A sharp end to sugary Wingless travels

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Drosophila melanogaster follicle stem cells are controlled by Wingless (Wg) ligands secreted 50 µm away, raising the question of how long-distance Wg spreading occurs. In this issue of *JCB*, Wang and Page-McCaw (2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201403084) demonstrate a potential mechanism by which the heparan sulfate proteoglycan Dally-like (Dlp) promotes Wg travel, whereas matrix Mmp2 (Metalloproteinase 2) impedes it by inactivating Dlp.

Tissues are maintained and patterned by stem cells that are controlled in part by signals derived from their niches (Losick et al., 2011). Follicle stem cells (FSCs), located in the germaria of each ovariole in *Drosophila melanogaster* ovaries, give rise to the epithelium that surrounds the egg chambers (Losick et al., 2011). FSCs are regulated by several signaling pathways, including Wingless (Wg), derived from the distal (≤50 µm) terminal filaments (TFs) and cap niche cells (Fig. 1; Losick et al., 2011). Because this signaling is long range, an unresolved issue is how Wg molecules spread. In this issue of *JCB*, Wang and Page-McCaw provide new insights into this process by identifying the heparan sulfate proteoglycan (HSPG) Dally-like (Dlp) and the matrix metalloproteinase Mmp2 as positive and negative regulators of long-range Wg signaling in the germarium, respectively.

In the *Drosophila* wing imaginal disc, Wg has been proposed to act as a morphogen, and a Wg gradient can be detected 50 µm from the source (Strigini and Cohen, 2000). The spreading of Wg in the wing disc requires the glypican Dlp that binds Wg and promotes Wg signaling in distal cells (Baeg et al., 2001, 2004; Kirkpatrick et al., 2004; Kreuger et al., 2004; Franch-Marro et al., 2005; Han et al., 2005; Yan et al., 2009). In the germarium, Wang and Page-McCaw (2014) find that Wg forms a gradient with highest concentrations at the cap/TF cells, whereas Dlp forms an inverse pattern with higher levels closer to the FSCs. They show that *Dlp* loss of function led to a reduction in extracellular Wg level, Wg signaling activity, and FSC proliferation, suggesting that, in the germarium as in the wing disc, Dlp is involved in retaining Wg at the cell surface and preventing its degradation.

In contrast, the authors found that extracellular Wg level and signaling and FSC proliferation (number of stalk cells between follicles, phospho–histone H3 staining, and mitotic clone frequency) are increased in *Mmp2* mutant germaria. Matrix metalloproteinases (MMPs) are extracellular Zn^{2+}-dependent endopeptidases that play pivotal roles in normal tissue remodeling and disease. MMPs have been shown to act on ECM proteins, including collagen, HSPGs, surface molecules, and signaling proteins (Kessenbrock et al., 2010). Mmp2, like Wg, is produced in germarium apical cells. The function of Mmp2 in Wg signaling is likely caused by its regulation of Dlp because Dlp accumulates in *Mmp2* mutant germaria at the TF and mutations in *dlp* suppress the *Mmp2* mutant phenotype.

Previous studies have suggested that Dlp is regulated at multiple layers. For example, in the wing disc, Dlp transcription is modulated by Wg and Hippo signaling (Han et al., 2005; Baena-Lopez et al., 2008), and Notum, a secreted member of α/β hydrolase family, has been shown to cleave Dlp at the level of its glycosylphosphatidylinositol anchor (Kreuger et al., 2004). Wang and Page-McCaw (2014) demonstrate a novel mechanism of Dlp regulation, whereby cleavage of Dlp at its N-terminal domain by Mmp2 causes Dlp to relocalize from the cell surface to intracellular vesicles, preventing its interaction with Wg. This finding is of particular interest because the core protein of glypicans, rather than their attached GAG chains, interacts directly with various signaling molecules. For example, the Dlp core protein interacts with Wg and Hedgehog (Hh), whereas the core protein of mammalian glypican-3 binds with high affinity to Sonic Hh (Capurro et al., 2008; Yan et al., 2009, 2010). Moreover, both *Drosophila* and mammalian glypicans are involved in Wnt, Hh, bone morphogenetic protein, FGF, and JAK/STAT (Janus kinase/signal transducer and activator of transcription) pathways (Filmus et al., 2008). Thus, uncovering the regulation of glypicans has a major impact on our understanding of signaling transduction in normal development and tumor progression.

In mammals, as in the fly ovary, important production sites for MMPs are the niche cells (Kessenbrock et al., 2010). Reminiscent of the study by Wang and Page-McCaw (2014), the HSPG syndecan-1 sequesters the chemokine CXCL1; upon lung injury, MMP7 is up-regulated, cleaving syndecan-1 and activating CXCL1, thereby inducing neutrophil migration (Li et al., 2002). MMPs can also cleave insulin growth factor (IGF) binding proteins (Fowlkes et al., 1995) and latent TGF-β binding protein (Dallas et al., 2002), releasing active IGF and TGF-β, respectively. In addition, MMP3 binds or cleaves

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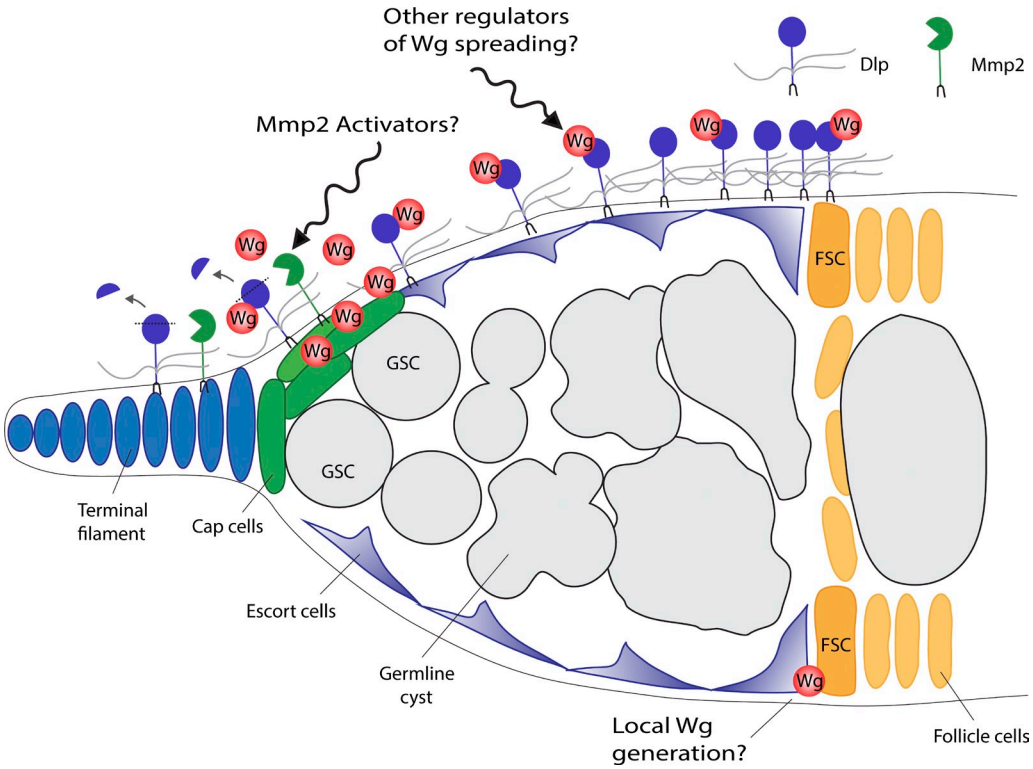

Figure 1. **Regulation of FSCs by Mmp2 and the glypican Dlp in *Drosophila* germarium.** Cap cells produce a long-range signal Wg to regulate the behavior of FSCs. Dlp mediates the transport of Wg from the cap cells to the FSCs to promote their proliferation. Dlp and Wg form opposing gradients in the germarium. Mmp2, expressed in the cap and TF cells, cleaves Dlp in its N-terminal domain and relocalizes Dlp from the cell surface to intracellular vesicles, preventing its interaction with Wg. It remains to be determined what signals regulate Mmp2 activity and what other factors mediate Wg spreading in the germarium. Also, Wg may be locally generated by escort cells (Sahai-Hernandez and Nystul, 2013). GSC, germline stem cell.

Wnt5b, a Wnt signaling inhibitor, increasing mammary stem cell function (Kessenbrock et al., 2013). Therefore, the work by Wang and Page-McCaw (2014) is relevant to mammalian systems in which HSPGs and MMPs act on multiple signaling pathways (Filmus et al., 2008; Kessenbrock et al., 2010).

The study by Wang and Page-McCaw (2014) raises several questions. First, is Dlp cleavage by Mmp2 required in vivo (only in vitro data were shown)? Second, given the evidence from mammals and *Drosophila* that HSPGs and/or MMPs affect numerous secreted factors (Filmus et al., 2008; Kessenbrock et al., 2010; Wang et al., 2010), does Mmp2 or Dlp act on other signaling pathways to affect FSCs or other cells or do they primarily act through Wg? Third, MMP activity is known to be regulated by proteinases, inhibitors, reactive oxygen species, localization, ECM stiffness, and signaling pathways (NF-κB, FGF, and leptin; Kessenbrock et al., 2010; Wang et al., 2010). Is Mmp2 activated by these or other signals (e.g., nutrition and systemic factors)? Fourth, what are the roles of Mmp2–Dlp interactions in other tissues? Fifth, is Wg spreading in the ovary dependent on other Wg binding factors, such as Swim, Wntless, Lipophorin, or others (Mulligan et al., 2012)? Sixth, it has been suggested that Wg may be produced by FSC-neighboring escort cells (Sahai-Hernandez and Nystul, 2013). As a membrane-tethered form of Wg can replace the endogenous Wg protein in the wing disc (Alexandre et al., 2014), it will be interesting to assess long-range Wg signaling in the ovaries of these flies.

In conclusion, Wang and Page-McCaw (2014) demonstrate beautifully the regulation of a signaling factor through proteinase–HSPG interactions. MMPs (Kessenbrock et al., 2010) and HSPGs (Blackhall et al., 2001) are altered in mammalian tumors, raising the question whether they act through similar mechanisms to influence tumor progression.

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