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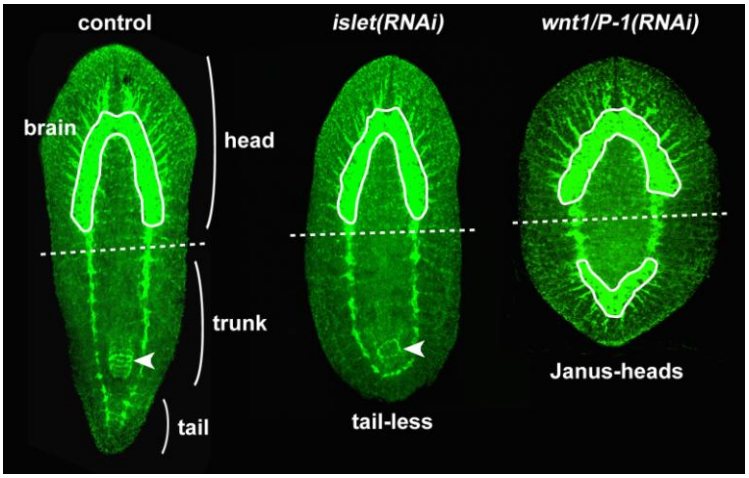
Planarian *Islet* homolog works in posterior differentiation

February 9, 2012 – Planarians are a group of small flatworms with significant capacity for regeneration, which enables them to reform entire bodies from individual amputated fragments. To do so, however, each fragment needs to have a sense of where it should be in the body, so that missing head and tail regions form as needed and in the appropriate location with respect to the rest of the regenerating body. This positional information is thought to be conferred by gradients in the expression of various genes that activate, modulate or inhibit cell differentiation. The Wnt signaling pathway, for example, is known to be necessary for the specification of posterior regions. The molecular mechanisms that underlie this function, however, are poorly understood.

A new study by Tetsutaro Hayashi and others in the Genome Resource and Analysis Unit (Fumio Matsuzaki, Unit Leader) now reveals an additional factor, encoded by a planarian LIM-homeobox gene known as *Djislet*, is required for the posterior differentiation of Wnt-expressing cells. Published in *Development*, this reports yields new insights into the coordination that occurs between stem and differentiated cells in an important model of animal regeneration.



Djislet(RNAi) animals show tail-less regeneration phenotype. *Djwnt1/P-1(RNAi)* animals show two-headed regenerants (Janus-heads phenotype). Arrowhead, pharyngeal neuron. Dashed lines indicate the amputation site.

The study began with the isolation of an apparent homolog of the vertebrate gene Islet from an EST (expressed sequence tag) library for the planarian species *Dugesia japonica*, which is indigenous to Japan. In vertebrates, the Islet family of LIM-homeobox transcription factors is known to be involved in the proliferation, maintenance and migration of stem and progenitor cells in a range of tissues. When Hayashi knocked down the function of this gene, dubbed *Djislet*, using RNA interference (RNAi), he found that its loss of function resulted in a tailless regeneration phenotype in fragments from any part of the body (head or trunk), suggesting that the gene might function in posterior differentiation and regeneration.

This suggested in turn the possibility of a functional link to the Wnt signaling pathway, which had previously been shown to be required for posterior specification and regeneration in another flatworm species, *Schmidtea mediterranea*. Analysis of the expression of these genes, *Djislet* and *Djwnt1/P-1*, showed co-localization at the midline of the blastema, the locus of regeneration in planarians, at day 3 of regeneration. Moreover, in *Djislet(RNAi)* worms, the expression of *Djwnt1/P-1* disappeared from the posterior blastema, suggesting that this gene functions downstream of the Islet function.

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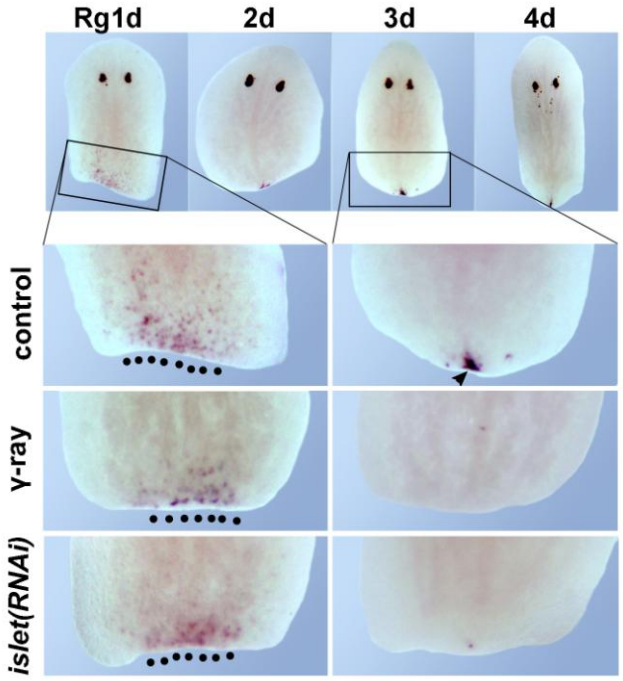

The expression patterns of *Djwnt1/P-1* mRNA in the head regenerants during posterior regeneration (from 1 to 4 days after posterior amputation). The expression of *Djwnt1/P-1* is eliminated by *Djislet(RNAi)* and γ-ray irradiation at day 3, but not day 1.

Interestingly, the loss of Islet function had a more limited effect than that of Wnt, in that *Djwnt1/P-1(RNAi)* worms show both tailless regeneration and occasional formation of two-headed regenerants (known as the Janus-heads phenotype), while *Djislet(RNAi)* animals regenerated without tails, but never with two heads. Examining gene expression in the process of *Dugesia* regeneration more closely, the lab found Wnt is expressed in two phases, at days 1 and 3, with the Islet homolog required only for the second. Importantly, this second phase is senstitive to γ-ray irradiation, suggesting that it involves stem cell-derived cells.

Hayashi next checked the effect of *Djislet(RNAi)* on a panel of genes known to function in the posterior blastema, and was able to groups these into three classes: Class I, which includes genes expressed in γ-ray-insensitive cells not regulated by *Djislet*; Class II genes which are partly downregulated by loss of *Djislet* function; and Class III, which are mainly expressed in γ-ray-sensitive cells and strongly downregulated by *Djislet(RNAi)* at day 3 of regeneration. These findings collectively point to a role for Islet via Wnt signaling in the maintenance and activation of posterior genes in differentiating blastema cells for tail formation.

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