worms as either the extrachromosomal array *zuEx123* or the chromosomally integrated array *zuIs145*. To test whether MOM-5::GFP could provide MOM-5(+) activity, both transgenes were crossed into a null *mom-5(ne12)* mutant strain. As described previously, *mom-5(ne12)* mutant embryos do not undergo appreciable tissue morphogenesis or body elongation and instead arrest as twitching masses of disorganized tissues (Figure 1A) [4]. In contrast, about 30% (n = 100) of the *mom-5(ne12); zuEx123* and *mom-5(ne12); zuIs145* embryos appeared to develop normally through the first half of embryogenesis (Figure 1B); these embryos began tissue morphogenesis but usually ruptured along the ventral midline as the body elongated. We next asked whether MOM-5::GFP could contribute to POP-1 asymmetry. For convenience, we describe cell stages by the number of descendants of the AB blastomere, the anterior blastomere of the 2 cell embryo. Previous studies have shown that *mom-5(ne12); mom-2(RNAi)* mutant embryos scored at the AB^{32} stage lack POP-1 asymmetry; divisions produce daughter cells that have equal, high levels of nuclear POP-1 [7]. In contrast, we found that daughter cells in *mom-5(ne12); mom-2(RNAi)*; *zuEx123* embryos showed the wild-type pattern of high/low POP-1 asymmetry in 46% of cell divisions scored at the AB^{32} stage (n = 24). Interestingly, in many cases where POP-1 asymmetry was not apparent, both daughter cells had equal, low levels of nuclear POP-1 rather than equal, high levels (see below).

When the transgenes were crossed into otherwise wild-type embryos, only faint, or no, expression of MOM-5::GFP was detectable before the AB^{32} stage by fluorescence microscopy or by staining with an antiserum for GFP (data not shown). When visible, MOM-5::GFP appeared dispersed throughout the cytoplasm and was not noticeably associated with the cell membrane or cortex. The low level of MOM-5::GFP may mean that the protein is expressed but unstable in early embryos or that high levels of early expression were selected against in the initial generation of transgenic animals. The level of MOM-5::GFP increased markedly in most cells beginning at the AB^{32} stage and persisted until late in embryogenesis (Figures 1C, 1D, and 2A). Within expressing cells, MOM-5::GFP was present in a diffuse distribution throughout the cytoplasm and was enriched on or near the cell plasma membrane (Figures 1C, 1D, and 2A). In addition, MOM-5::GFP often was enriched in prominent cytoplasmic puncta (Figures 1D and 2A). Costaining experiments showed that these puncta were closely associated with, but distinct from, centrosomes (short arrows in Figure 2A). These puncta may correspond to large secretory vesicles; however, their positions are noteworthy because MOM-5 has an essential role in determining centrosome position/spindle axis for certain embryonic blastomeres [4, 5, 8]. Most cells showed comparable levels of membrane-associated MOM-5::GFP, with the following exceptions. First, the

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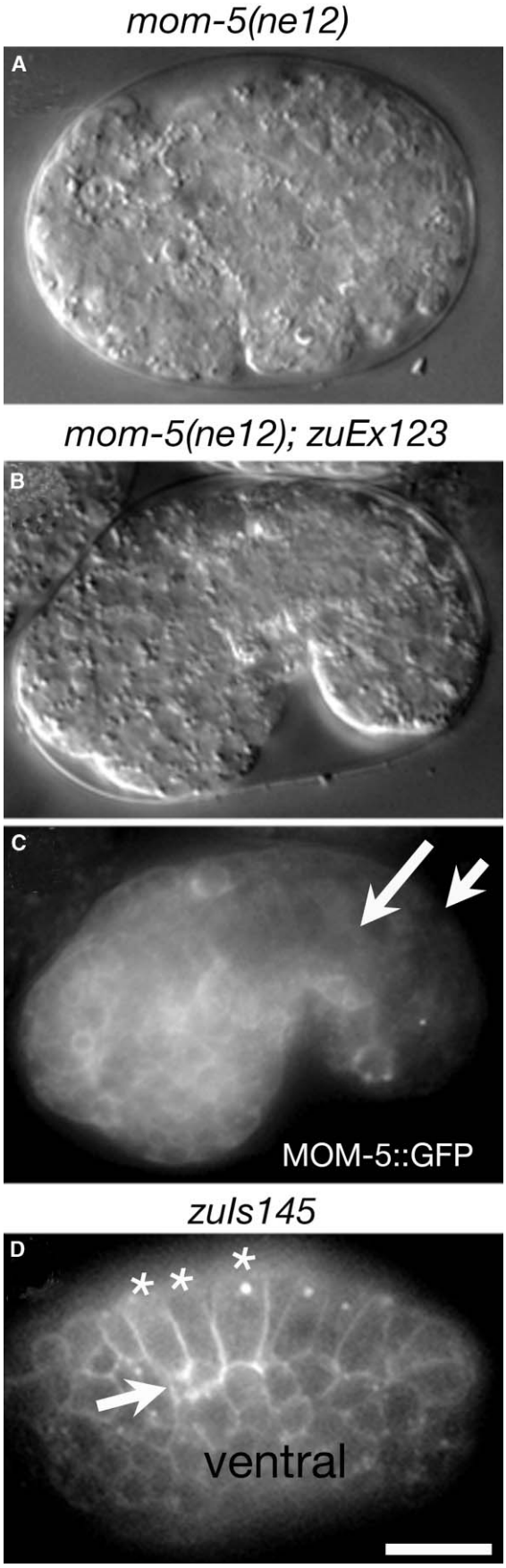

descendants of the embryonic "founder" blastomeres called E and C reproducibly showed less MOM-5::GFP expression than other cells (arrows in Figure 1C). Second, postmitotic epidermal cells (ventral hypodermal cells) that spread across and enclose the ventral surface of the embryo showed a transient enrichment of MOM-5::GFP at their actin-rich leading edges (arrow, Figure 1D). Finally, several embryos contained small groups of cells of variable identity with exceptionally high levels of MOM-5::GFP (data not shown); because similar levels of expression were not observed in the same cells in other embryos at the same stage, we consider this a likely artifact of transgene expression. Several embryos that were costained for both GFP and POP-1 also showed exceptionally high levels of MOM-5::GFP expression in one or more groups of cells of variable identity (Figure 2B). Interestingly, cells within these groups had low, relatively uniform levels of nuclear POP-1 (red nuclei in Figure 2B). In contrast, cells in the same embryo with lower levels of MOM-5::GFP showed the wild-type pattern of high/low POP-1 asymmetry in daughter cells (red nuclei in Figure 2C). These observations suggest that abnormally high levels of MOM-5::GFP might inappropriately trigger the nuclear export of POP-1 from anterior daughter cells.

Although MOM-5::GFP appeared to be distributed uniformly along the membranes of most cells throughout the cell cycle, we observed that cells in prophase occasionally showed a slight enrichment of MOM-5::GFP toward the posterior pole (arrow, Figure 3A). In an effort to enhance visualization of MOM-5::GFP, we asked whether its distribution might be limited by competition with endogenous MOM-5. Because the *mom-5::gfp* transgene was constructed with a heterologous 3′UTR, we used dsRNA corresponding to the 3′UTR of endogenous *mom-5* in an effort to deplete endogenous MOM-5 specifically (Experimental Procedures). We found that wild-type worms exposed to this dsRNA produced only a small percentage of embryos resembling *mom-5* mutant embryos, suggesting that this treatment only partially depleted MOM-5. Nevertheless, *zuIs145* and *zuEx12*3 worms exposed to the dsRNA showed markedly enhanced membrane-localization of MOM-5::GFP

Figure 1. MOM-5::GFP Provides Partial Phenotypic Rescue of *mom-5* Mutant Embryos

(A) Nomarski photomicrograph of a terminal stage *mom-5(ne12)* embryo.

(B and C) Lateral views through the middle of a *mom-5(ne12)* mutant embryo expressing MOM-5::GFP. In (C), MOM-5::GFP is visible in cells throughout the head (left), in anterior dorsal epidermal cells (top left), and in ventral neural precursors (bottom). The posterior dorsal epidermal cells (descendants of the C blastomere, short arrow) and intestinal cells (long arrow) have relatively low levels of MOM-5::GFP.

(D) Ventral view of embryo at the start of ventral enclosure. Asterisks indicate three epidermal cells on the left side of the body that have flattened and begun to move toward the ventral midline, spreading across neural precursors. Note concentration of MOM-5::GFP at the leading edges (arrow) of these cells. An example of a large cytoplasmic punctum of MOM-5::GFP in visible below the third asterisk. All embryos oriented with anterior to left. Scale bar: 10 μm.

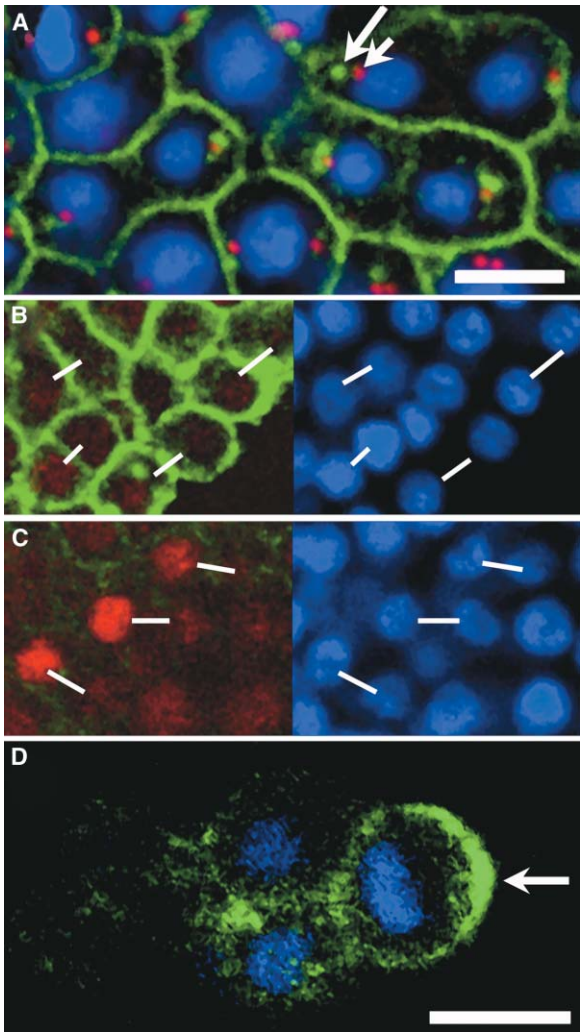

Figure 2. MOM-5::GFP Levels and POP-1 Asymmetry

(A) Confocal image of cells in an embryo fixed and immunostained for MOM-5::GFP (green) and for centrosomes (red dots, indicated by short arrows). Long arrows point to large puncta of MOM-5::GFP in the cytoplasm.

(B–C) Different regions of the same embryo photographed at the same exposure after immunostaining for MOM-5::GFP [green in (B) and (C)] and POP-1 [red in (B) and (C)]; the right half of each panel shows the DAPI-stained cells (blue). Sister cells were identified by costaining with an additional antiserum that recognizes midbodies (not shown, [7]). In (C), the level of nuclear POP-1 in each of the anterior daughters (left nucleus in each sister pair) is comparable to wild-type.

(D) Cluster of cells derived from a single, sequentially isolated AB^{32} cell that was allowed to divide 2–3 times before fixation and immunostaining for MOM-5::GFP (green); the DAPI-stained image is superimposed in blue. The nucleus of the cell on the right has the asymmetric bar-shape typical of early metaphase nuclei. The orientation of this metaphase figure indicates that the cell will divide perpendicular to the cluster, with the contact-free pole indicated by an arrow. All images are of *zuIs145; mom-5 3′UTR (RNAi)* embryos. Scale bars: 5 μm.

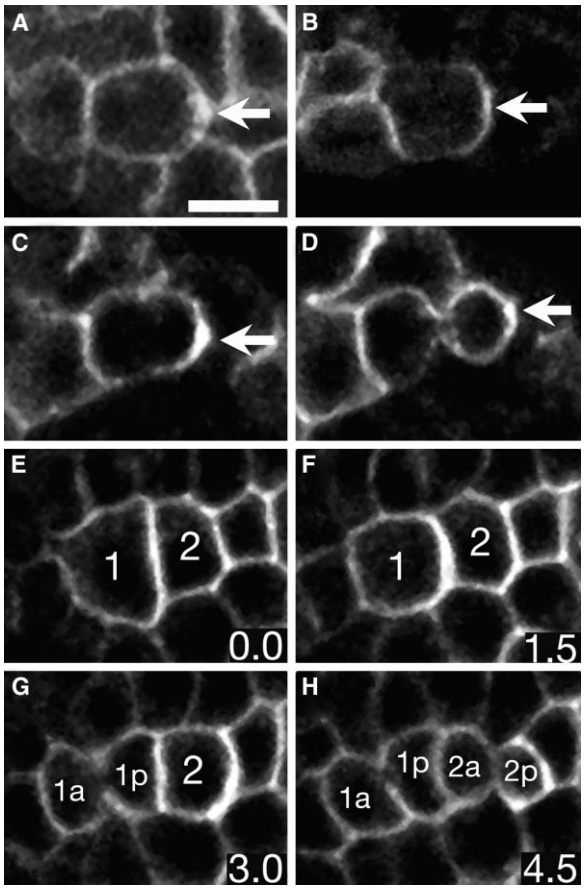

Figure 3. MOM-5::GFP Asymmetry in A/P-Dividing Blastomeres

High magnification, confocal fluorescence images of MOM-5::GFP in embryonic cells either without depletion of endogenous MOM-5 (A) or after depletion (B–H). Embryos are oriented with anterior to the left, and arrows indicate the posterior poles of cells. Cells in (A) and (B) are just prior to division; in (B), the cell is flanked by nonexpressing cells on its lateral and posterior sides. (C and D) Cell division. (E–H) Cell divisions of adjacent cells, numbered 1 and 2; elapsed time is shown at the bottom right of each panel in minutes. Cell 1 is ABarpaaaa and cell 2 is ABarpaaap. The latter cell divides unequally to produce a small, posterior daughter [2p in (H)] that undergoes programmed cell death in normal development. We observed a similar, posterior localization of MOM-5::GFP prior to the unequal division of ABalapapa, where the anterior daughter is small and undergoes programmed cell death (data not shown). Scale bar: 5 μm.

(compare Figures 3A and 3C). We observed a similar enhancement of MOM-5::GFP localization in *mom-5(ne12); zuIs145* and *mom-5(ne12); zuEx123* mutant embryos (data not shown). All of the results described below incorporate one of these strategies to reduce endogenous MOM-5 (Experimental Procedures).

We found that cells in late prophase showed a small but consistent enrichment of MOM-5::GFP toward their posterior pole. This posterior enrichment was particularly evident in mosaic embryos where expressing cells were partially surrounded by nonexpressing cells (Figures 3B and 3C). In regions of the embryo where all cells expressed MOM-5::GFP equally, asymmetry was most apparent when cells did not divide synchronously with their neighbors (Figures 3E–H; Supplemental Movie S1). When divisions were synchronous, we presume that high posterior MOM-5::GFP in one cell masked the low

anterior level in the neighboring cell. Cell cycles in the embryo range between about 15 and 25 min, and MOM-5::GFP appeared to localize to the posterior pole approximately 3–5 min before cell division (Figures 3E–H; Supplemental Movie S1). This transient asymmetry resulted in the posterior daughter inheriting slightly more MOM-5::GFP than the anterior daughter (Figures 3D and 3H). The a/p asymmetry in MOM-5::GFP distribution was reiterated in daughter cells monitored through successive cell divisions (data not shown). MOM-5::GFP asymmetry was most apparent in dividing cells on the surface of the embryo, but could also be detected in internal cells such as those forming the pharyngeal primordium (data not shown). MOM-5::GFP asymmetry was visible in the divisions of about 80% of the surface cells analyzed (n=152), and in all cases MOM-5::GFP was enriched toward the posterior pole. Although MOM-5::GFP often was distributed across the entire posterior surface of a dividing cell, in many cases it appeared to be concentrated into a distinct posterior focus prior to division (see Supplemental Movie S1). It is possible that some of these posterior foci are associated with midbody remnants from earlier cell divisions; however, in many cases the foci were present between distantly related cells.

MOM-5::GFP Is Localized Asymmetrically in Dividing Larval Cells

POP-1 asymmetry has been observed in several a/p cell divisions that occur during larval development [2, 9, 10]. We observed variable MOM-5::GFP expression from the *zuIs145* transgene in many larval cells, including cells of the gonad, vulva, tail, nervous system, and hypodermis. At about 5 hr after hatching, MOM-5::GFP was prominent in lateral skin cells called V1 through V6 and T, and in a neuroblast called QL. Each of these cells undergoes an a/p division to produce daughters that differ in fate; POP-1 asymmetry has been observed between the daughters of the lateral cells [2], and POP-1 function has been shown to be required for the proper development of the QL descendants [9]. In each of these cells, MOM-5::GFP was present as apparently random puncta on, or near, the cell surface (V5 in Figure 4A) and in the cytoplasm (V6 in Figure 4A). In contrast to the rapid embryonic cell divisions, the larval cell cycles can occupy several hours. Approximately 30 min prior to cell division, MOM-5::GFP appeared to accumulate toward the posterior pole of the cell (arrow in Figure 4B; Figure 4D) and in cytoplasmic puncta near the centrosomes (arrowheads in Figures 4B, 4E, and 4F). Upon cleavage, the posterior daughter contained a greater quantity of MOM-5::GFP than did the anterior daughter (Figures 4C and 4F). Because MOM-5::GFP expression is driven by a heterologous promoter, we do not yet know whether MOM-5 normally is expressed in these cells; *mom-5* is one of four *frizzled*-related genes in *C. elegans*, and other family members have been shown to function postembryonically (for review, see [11]). At least one family member, LIN-17/Frizzled, does not appear to be localized asymmetrically in larval cells [12]. Nevertheless, our results demonstrate that larval cells, like embryonic cells, have the ability to localize a Frizzled-related receptor prior to cell division.

MOM-2/Wnt-Signaling and WRM-1/β-Catenin Are Not Essential for MOM-5::GFP Asymmetry

MOM-2/Wnt is thought to act through WRM-1/β-catenin [4]. In the absence of WRM-1, embryonic cells are unable to generate POP-1 asymmetry after division; nuclear POP-1 levels remain high in the posterior daughter cells [3, 4, 7]. Experimental studies have shown that MOM-2/Wnt signaling polarizes cells before they divide [6]; however, it is not known whether WRM-1/β-catenin functions in polarizing the parental cell itself or functions solely to transduce the polarity of the posterior daughter. In the latter role, WRM-1/β-catenin has been shown to bind the kinase LIT-1/Nemo and to stimulate its ability to phosphorylate POP-1 [13], presumably promoting the export of POP-1 from the nucleus of posterior daughter cells [3]. We examined *wrm-1(RNAi); mom-5(ne12); zuEx123* embryos and observed MOM-5::GFP expression similar to that of *mom-5(ne12)*; *zuEx123* embryos; about 83% (n = 37) of the dividing cells scored at and after the AB^{32} stage showed MOM-5::GFP asymmetry, with each of these showing posterior enrichment. These results are consistent with the view that MOM-5 functions upstream of WRM-1/β-catenin for POP-1 asymmetry.

Individual cells at or before the AB^{8} stage do not divide into daughters with POP-1 asymmetry unless they contact a signaling cell that expresses MOM-2/Wnt [7]. Beginning at the AB^{16} stage, however, cells show an ability to generate POP-1 asymmetry that does not require MOM-2/Wnt and that appears to be independent of Wnt signaling in general. For example, AB^{16} and later cells in *mom-2; mom-1* double mutant embryos are able to generate POP-1 asymmetry [7]; *mom-1* encodes the only *C. elegans* protein closely related to Porcupine, a protein required for Wingless/Wnt secretion in *Drosophila* [4]. In functional assays for Wnt signaling, neither an isolated AB blastomere nor its descendants are able to substitute for embryonic cells that express MOM-2/Wnt [6, 7]. Nevertheless, if the AB blastomere is isolated and its descendants are separated sequentially after each of the first five divisions (equivalent to the AB^{32} stage in intact embryos), the resulting cells can divide into daughters with POP-1 asymmetry [7]. We therefore wanted to determine whether MOM-5::GFP asymmetry showed a similar, apparent independence from Wnt signaling. Individual AB^{32} cells were isolated by sequentially separating parental cells as above, then allowed to undergo two to three additional divisions. The resulting small clusters of cells were fixed and stained with an antiserum that recognizes GFP and with DAPI to visualize DNA (n = 22). Similar to our observations on intact, living embryos, we found that MOM-5::GFP appeared to be distributed uniformly in interphase cells, but localized asymmetrically during late prophase and metaphase to one of the two poles (Figure 2D; 27/28 cases).

With cell divisions polarized by Wnt signaling, the daughter cell proximal to the signaling cell always has low POP-1 (see above). Although isolated clusters of AB descendants do not exhibit Wnt signaling, cell contacts within the cluster appear to provide an alternative polarity cue [7]. This latter cue orients POP-1 asymmetry in the opposite direction to Wnt signaling; when cells di-

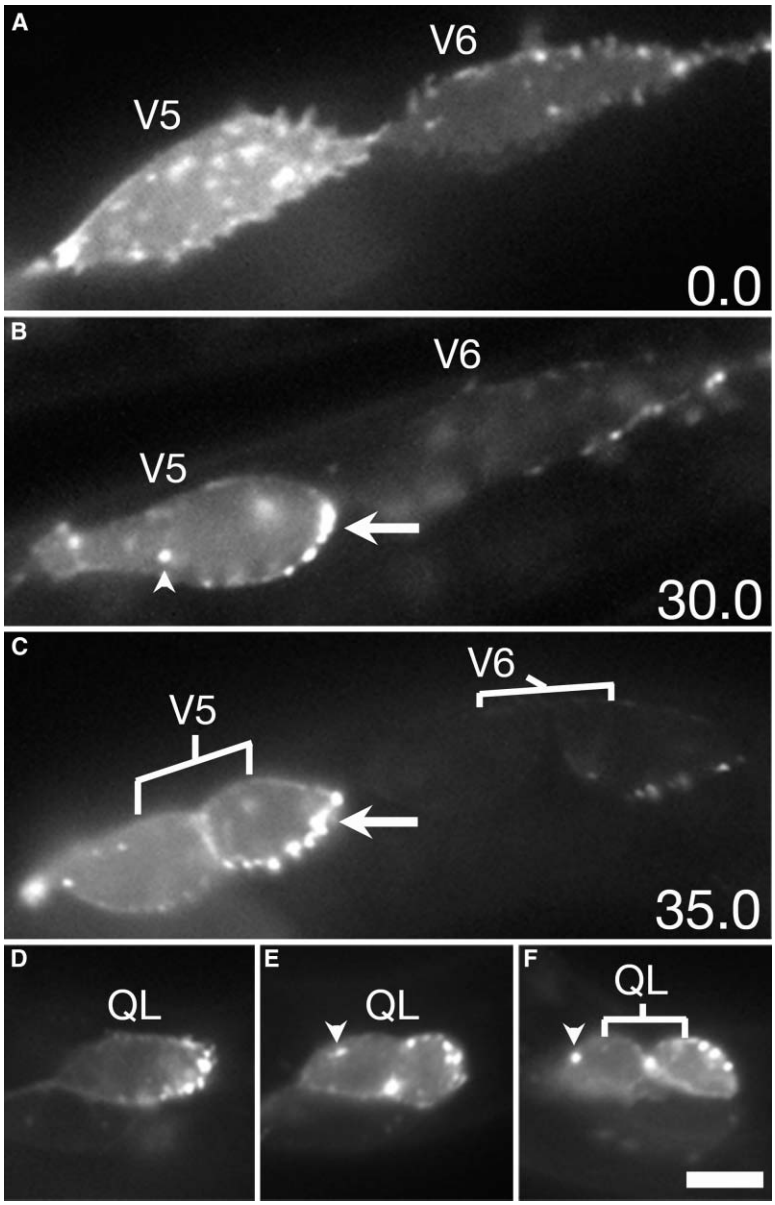

Figure 4. MOM-5::GFP Asymmetry in Larval Cells

(A–C) MOM-5::GFP localization in skin (hypodermal) cells called V5 and V6; elapsed time in minutes is indicated at bottom right. In the larva shown, expression in V5 is much higher than in V6. The focal plane in (A) is at the surface of V5 and through the interior of V6; all other images show the interior of both V5 and V6.

(D–F) MOM-5::GFP expression in the QL neuroblast before (D), during (E), and after (F) division. Arrowheads in (B), (E), and (F) indicate centrosome-associated, cytoplasmic puncta of MOM-5::GFP. Scale bar: 5μm.

vide perpendicular to the cluster, the daughter proximal to the cluster always has high POP-1, while the contact-free daughter has low POP-1 [7]. In our present experiments on isolated AB descendants, MOM-5::GFP invariably was enriched at the contact-free pole of cells that divided perpendicular to the cluster (Figure 2D; 27/27 cases). Thus, in both cultured cells and in intact embryos, an asymmetrical high level of MOM-5::GFP is correlated with the daughter that has low POP-1.

We have not been able to detect an asymmetry in the distribution of MOM-5::GFP in cells before the AB^{32} stage, and these cells normally are polarized by direct contact with posteriorly localized, MOM-2/Wnt-expressing cells. Thus, it is possible that MOM-5 and/or other Frizzled proteins are not localized asymmetrically in these early cells and that an asymmetry in Frizzled activity is achieved solely by the asymmetrical presentation of MOM-2/Wnt. In older cells at and after the AB^{32} stage, posteriorly localized MOM-5/Frizzled might be activated independent of ligand or activated by a uniformly distributed ligand. Why might early and older embryonic cells have different mechanisms for generating an asymmetry in MOM-5/Frizzled activity? It is likely that early signaling has a role in defining the a/p axis that orients subsequent MOM-5 asymmetry, since embryos depleted of MOM-2/Wnt contain some cells with reversed POP-1 asymmetry [7]. In addition, if the asymmetric localization of MOM-5 requires transport to the posterior pole, such transport might be difficult in the early embryonic cells. The early, but not later, cells show a pronounced flow or "capping" of their cortical actomyosin cytoskeleton toward their apical surfaces ([14] and E. Munro, personal communication). In most of the early cells, this flow is oriented either opposite or perpendicular to the posterior pole.

MOM-5/Frizzled is the first component of the POP-1 asymmetry pathway that has been shown to localize

asymmetrically prior to cell division. In addition, we have shown that MOM-5::GFP localizes to the leading edges of epidermal cells during ventral enclosure. These asymmetries suggest a possible molecular parallel to *Drosophila* Frizzled and the Planar Cell Polarity (PCP) pathway. Frizzled is localized asymmetrically in the wing epithelium [15] and is localized to the leading edges of epidermal cells during dorsal enclosure [16]. However, we have not yet been able to demonstrate by RNAi experiments that *C. elegans* homologs of core components of the PCP pathway, such as Prickle or Flamingo/Starry Night, have roles in polarity, and a presumptive null mutation in the only apparent homolog of Van Gogh/Strabismus is homozygous viable (B0410.2; S. Mitani, personal communication). Anterior/posterior MOM-5::GFP asymmetry is present in *C. elegans* cells that have very short cell cycle times, between 15 and 25 min, and that are not organized into distinct epithelia; these cells lack adherens junctions and many change their neighbors markedly as they move over the surface of the embryo or enter the interior during gastrulation. In contrast, the asymmetrical accumulation of PCP proteins in *Drosophila* wing cells occurs over several hours, and many of the PCP components are associated with adherens junctions [15, 17]. Future studies should clarify whether these differences necessitate additional or distinct mechanisms for cell polarity in *C. elegans* embryos.

Experimental Procedures

Strains and Alleles

Nematodes were cultured as in Brenner [18]. The following mutant alleles and chromosomal rearrangements were used: LGI, *mom-5(ne12), dpy-5(e61)*; LGIII, *unc-119(ed3)*. Transgene insertion *zuIs145* and extrachromosomal array *zuEx123* were created in this study and are described below. Levels of endogenous MOM-5 were reduced either by mutation (*mom-5(ne12)* genetic background) or by RNAi directed against the 3′-UTR of the *mom-5* gene (see below). Both techniques were used for analysis of MOM-5::GFP expression in intact embryos, except in *wrm-1(RNAi)* embryos, where only mutation was used. 3′-UTR RNAi was solely used in isolated blastomeres, and neither technique was utilized for larval cell analysis.

Plasmid Construction and Worm Transformation

Standard techniques were used to manipulate and amplify DNA. Briefly, a *nmy-2::mom-5::gfp* transgene was created by modification of a previously described *nmy-2::gfp* transgene [14]. A full-length *mom-5* cDNA was isolated from adult *C. elegans* RNA by RT-PCR and fused to the *nmy-2* promoter and to the gene encoding GFP as follows: *nmy-2*/linker/5′ *mom-5*: gcggtaataatg/ggcgcgcca/catcgacat and 3′ *mom-5*/linker/*gfp*: aatatgagg/ggaggaagtggtcctgcaggaggt/atgagtaaa. Error-free constructs were identified by sequencing and used for transformation. Strains expressing MOM-5::GFP were obtained by microparticle bombardment of *unc-119* worms and extrachromosomal arrays were integrated by γ-irradiation [19]. We determined that the transgene was expressed embryonically, as well as maternally, by crossing heterozygous transgenic males into wild-type hermaphrodites and scoring GFP in the resulting cross-progeny.

Imaging and Analysis of Live Embryos and Larvae

Confocal images were acquired on either a Leica TCS SP or Zeiss LSM 510 META scanning confocal microscope; optical sections of embryos mounted on 4% agarose were collected at 1.5 min intervals. Confocal images were constructed by combining two adjacent z-slices, 0.5 μm apart, with the exception of Figures 2A–2D, which are single z-slices. For analysis of postembryonic stages, larvae that were hatched in M9 at 26°C were placed on culture plates containing food and collected at various time points. Prior to analysis (30 min), larvae were placed in a solution of 0.1% Tricaine and 0.01% Levamisole in M9. After 20 min, the anaesthetized larvae were mounted on agar pads with an additional 20 μL of the anesthetic solution.

Immunostaining and Cell Isolations

Embryos and cultured blastomeres were fixed and stained for POP-1, DAPI, and midbodies as described previously [7]. Cultured blastomeres were stained overnight at room temperature for GFP (1:1000 dilution rabbit anti-GFP, Abcam ab6556). Embryos were prepared for centrosome immunostaining by freeze-cracking [20] followed by 5 min in ice-cold DMF (N,N-Dimethylformamide, Sigma-Aldrich); a 1:10 dilution of mouse monoclonal IFA primary antibodies [21] was used.

dsRNA-Mediated Interference (RNAi)

Standard techniques were used to synthesize double-stranded RNA (dsRNA) for *mom-2* and *wrm-1* as described [7]. For dsRNA of the mom-5 3′UTR, a 570 base sequence was chosen beginning 120 bases downstream of the stop codon. L3 or L4 hermaphrodites were soaked overnight with dsRNA and 3 mM spermidine [22].

Supplemental Data

A supplemental movie is available with this article online at http://www.current-biology.com/cgi/content/full/14/24/2252/DC1/.

Acknowledgments

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