Gut Reaction to Minireview
Wnt Signaling in Worms

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The demonstrations in two papers in this issue of Cell (Rocheleau et al., 1997; Thorpe et al., 1997) of the involvement of a Wnt pathway in very early embryogenesis in Caenorhabditis elegans provides another significant step toward the ambitious but realistic goal of understanding all of the basic strategies used to control embryogenesis in this model organism. At the same time, they challenge some of the prevailing models of Wnt signaling, suggesting that interactions among Wnt pathway components may vary in different developmental processes. With these papers, as well as the earlier reports on Wnt pathway genes li-4, lin-17, and pops-1 (Herman et al., 1995; Lin et al., 1995; Harris et al., 1996; Sawa et al., 1996) and new studies on Wnt pathway genes reported in recent meetings, worm breeders have become a significant force in the army of Wnt researchers. They have also illustrated how different systems can provide important new complementary insights.

Signaling at the Four-Cell Stage Polarizes a Blastomere

The establishment of tissue specificity in the early C. elegans embryo is directed by the combination of asymmetric cell divisions guided by intrinsic mechanisms and cell-cell interactions (reviewed by Schnabel and Priess, 1997). The fertilized egg, polarized by the entrance of the sperm, divides unevenly and generates an unpolarized cell AB and a polarized cell P2. Some evidence suggests that a signal from P2 to AB specifies the anterior-posterior differences of AB descendants. During the second round of divisions, the P1 cell again generates an unpolarized cell EMS and a polarized daughter cell P2, whereas AB generates two cells (ABp and Aba) with equal developmental potential. While the cell fate of P2 continues to be dictated, at least in part, by intrinsic factors, the cell division patterns and differential fates of ABp and EMS are instructed by P2-originated cell signaling at the four-cell stage (Figure 1). A signal from P2 to ABp breaks the equivalence between ABp and Aba, establishing the dorsal/ventral, differential fates of their descendants. This signaling event is mediated by the APX-1 (Delta-like) and the GLP-1 (Notch-like) proteins. A second signal from P2 polarizes its sister cell, EMS, which results in the two daughters of EMS, E and MS, having strikingly different cell fates. This P2-EMS signaling event has now been shown to involve a Wnt pathway.

During normal embryogenesis, the descendants of MS primarily generate mesodermal tissues, such as pharyngeal tissue and body wall muscle, while the descendants of E are endodermal cells that make the intestine (gut). E cell fate depends upon an inductive signal originating outside of EMS, since when an EMS blastomere is isolated and allowed to develop alone, both of its daughter cells differentiate like MS and no longer produce gut (Goldstein 1993). Further nongenetic manipulations of cultured embryonic cells have demonstrated that P2 is the source of this signal and that the position of P2 relative to EMS determines which daughter of EMS produces gut.

mom Genes Define a Wnt Pathway That Induces Cell Polarity and Gut Differentiation

The pioneering studies using cultured blastomeres were followed by classical genetic screens for mutant embryos exhibiting the gutless phenotype expected from disrupting P2-EMS signaling (E transformed to MS) (Rocheleau et al., 1997; and Thorpe et al., 1997). The two research groups isolated 29 such mutations that define five mom (more mesoderm) genes. Three of the genes have been cloned and found to encode components in the Wnt signal transduction pathway.

Figure 1. Cell-Cell Signaling in Four-Cell Embryo of C. elegans
See the text for explanation and Schnabel and Priess (1997) for a detailed review.
the gonad of C. elegans hermaphrodites, has recently been shown in many laboratories to generate loss-of-function mutant phenotypes (refs. in Rocheleau et al., 1997). For example, injections of RNA of other Wnt pathway genes like mom-2, mom-5, and pop-1 result in similar phenotypes as those of mutations in these genes. RNAi is now commonly used to probe functions of cloned genes in early developmental events. How RNAi disrupts gene activity is currently not understood, although it has been observed in several cases that the protein is not expressed in RNAi-treated embryos. Injections of wrm-1 or apr-1 RNA, which presumably suppress the endogenous genes’ activities, cause the same gutless phenotype as that of mom mutants, suggesting both genes act positively in this wnt-mediated signaling pathway. In order to confirm these RNAi observations, and to study further the functions of wrm-1 and apr-1, it will be still desirable to have true genetic mutations in these genes.

One other gene also involved in specifying cell fates of E and MS is pop-1 (Lin et al., 1995). pop-1 mutants have a phenotype opposite to that of the mom mutants in gut induction, as both E and MS cells adopt an E-like fate and produce gut tissue. pop-1(+/-) activity thus normally suppresses the E cell fate. pop-1 encodes an HMG-domain protein similar to the TCF and LEF-1 proteins discovered in vertebrates and more recently in Drosophila as downstream factors in the Wnt pathway (Figure 2). In double mutants, the pop-1 mutant phenotype (extra gut) is completely epistatic to the gutless (B) current prevail model for genetic (A) and molecular (D) functions of downstream Wnt pathway components during gut induction in C. elegans (based on Rocheleau et al., 1997, and Thorpe et al., 1997). Since the mother cell EMS is already polarized for gut potential prior to its division, POP-1 distribution is likely already polarized in EMS, and the difference is then carried to the daughters.

**Different Models for Functions of β-catenin, TCF, and APC Gene Families**

In recent years, major advances have been made in understanding Wnt signaling pathways in Drosophila and in vertebrates that control a variety of cell differentiation and pattern formation decisions (e.g., reviewed by Peifer, 1996; Moon et al., 1997; Nusse, 1997). A prevailing model of the actions of downstream Wnt-signaling components is depicted in Figure 2A and 2C. In the absence of a Wnt signal, β-catenin/Arm level is low due to degradation promoted by GSK-3 kinase and APC. In the presence of a Wnt signal, an Fz-like receptor is activated and it, in turn, activates Dishevelled (Dsh). Dsh then inactivates the GSK-3 kinase, resulting in a high level of β-catenin. β-catenin then interacts with TCF/LEF1. The complex translocates to the nucleus to activate transcription of Wnt-responsive genes. The evidence for the positive role of TCF/LEF1 in Wnt signaling is summarized below (see Nusse, 1997, for references). TCF and LEF1 contain an HMG-box that binds to DNA and causes bending of the helix. Binding between TCF and β-catenin appears to promote β-catenin translocation to the nucleus, and β-catenin appears to be a coactivator when forming the complex with TCF. In functional tests using Xenopus embryos, injection of LEF1 RNA into ventral blastomeres induces the formation of a second body axis as does Wnt. More convincing data supporting a positive role of TCF in Wnt signaling comes from the more recent genetic work on the Drosophila gene named pangolin (pan) or dTCF (Brunner et al.,...
1997; van de Wetering et al., 1997). Loss-of-function mutations in pan result in phenotypes similar to that of loss of wingless (wg) functions. Since pan mutations suppress the phenotype of constitutively activated arm, Pan (TCF) is thus required for Arm (β-catenin) to transduce the Wg signal.

The genetic properties of pop-1 (TCF) clearly do not fit the above model (Figure 2). In C. elegans, wrm-1 (Arm) inactivates pop-1 (TCF), whereas in Drosophila, arm activates pan (TCF). Such a contradiction suggests that the same proteins may execute different roles under different developmental circumstances or different subtypes of the protein families may function differently. It could be speculated that in the case of C. elegans gut induction, POP-1 protein, either by itself or by forming a complex with another unknown protein, functions to repress transcription of genes specific for the gut fate. The Wnt signal from the P2 cell may activate WRM-1 in the posterior half of EMS, and binding of WRM-1 to POP-1 would then form a nonfunctional, perhaps cytoplasmic, complex. The E cell would then differentiate into gut by a default mechanism (Figure 2D). Such a scheme may be consistent with some work done in other systems. In Xenopus, a mutant XTCF3 with its β-catenin binding domain deleted was shown to suppress normal axis formation (Molenaar et al., 1996). Merriam et al. (1997) also showed that cytoplasmically anchored plakoglobin (another vertebrate homolog of Arm) can nevertheless induce a phenotype similar to that induced by Wnt.

In the vertebrate model, APC plays a negative role in Wnt signaling as it functions to promote β-catenin degradation by forming a protein complex with β-catenin and GSK-3. Mutations in APC block such downregulating processes in mammalian cells, resulting in a high level of cytoplasmic β-catenin activity (Peifer 1996). In contrast, a positive role has been proposed for C. elegans apr-1 (APC) in gut induction (see above, Figure 2B and 2D). A positive role of APC may also be suggested by the unexpected result in Xenopus (Vleminkx et al., 1997) that APC induces a duplication of the body axis similar to that induced by Wnt. Given that this effect appears to require cytosolic β-catenin, APC may act together with β-catenin to promote signaling (Peifer, 1996). APC thus may also exert different functions under different circumstances. In C. elegans, APR-1 (APC) may act with WRM-1 (Arm) to down-regulate POP-1 (TCF) (Figure 2D).

**Multiple Signaling Input in Gut Induction**

One striking feature of the genes involved in worm gut induction is the difference in the penetrance of their mutant phenotypes (Rocheleau et al., 1997; Thorpe et al., 1997). Although lesions in many mom mutants suggest they are null or severe loss-of-function mutations, the percentage of the mutant embryos with the gutless phenotype is low for some genes. For example, less than 10% of the mom-5 (Fz) mutant embryos and only 26% of apr-1(RNAi) embryos lack endoderm, suggesting that there are other signaling molecules that can mediate P2-EMS induction. Since disrupting both the apr-1 (APC) and mom-5 (Fz) genes causes a highly penetrant gutless phenotype, apr-1 may act in parallel to mom-5 to induce the E cell fate. These two signaling branches may converge on the wrm-1 (Arm) gene since disrupting wrm-1 gene activity results in a completely penetrant gutless phenotype (Rocheleau et al., 1997; Figure 2B and 2D).

Another puzzling phenomenon is that mom-5 (Fz) mutants have a much lower penetrance of the phenotype (5% for allele zu193) than do mom-2 (Wnt) mutants (39% for allele ne414). The double mutant with both zu193 and ne414 is surprisingly similar to the weaker mom-5 single mutant. This result suggests that the MOM-5 (Fz) receptor has a negative role in the absence of the MOM-2 (Wnt) signal (e.g., by responding to a different signal that antagonizes WRM-1 activity). Wnt signaling processes, like many other signaling pathways, are likely to be networks that involve cross-talk and feedback loops, rather than simple linear cascades.

**Multiple Decisions Controlled by Multiple Wnt Signaling Pathways**

wnt genes and several components in the wnt pathway exist as multigene families in vertebrates, Drosophila and C. elegans. For example, there are at least 16 wnt-like and eight fz-like genes in the mouse (Moon et al., 1997) and at least five wnt-like and four fz-like genes in C. elegans. Multiple genes also appear to encode Dsh and Arm homologs. Although some family members might have redundant roles, in many cases, functional studies have indicated that different members function in distinct developmental decisions. Current work on mom-2 (Wnt) and mom-5 (Fz) in C. elegans has defined developmental functions that are distinct from those of the previously described lin-44 (Wnt) and lin-17 (Fz) genes (Herman et al., 1995; Harris et al., 1996; Sawa et al., 1996).

lin-17(Fz) mutations disrupt the asymmetry of cell division in unrelated cells in many different tissues (Figure 3A). lin-17 (Fz) appears to function in the mother cell to polarize the cell and thus plays a role similar to that of mom-5 (Fz) in the induction of EMS polarity. However, lin-44 (Wnt) mutations cause reversals, but not elimination, of the polarity of division of a subset of those cells affected by lin-17 (Fz) mutations (Figure 3A). To explain the difference in the phenotype by these two genes, Sawa et al. (1996) proposed the existence of additional signal(s) for the lin-17 receptor (Figure 3B). In their
model, LIN-17 would be activated only in the posterior half of the cells, presumably by the directional signal of LIN-44 (Wnt). However, LIN-17 receptor would also be activated in the anterior half of the cells by another Wnt-like signal (X). The effect of this unidentified anterior signal is proposed to be overridden by the LIN-44 (Wnt) signal. When the lin-17 (Fz) gene is mutated, the asymmetry of division and the major difference between the two daughter cells are abolished. The role of LIN-44 in this model is consistent with its cell-nonautonomous function and its expression in cells that are located posterior to the cells receiving the signal (Herman et al., 1995).

Many wnt genes (e.g., the wingless gene in the fly) function in multiple developmental events, and this may also be the case for the mom genes. Some mom genes appear to function to orient mitotic spindles in many blastomeres during early development. For example, mom-1 (Porc), mom-2 (Wnt), mom-5 (Fz) and mom-3 (uncloned) mutants all have fully penetrant defects in mitotic spindle orientation of the ABar cell, a granddaughter of AB (Rocheleau et al., 1997; Thorpe et al., 1997), a phenotype that suggests a role for the Wnt signaling in the regulation of cytoskeletal organization during mitosis. Since disruption of wrm-1 (Arm), apr-1 (APC), mom-4 (uncloned), and pop-1 (TCF) does not result in a mitotic spindle phenotype in ABar, this suggests that the wnt pathway takes a different turn at a point somewhere between the receptor (MOM-5) and WRM-1. Since normal spindle orientation in early blastomeres does not depend upon embryonic transcription, it is likely that the Wnt signal does not involve a DNA target (Rocheleau et al., 1997). This process could have some similarity to tissue polarity determination in Drosophila where fz, dsh, and rhoA, but not other downstream factors of the wnt pathway, were found to play roles (Drasnow et al., 1995; Strutt et al., 1997).

Conclusions
Current studies using C. elegans genetics are expected to generate more exciting results about the functions of Wnt signaling pathways in various developmental events. While the research projects will continue to aim at understanding the cellular and molecular mechanisms of cell signaling events in directing development, some cell signaling events such as the P2-to-EMS signaling will also be excellent assay systems to dissect the Wnt signal transduction pathways. Not only do these genetic and molecular analyses increase our knowledge of the functions of the known components of the Wnt pathways, but they might also reveal new components of the pathways, as the cloning of mom-3 and mom-4 is likely to prove.

Selected Reading