Heparan sulfate proteoglycans in the stem cell niche : Lessons from *Drosophila*

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Profile

Hiroshi Nakato received his Ph.D. degree in Biology from Tokyo Metropolitan University (1993). He has been studying the function of heparan sulfate proteoglycans (HSPGs) in development using *Drosophila* genetics for more than 15 years. He first became interested in this class of molecules when he was a postdoctoral fellow in Dr. Scott Selleck's lab at the University of Arizona (1993-1995). During his postdoctoral studies in the Selleck lab, he identified the *division abnormally delayed* (*dally*) gene, which encodes a *Drosophila* HSPG of the glypican type, as a regulator of cell division patterning in the developing CNS. He continued genetic and biochemical studies of HSPGs and HS modifying enzymes in *Drosophila* after becoming a faculty member of Tokyo Metropolitan University (1995) and the University of Arizona (2001). Dr. Nakato is currently an Associate Professor in the Department of Genetics, Cell Biology and Development at the University of Minnesota (2003-). He currently serves as a reviewer in the NIH Intercellular Interactions (ICI) Study Section (2010-2014). He is also the President of the Minneapolis Japanese School, a nonprofit organization supported by the Japanese government. Dr. Nakato's main research interests are elucidating the mechanisms by which HSPGs regulate molecular recognition events during developmental processes, such as morphogen gradient formation, stem cell regulation, and wound healing.

Stem cells are defined by their unique potential to self-renew and differentiate into mature cell types. Stem cells typically reside in specialized microenvironments called 'niches', which support their ability to retain the characteristics of a stem cell. The molecular basis underlying the regulation of the stem cell niche is poorly understood. For example, the contact between niche cells and stem cells is a key component of niche control in many stem cell systems, but the molecular foundation of this contact is unknown. It is critical to explore the regulatory networks controlling stem cell niches for the advancement of cancer therapies, studies on aging, and ultimately, for the understanding of a universal mechanism that controls animal development. We study the stem cell niche in the genetically tractable model organism *Drosophila*, in which specific cell type markers as well as elegant genetic techniques, are available.

We have demonstrated that heparan sulfate proteoglycans (HSPGs) are essential regulators of the germline stem cell (GSC) niche in the *Drosophila* ovary[1]. In the female GSC niche, Decapentaplegic (Dpp; a *Drosophila* BMP), regulates the asymmetric division of GSCs. However, the mechanism by which this secreted molecule differentially regulates the fate of the two daughter cells has been a mystery. We found that Dally, a *Drosophila* HSPG of the glypican type, is specifically expressed in the niche cells contacting the stem cells (red in Figure 1). Dally activates Dpp signaling *in trans* in a directly contacting GSC. This *trans* co-receptor activity of Dally explains the contact-dependency of the GSC niche: when a GSC divides, the daughter cell directly contacting the Dally-expressing niche cells remains a GSC (Dpp signaling is ON), but the other daughter cell, which has lost contact, will differentiate (Dpp signaling is OFF) (Figure 1, wild-type). In a *dally* mutant ovary, the daughter cell directly contacting the niche cells cannot activate Dpp signaling (OFF), thus GSCs are lost to differentiation (Figure 1, *dally* mutant). When *dally* is ectopically expressed in somatic cells outside the niche, all germ cells contacting ectopic Dally are maintained as GSCs by ectopic activation of Dpp signaling (ON) (Figure 1, ectopic *dally*). Thus, the contact-dependent signaling by HSPGs provides a mechanism to define the physical space of the stem cell niche.

To further investigate the molecular mechanisms by which HSPGs regulate contact-dependent BMP signaling, we developed a novel *in vitro* assay system using *Drosophila* S2 tissue culture cells and analyzed HSPG trans co-receptor activity[2]. In this "single-cell BMP-HSPG *trans* signaling assay", "signal-sending cells" (expressing Dally) and "signal-receiving cells" (expressing BMP receptors) are co-cultured. Contact between the sending and receiving cells is visualized by the split-GFP complementation system, and BMP signal activation in the receiving cells is monitored by phosphorylation of Mad protein. Using this single-cell assay system, we demonstrated that HSPGs *in trans* enhance BMP signaling in S2 cells in a contact-dependent manner. The previously known mechanism for contact-dependent signaling is

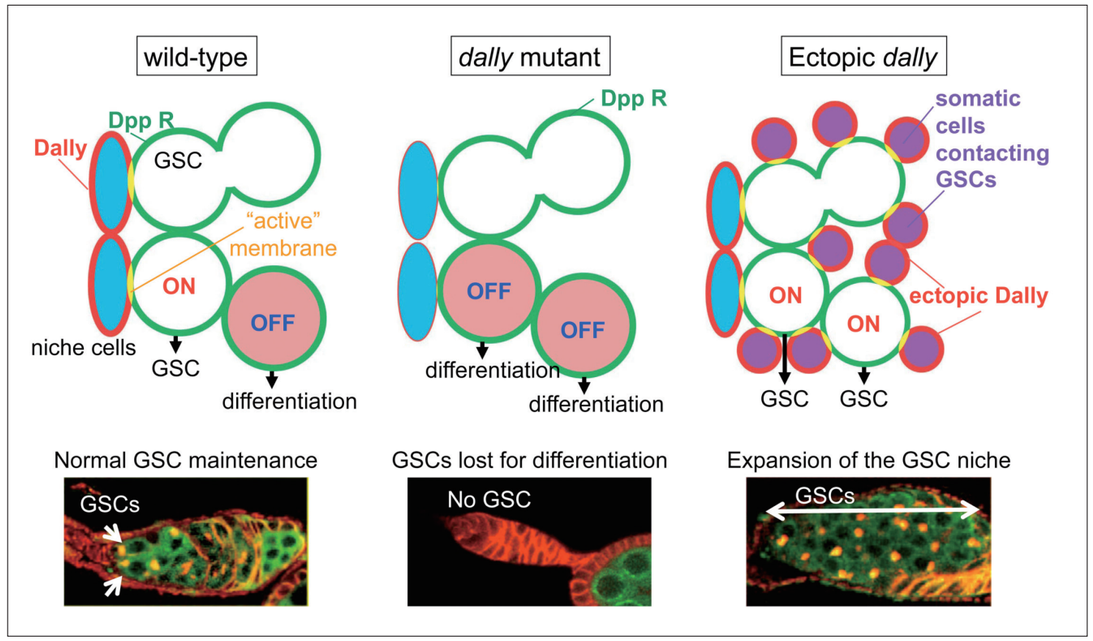

Figure 1. Contact-dependent Dpp signaling in the *Drosophila* germline stem cell niche.

communication mediated by membrane-bound ligands, such as the ligand proteins for the Notch receptor. Our study on the new function of Dally provides a novel mechanism to achieve contact-dependent signaling, in which complementation of signaling complex components can spatially restrict the physical field of signaling. Importantly, this system is used in a specific, extremely important *in vivo* context: the stem cell niche. I will present the molecular mechanisms for this novel signaling system between contacting cells, revealed by a combination of *in vitro* and *in vivo* analyses.

Functional analyses of HSPGs in other *Drosophila* stem cell systems are in progress. We found that HSPGs are critical for proliferation and maintenance of the male GSCs, neural stem cells in the developing CNS, and adult midgut stem cells. Thus, our study highlights the universal and fundamental roles of HSPGs in the stem cell niche.

References

1) Hayashi Y, Kobayashi S, and Nakato H. *Drosophila* glypicans regulate the germline stem cell niche. *J. Cell Biol.* (187):473-480, 2009.
2) Dejima K, Kanai MI, Akiyama T, Levings D, and Nakato H. Novel contact-dependent bone morphogenetic protein (BMP) signaling mediated by heparan sulfate proteoglycans. *J. Biol. Chem.* (286): 17103-17111, 2011.