



DPP SIGNAL REGULATION THROUGH O-LINKED GLYCOSYLATION

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The research thus far has identified an interaction between glycosylation activity and Dpp signal regulation during *Drosophila* dorsal closure, but the mechanism of this interaction has yet to be well described. Prior studies have predicted that the glucose derived O-GlcNAc modification has an influence on the level of Dpp signaling in the dorsal lateral epidermis. There is a single O-GlcNAc transferase (OGT) in *Drosophila* called Sxc, so it is reasonable to suspect that it may play a direct role in modulating the Dpp pathway during embryonic dorsal closure (DC). We set out to determine if Sxc does indeed interact with the Dpp pathway, and then characterize that interaction.

Our lab performed an RNAi screen targeting GlcNAc transferases, and discovered that *sxc* mutants displayed a loss-of-function phenotype similar to *mmv* mutants, as well as similar patterning defects. It is clear that *mmv* loss-of-function mutants have an effect on Dpp expression while *sxc* mutants do not. These results indicate that Sxc functions as a Dpp signal antagonist.

It was hypothesized that Sxc may be interacting with a Dpp receptor. The ectopic Dpp signaling phenotype seen in *sxc* is lost in *sxc sax* double mutants. This finding demonstrates that Type I Dpp receptor Sax must be present in the epidermis in order for the *sxc* phenotype to be observed, and implies that Sxc is interacting with the Sax receptor in some way to regulate the Dpp signaling pathway. Lysates from embryos expressing Sxc^{RNAi} were analyzed and blotted for O-GlcNAc, and these lysates showed no band in an immunopurified pellet and hence are not glycosylated. These observations indicate that the OGT Sxc is responsible for O-GlcNAcylating the Sax receptor during DC.

The results reveal a novel role for *sxc* during *Drosophila* embryogenesis, in that Sxc functions to antagonize the Dpp signal during *Drosophila* DC. The *sxc* mutants have cuticle phenotypes that are identical to those seen in *mmv*, and display ectopic downstream patterning prior to DC. The *sxc* loss-of-function phenotype can be rescued by an additional loss of *sax*, which predicts a unique signaling relationship between Sxc and the Sax receptor. Even though there is another Type I Dpp receptor (Tkv), the results show that this relationship with Sxc only occurs with Sax. Sxc interacts with Sax by a post translational O-linked attachment of GlcNAc to restrict its activity. As glycosylation is frequently used as a mechanism for deactivating and blocking phosphorylation sites, it is predicted that this modification takes place at residues normally targeted by kinase activity.

