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Session 4 "Development, KO"

## Molecular Mechanisms of Glypican Co-receptor Function: The Role of *Drosophila* Dally in Dpp Signaling

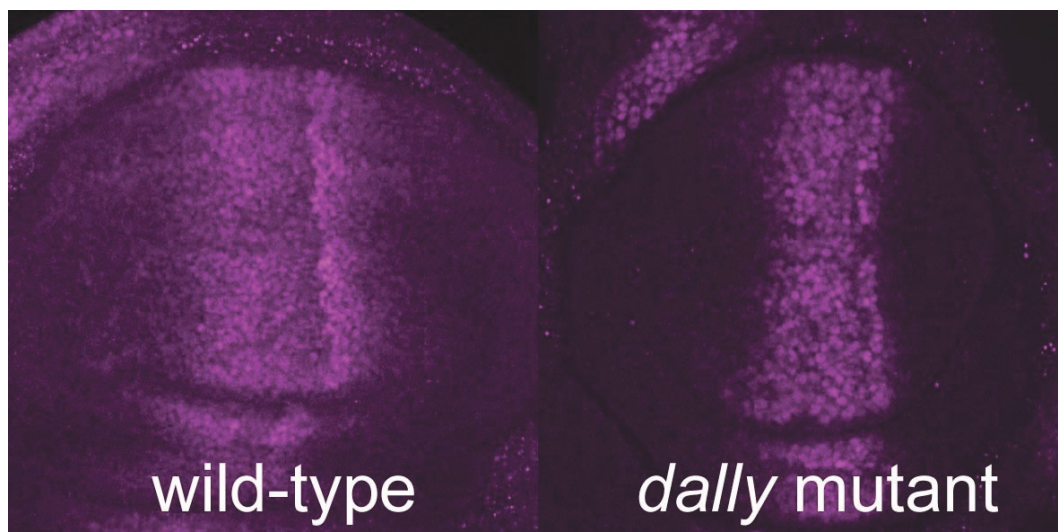
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Hiroshi Nakato received his Ph.D. degree in Biology from Tokyo Metropolitan University (1993). He began working on *Drosophila* heparan sulfate proteoglycans (HSPGs) during his postdoctoral studies in Dr. Scott Selleck's lab at the University of Arizona (1993-1995). He continued genetic and biochemical

studies of proteoglycans and heparan sulfate 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 Assistant Professor in the Department of Genetics, Cell Biology and Development at the University of Minnesota. His main research interests are elucidating the role of HSPGs in development. Another goal of his research is to understand the mechanisms by which HSPG function is regulated through modification events of glycosaminoglycan chains.



Dpp morphogen activity gradient in the wild-type (left) and *dally* mutant (right) wing discs. The Dpp activity gradient was monitored using an antibody, which specifically recognizes the phosphorylated form of MAD (pMAD). Note that *dally* mutant discs show abnormally low levels of pMAD except the central part.

Morphogens are extracellular signaling molecules that form concentration gradients across developmental fields and specify different cell fates in a concentration dependent manner. Members of the bone morphogenetic protein (BMP), Wnt, and Hedgehog growth factor families behave as morphogens, providing a fundamental mechanism of generating patterns during tissue assembly. An in-depth understanding of how tissues are assembled and repaired requires insight into the molecular mechanism of morphogen gradient establishment and maintenance. Although recent studies showed that heparan sulfate proteoglycans (HSPGs) play key roles in morphogen signaling and distribution, very little is known about the molecular basis of this control.

Dally, a *Drosophila* member of the glypican family of HSPGs, regulates gradient formation of Decapentaplegic (Dpp), a *Drosophila* BMP, in the developing wing. It affects the shape of the Dpp ligand gradient (protein distribution) as well as its activity gradient (spatial patterns of signaling activity). In order to elucidate the molecular mechanism by which HSPGs contribute to the morphogen gradient formation, we studied how Dally controls distribution and signaling of Dpp morphogen. We found that Dally forms a complex with Dpp and enhances Dpp signaling in a cell autonomous fashion. These findings are consistent with the idea that Dally serves as a “co-receptor” for Dpp.

In order to understand the roles of co-receptors in the gradient formation of morphogens, two molecular genetic approaches are useful. First, one can use mutant cells deficient for a co-receptor to observe the consequence of losing this molecule. Recent experiments using the genetic mosaic system showed that Dpp is lost inside and behind HS-deficient clones. This phenomenon can be explained by

either blocking Dpp movement (Dpp can not enter the HS-deficient cells) or Dpp destabilization (Dpp is rapidly degraded in HS-deficient cells). As an alternative and a complimentary approach, one can study behavior of a mutant morphogen molecule which lacks ability to interact with co-receptors but retains all other activities of the wild-type form. We generated a truncated form of Dpp (deltaNDpp), which lacks a short domain (7 amino acid residues) at the N terminus essential for interacting with Dally. Our results demonstrated that deltaNDpp shows the same signaling activity and protein stability as wild-type Dpp *in vitro* but has a shorter half-life *in vivo*: it is more quickly internalized by cells for degradation than Dpp and therefore fails to form a normal gradient in tissues. These results strongly suggest that Dally stabilizes Dpp in the extracellular matrix.

This finding was further supported by a different set of experiments in which we analyzed the relationship between the roles of receptor and co-receptor in morphogen signaling. Dally and Thickveins (Tkv), a type I receptor for Dpp, share common properties as components of the Dpp signaling complex; they both autonomously enhance Dpp signaling, and limit migration of Dpp by binding to Dpp protein. Nevertheless, they genetically behave opposite of one another in wing development. *tkv* and *dally* suppress each other’s effects on the Dpp activity gradient. These results indicated that Dally co-receptor has an activity to antagonize the effect of Tkv receptor on Dpp signaling. Given that Tkv can down-regulate Dpp signaling by receptor-mediated endocytosis of Dpp, *dally* may inhibit this process. Based on these observations, we propose a model in which Dally serves as a co-receptor for Dpp and regulates its distribution and signaling by disrupting internalization of the Dpp-receptor complex.

**Keywords :** Morphogen, co-receptor, Decapentaplegic (Dpp), Dally, *Drosophila*