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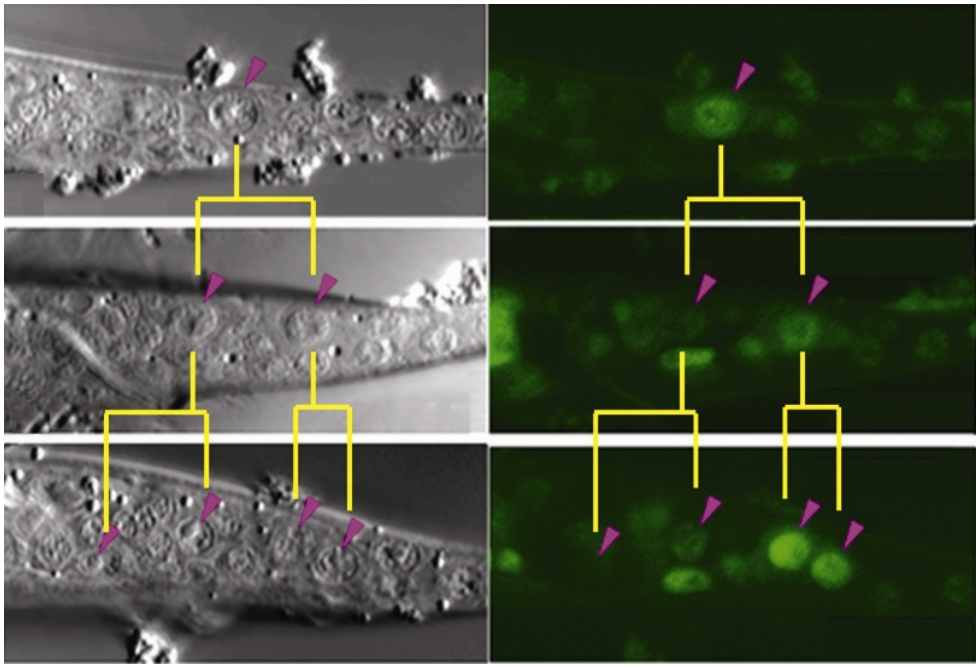
Wnt signaling and Hox regulate PSA-3 to determine cell fate in *C. elegans*

July 10, 2006 – A diversity of cell fates is generated during animal development through processes including asymmetric cell division, in which cells' identities may be fixed even before they are born by the unequal allocation of fate-determining proteins to opposite sides of a cell prior to mitosis. This form of predestination is at work in the roundworm *C. elegans* just as it is in larger, more complex organisms. Surprisingly, in *C. elegans*, most of cell asymmetry is the outcome of divisions regulated by the Wnt-MAPK signaling pathway, as mediated by POP-1. This general mechanism, however, cannot fully explain the spatio-temporally specific cell fates generated through asymmetric division.

Now, Yukinobu Arata and colleagues in the Laboratory for Cell Fate Decision (Hitoshi Sawa; Team Leader) report that cell specialization arises as the net regulatory effect of Wnt signaling and positional identity cues on a novel factor, PSA-3. "This is a neat demonstration of how cooperation between a general mechanism for asymmetric cell division and position-specific factors can lead to discrete outcomes during development," says Arata.



Asymmetric expression of psa-3 in the tail region of C. elegans as visualized by differential interference contrast (DIC) microscopy (left) and GFP labeling (right)

Recent work from the Sawa lab had shown the role a Wnt-related factor, POP-1, plays in determining the polarity of both embryonic and postembryonic cells in *C. elegans*. But the mechanism appeared to be general to a wide variety of asymmetrically dividing cells, suggesting that additional factors must help guide such Wnt-polarized cells to their ultimate fates. Arata et al. focused on at a single cell type, the asymmetrically dividing T cell in the roundworm tail, which ordinarily divides to give rise to a hypodermal anterior and a neural posterior daughter. Screening for mutations that disrupted this pattern, they identified a trio of mutants for the genes *psa-3*, *ceh-20* and *nob-1*, all of which yielded posterior daughters lacking structures known as phasmid sockets, which characterize cells in the neural lineage. Of these, *nob-1*and *ceh-20* had previously been identified as roundworm homologs of the

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positional specification genes *Hox* and *Pbx*, respectively. *psa-3*, however, showed up only as a presumptive entry in databases of *C. elegans* genes.

Analysis of its sequence revealed that *psa-3* shared similarities with genes in the Meis family of transcription factors, and even more intriguingly, contained a domain that appeared to be a POP-1 binding sequence. The team created a GFP *psa-3* construct to allow them to follow its activity in T cells, and found that *psa-3 was* gradually upregulated in the posterior descendants, following the initial T cell division. In worms engineered with a nucleotide substitution in the putative POP-1 binding sequence, this effect was lost, indicating to the team that *psa-3* expression in posterior T cell daughters is regulated by its interaction with the *C. elegans* Wnt signaling pathway.

It remained to be seen, however, how *psa-3*'s effect could be kept specific to posterior descendants of T cells, when POP-1 was known to operate in the asymmetric divisions of all seam cells, of which T cells are only a subset. Roundworms with mutations in the *Hox* equivalent *nob-1* and the *Pbx* homolog *ceh-20* showed decreases in *psa-3* in the posterior lineage. Searching for the regulatory elements responsible, they found that the fourth *psa-3* intron contained a binding sequence for NOB-1, and that this interaction appeared to be facilitated by CEH-20, indicating that the tail Hox factor, NOB-1 allows *psa-3* to function selectively in posterior descendants of T cells. And interestingly, the Sawa team found that *psa-3* itself functioned in localizing CEH-20 to the nucleus in posterior T cells, suggesting a regulatory circuit in which *psa-3* and *ceh-20* cooperate to maintain each other's functions. These findings were borne out in vivo, where the results of heat shock rescue experiments indicated that PSA-3, NOB-1 and CEH-20 work as co-factors in regulate cell fate in the posterior lineage after asymmetric T cell division.

"It appears that similar mechanisms for asymmetric division are used repeatedly during the development, not only of *C. elegans*, but many other organisms as well," notes Sawa. "It will be interesting to find out whether Hox proteins cooperate with a common mechanism for asymmetry to specify diverse cell fates in other species as well."

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