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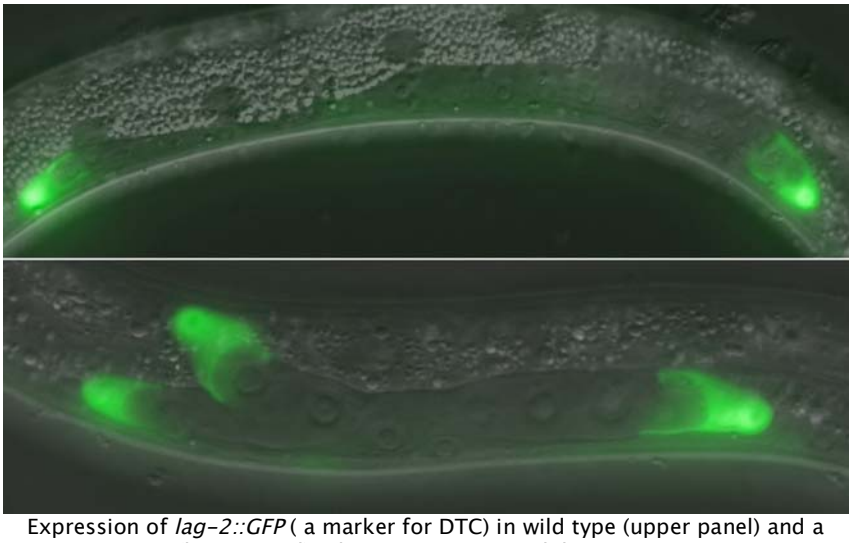
Key to quiescence? New role for cell-cycle regulators in inhibiting proliferation of uncommitted cells

May 7, 2007 – In many biological contexts, proliferation and differentiation stand at odds, as dividing cells tend to be undifferentiated and terminally differentiated cells tend not to divide. Cell cycle regulators frequently play a part in governing this diametric relationship, but little is known of what roles such factors might play in maintaining the uncommitted state of quiescent (non-proliferating) cells.

This question has now been addressed in a study by Masaki Fujita and others in the Laboratory for Cell Fate Decision (Hitoshi Sawa; Team Leader), which looked at how a tightly maintained balance of the activity of cell cycle regulators keeps such cells from differentiating in the roundworm, *C. elegans*. In an article published in the open access journal *PLoS One*, the team describes how the interplay between the worm homologs of a triad of cell cycle regulators—cyclin E, CDK2 and CKI—simultaneously prevents differentiation and division in uncommitted, quiescent cells.



Expression of *lag-2::GFP* (a marker for DTC) in wild type (upper panel) and a *cye-1* mutant (lower panel). The cye-1 mutant exhibits supernumerary DTC.

The *C. elegans* gonad develops from a pair of precursor cells, called Z1 and Z4, which undergo two rounds of division to yield four progeny, the most distal of which (Z1.aa and Z4.pp) differentiate into the distal tip cells, or DTCs, that lead the developing gonad as it follows its U-shaped migratory path through the larval body. Fujita found that worms lacking the gene *cye-1* (the *C. elegans* homolog of *cyclin E*) frequently developed extra DTCs, suggesting a role for this gene in regulating differentiation. By laser-ablating daughter and granddaughter cells in the Z1 and Z4 lineages, individually and in various combinations, the team determined that the supernumerary DTCs in the *cye-1* mutants are generated from the sisters of the normal DTCs (Z1.ap and Z4.pa).

Cyclin E is known to function cooperatively with a second cell-cycle protein, CDK2, in other organisms, so Fujita et al. next examined the effects of its loss of function by inhibiting the roundworm gene using RNAi. As with the *cye-1* mutants, the *cdk-2* knockdown worms showed the extra DTC phenotype, in a manner suggesting that the two factors act in partnership.

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Cell-cycle regulators such as cyclins and cyclin-dependent kinases are typically kept in check by factors known as CKIs. On analyzing the patterns of expression of *cye-1* and the roundworm CKI, *cki-1*, in worms in which the protein products of these genes had been engineered to fluoresce green, the Sawa team found that they were expressed asymmetrically, with *cye-1* levels higher in the Z1.ap and Z4.pa cells (which are normally quiescent) and *cki-1* expression stronger in the Z1.aa and Z4.pp cells that normally differentiate into DTCs. Using a temperature-sensitive mutation of *wrm-1* (roundworm *β-catenin*) to allow them to initiate its loss of activity after the division of the Z1/Z4 cells, they found that this unequal distribution is under the control of the Wnt/MAPK pathway, as is the case in a number of other asymmetric cell divisions in *C. elegans*.

Given the higher levels of *cki-1* in the quiescent sisters of the DTCs, the lab next looked at a possible interaction between this gene and the partnership of *cye-1* and *cdk2*. In worms in which *cki-1* alone had been knocked down, the majority of animals showed extra cell divisions, but when this interference was combined with either *cye-1* mutation or *cdk-2* RNAi, no such phenotype occurred, suggesting that *cki-1* inhibits the proliferation of the Z1.ap and Z4.pa cells, preventing *cye-1*/*cdk-2* from triggering cell division but leaving their activity high enough to prevent terminal differentiation.

A similar mechanism appears to be at work in at least one other cell lineage exhibiting asymmetric cell divisions, as usually quiescent seam cells in *cye-1* mutant worms adopted an abnormal syncytial fate, indicating that *cye-1* is needed to repress terminal differentiation in multiple quiescent cell types. “As many stem cells are quiescent, the cell cycle regulators we identified as functioning in this context may also serve to maintain the undifferentiated state of stem cells in other organisms,” notes Sawa, highlighting the potential importance of this new role for cyclins, CDKs and their inhibitors in maintaining quiescence.

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