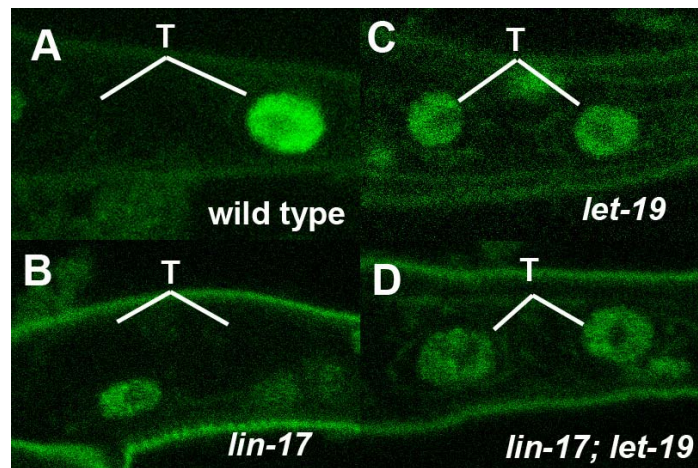


Asymmetric T cell division by dint of Wnt

March 25, 2005 – Asymmetric division, in which a cell divides into daughter cells of different natures, is an indispensable process in the development of multicellular organisms. Every embryo begins its development as a single fertilized egg, dividing first to form a small cluster of very similar cells which later go on to differentiate into cells of myriad types, enabling the construction of complex tissues and organs. From the very first step of ontogeny, the ability to generate cells of different types from a single ancestor is central – indeed, without asymmetric cell division, the embryo would be no more than an undifferentiated clump of identical cells.

This asymmetry is achieved in different taxa by various means. The fruit fly *Drosophila*, for example, allocates the transcription factor Prospero to only one of two daughter cells during its neurogenesis, marking it as a ganglion mother cell directly responsible for generating neurons. The daughters deprived of Prospero, meanwhile, retain their identity as neuroblasts, progenitor cells capable of further self-renewal in subsequent rounds of cell division.

Asymmetric cell division is important in many aspects of development in the nematode *C. elegans* as well, including the differentiation of a lineage of cells found in the tail region, named T cells. In a report published in the March 25 issue of the journal *Development*, Hitoshi Sawa and colleagues in the Laboratory for Cell Fate Decision, and in labs at Osaka, Kobe and Keio Universities, describe the requirement for a pair of molecules, LET-19 and DPY-22, thought to contribute to the formation of a multicomponent complex at work in T cell asymmetric division.



T cells expressing *tlp-1::GFP* fusion protein. Wildtype cells (A) show expression in only a single daughter cell, while in *lin-17* mutants (B) expression is absent in both daughters. Patterns in *let-19* single mutant (C) and *lin-17/let-19* double mutant, in which both daughters express the fusion protein, are similar, suggesting *let-19* functions downstream of *lin-17*.

LET-19 and DPY-22 seem to achieve their asymmetry-regulating function by the transcriptional repression of target genes in the Wnt signaling cascade, a molecular pathway known to play roles many aspects of development in a diverse menagerie of organisms, including *C. elegans*. In T cells, for instance, the factor TLP-1 has recently been shown to be regulated by the Wnt pathway resulting in its unequal expression

in T lineage daughters. Loss of function of the genes *lin-44* or *lin-17* (which respectively encode proteins homologous to the Wnt ligand and its receptor Frizzled) interferes with normal asymmetric division, causing the failure of T.p cells to generate the phasmid socket cells that would usually be included in their progeny. But the molecular means by which Wnt regulates the transcription of target genes in to achieve this asymmetry remains obscure.

Sawa and colleagues first identified *let-19* and *dpy-22* in an analysis of worms in which the phasmid socket cells were absent. These phenotypes showed loss of asymmetry in T cell daughters similar to that seen in *lin-17/frizzled* mutants. Seeking to determine the mechanism by which *let-19* and *dpy-22* operate in this process, Sawa et al. looked at their relationship to a pair of genes, *pop-1* and *tlp-1*, known to be expressed differentially in T.a and T.p cells, the anterior and posterior progeny of T cell division. In T.p cells, the localization of POP-1 is controlled by Wnt signaling, so the team used a construct fusing POP-1 with green fluorescent protein (GFP), allowing them to track the protein's distribution within cells. These studies revealed that mutations of neither *let-19* nor *dpy-22* affected POP-1 localization, ruling out the possibility of a regulatory role for these genes in that process.

They turned next to *tlp-1*, whose protein product is a downstream factor in the Wnt pathway expressed specifically in T.p but not in T.a cells. A study in *lin-17* mutants showed the expected defects in *tlp-1* expression. Looking at loss-of-function mutations for *let-19* and *dpy-22*, Sawa found that in either of these mutants, *tlp-1* expression could be seen in both the T.a and the T.p daughter cells, reflecting the loss of their normally unequal distribution. Worms in which both *lin-17* and *let-19* were mutated showed the same phenotype as the *let-19* single mutant, indicating that *let-19* works downstream of the Wnt signal. Further studies using other cell lineages failed to produce any effects on patterns of *tlp-1* expression.

Pursuing the *let-19* and *dpy-22* connection to the Wnt cascade, Sawa next examined their effects in Pn.p cells, a series of 11 cells found running down the anterior-posterior axis on the ventral (belly) side of the nematode. P1.p and P2.p, the anteriormost of the cells, and P9.p~P11.p, the last three in line, undergo fusion with the hypodermis, leaving the remaining six cells in the center to act as precursors of one of the worm's organs of reproduction, the vulva. In this cohort, the gene *bar-1* encodes the *C. elegans* homolog of β -catenin, which serves to maintain the expression of LIN-39/Hox, an inhibitor of cell fusion. In *bar-1* mutants, fusion occurs ectopically, leading to a reduction in the number of vulval precursors. The team found that both the *let-19* and *dpy-22* mutants underwent less frequent cell fusion and seemed to repress the *bar-1* phenotype characterized by ectopic fusion. Contrastingly, *let-19* mutations did not suppress the *lin-39* phenotype, suggesting that *let-19* and *dpy-22* work by repressing *lin-39* downstream of *bar-1*. They looked for further involvement in other phenomena where Wnt signaling is a factor, such as endodermal induction and the formation of distal tip cells at the leading edge of the migrating gonad, but found that *let-19* and *dpy-22* function is specific to Pn.p and T cells.

The gene products of *let-19* and *dpy-22* are homologous to mammalian MED13 and MED12, respectively, both of which are components of transcriptional regulatory "Mediator" complexes, which have been shown to play roles in the activation or repression of numerous genes. Two mammalian Mediator complexes – CRSP and ARC-L – are known, the latter being a larger, transcriptionally active complex that contains both MED12 and MED13. Co-immunoprecipitation assays demonstrated that

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LET-19 and DPY-22 bound other putative Mediator complex components, leading the team to surmise the existence of a pair of Mediator complexes in *C. elegans* as well, with LET-19 and DPY-22 acting as components of the counterpart to mammalian ARC-L.

The results of this thoroughgoing investigation point clearly to roles for LET-19 and DPY-22 in both the asymmetric division of T cells and the regulation of Pn.p cell fusion by the inhibition of Wnt target genes. They speculate that activation of the Wnt (LIN-44/LIN-17) pathway results in the conversion of the ARC-L like complex to one that resembles CRSP by causing the release of LET-19 and DPY-22, which suppress the expression of *tlp-1*. Normal asymmetry may arise in T cells where the ARC-L-like complex inhibits *tlp-1* in the anterior T.a cells, while the CRSP-like version lacking LET-19 and DPY-22 allows *tlp-1* to be activated in the T.p posterior daughter. Similarly, in Pn.p cell fusion, LET-19 and DPY-22 may inhibit the expression of *lin-39* in the absence of Wnt signaling, and conversely activating *lin-39* (and thereby repressing cell fusion) in the presence of Wnt signal. Although the means by which Wnt signaling and responsiveness are regulated remain to be worked out, the Sawa study has shown how this prevalent and much-studied cascade may act as a kind of switch by adjusting the makeup of a protein complex responsible for regulating gene transcription.