Protein Cell 2011, 2(1): 2–4
DOI 10.1007/s13238-011-1005-6

Protein & Cell

NEWS AND VIEWS

# Smurfs have "fused" into the asymmetric division of stem cells

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The asymmetric cell division is the way in which a stem cell divides into one daughter stem cell and one differentiated daughter cell. This process is one of the key principles of developmental biology that ensures the perpetual supply of stem cells while allowing a particular cell lineage to be populated. During *Drosophila* oogenesis, the fate of the daughter stem cell produced from the asymmetric division of germline stem cells (GSCs) is specified by Decapentaplegic (Dpp), but the other daughter cell has almost equal access to the Dpp signal. How Dpp signaling is inactivated in the differentiated daughter cell has been a deep mystery until a recent study, led by Dahua Chen of the Institute of Zoology, China (Xia et al., 2010), showed that the Dpp receptor, Thick vein (Tkv), is degraded specifically in the differentiated daughter cell by an HECT-domain ubiquitin E3 ligase, Smurf, in conjunction with a serine-threonine kinase Fused (Fu). This finding is as much revealing as it is surprising, because up until now Fu is considered a core member of the Hedgehog signaling pathway. Whether Fu serendipitously steps onto the turf of Dpp signaling or its regulation of Tkv degradation foretells an elaborate coordination between two important cell-cell communication systems will surely be a hot button issue for future studies.

The *Drosophila* ovary is a fascinating organ for studying regulation of stem cell fate decisions by surrounding stromal cells (Fig. 1). Each adult ovary is composed of about 15 linear strings of follicles called ovarioles. Follicles are formed in the most anterior part of the ovariole known as germarium, mature progressively toward the posterior, and eventually bud off. The germarium can be morphologically divided into 3 distinctive regions; region 1 contains 2–3 GSCs residing in the anterior part of the germarium encasement. A stack of terminal filament cells (TF) at the anterior apex of the germarium wall and a single ring of adjoining cap cells (CPC) form a stromal niche, which instructs the contacting daughter cell produced from the asymmetric division of GSCs to maintain the stem cell fate. The other daughter cell, which is positioned posterior to the daughter stem cell and in contact with the cap cell and the inner germarium sheath cell (IGC), becomes the differentiated cystoblast (CB). Each cystoblast undergoes precisely 4 rounds of mitosis with incomplete cytokinesis to give rise to 16 interconnected cystocytes in region 2a. This 16-cell syncytium is enveloped by somatic prefollicular cells in region 2b, and is joined by additional somatic cells as the maturing follicle prepares to bud off in region 3. Only one of the 16 cystocytes will become the oocyte, while the rests form the nurse cells. The stromal niche surrounding GSCs secretes many morphogenic signals

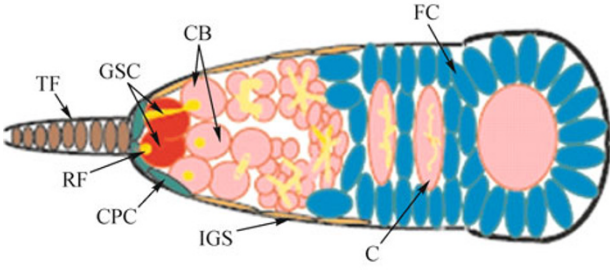


**Figure 1. Diagram of a *Drosophila* germarium structure in cross section.** GSCs, germline stem cells (red); CBs, cystoblasts (pink); TFs, terminal filament cells (brown); CPCs, cap cells (green); IGS, inner germline sheath cells (orange); FC, somatic follicular cells (blue); and C, germline cysts (pink oval). From Xie, T. and Spradling, A. Science 290: 328–330, 2000. Reprinted with permission from AAAS.

including Hedgehog, Wingless, and Dpp, each with a different role. Over 10 years ago, Ting Xie and Allan C. Spradling at the Carnegie Institute of Washington showed that ectopic expression of Dpp in the ovary resulted in the germarium filled with large germline cells, a phenotype reminiscent of what was shown in *bag of marbles* (*bam*). Bam is a stem cell differentiation factor that is specifically expressed in CBs but not in GSCs. The large germline cells induced by Dpp were actually germline tumors, so this observation established the crucial role of Dpp in specifying the germline stem cell fate by suppressing Bam expression (Xie and Spradling, 1998). However, why does not the posterior daughter cell that is destined to be cystoblast respond to Dpp?

To address that question, Chen’s group decided to turn on Dpp signaling in CBs autonomously by ectopic expression of a constitutively activated form of Dpp receptor, Tkv(ca), from a *bam* promoter using the binary UAS (Upstream Activating Sequence) system. To their dismay, this approach permitted the germline to develop normally. After checking a few controls to make sure that the *bam* promoter was active, they found that Tkv(ca) was not expressed in CBs, and instead it was degraded. To investigate what caused Tkv(ca) degradation, they conducted mass spectrometry analysis and identified Fu as one of interacting proteins. It appears that the cytoplasmic domain of Tkv can specifically bind to either the N- or the C-terminus of Fu. Chen’s group demonstrated that in *fu* mutants as well as in flies in which *fu* was inactivated by a microRNA-based RNAi strategy, the germarium accumulated tumorous GSC-like cells, much like the effect caused by ectopic Dpp expression. Since this phenotype could be overcome by increasing Bam expression, Fu must act upstream of Bam as a negative regulator of Dpp signaling in the ovary. *Drosophila* Dpp is homologous to mammalian bone morphogenetic protein 2 (BMP2) and 4 of the transforming growth factor-beta superfamily, which signals through a complex of type I and type II receptors. It is known that the mammalian type I receptor as well as the *Drosophila* counterpart, Tkv, are targeted to ubiquitination and proteasomal degradation by the E3 ubiquitin ligase, Smurf. In the absence of dSmurf, the mutant *Drosophila* ovary exhibits a similar tumorous phenotype as seen in *fu* mutants (Casanueva and Ferguson, 2004). To their prepared minds, this similarity implied underlying mechanistic connection. Lo and behold, Fu, Smurf, and Tkv indeed can form a complex to promote Tkv ubiquitination. Thus, the answer to unresponding CBs seems to lie in the ability of dSmurf and Fu complex to blunt Dpp signaling by removing Dpp receptor Tkv, thereby allowing the expression of Bam to promote differentiation.

The implication of *fu* in the Dpp control of GSC asymmetric division, although surprising, actually solved another mystery of a seldom studied phenotype of *fu* associated with the germline development in the *Drosophila* ovary. It has been known since the 1950s that the ovaries of certain *fu* mutant alleles are filled with the same kind of germline tumors described above (Besse et al., 2005). However, although Hedgehog is part of the stromal niche signal, its influence in the ovary seems to be restricted to the proliferation of somatic prefolliclular cells (Forbes et al., 1996a), because the transcription factor Cubitus interruptus is not expressed in the germline cells (Forbes et al., 1996b; Narbonne-Reveau et al., 2006). So, the ovarian tumor phenotype of *fu* appears to be unrelated to its classical role in the Hedgehog signaling as demonstrated in imaginal disc patterning as well as segmentation of the embryos. For this reason, this part of *fu* function was largely overlooked if not for the effort from a single laboratory led by Anne-Marie Pret of the Center de Génétique Moléculaire, France. Now, it is clear that the germline function of *fu* is by way of antagonizing Dpp signaling. Prior to the publication of Chen’s study, the function of *fu* was intimately wedded into the Hedgehog signaling pathway. It is not clear whether the new finding of *fu* functioning in Dpp signaling is only an opportune breach of its fidelity to Hedgehog or the revelation of a norm of its functional promiscuity. Perhaps, evolution has already given a clue. Despite that the identity and function of many other components of the Hedgehog pathway are highly conserved between insects and mammals, the mammalian kinase (i.e. STK36) that comes closest in resembling the *Drosophila* Fu is not involved in Hedgehog signaling at all (Wilson et al., 2009). Instead, a role of STK36 in antagonizing BMP signaling appears to be conserved in evolution as Chen’s group showed that STK36 physically interacts with both Smurf proteins and ALK3, the type I BMP receptor, and knockdown of STK36 reduced ALK3 ubiquitination and enhanced BMP signaling responses in cultured mammalian cells and, possibly, in zebrafish.

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