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Progress Report:

Abstract

1. Objectives: A major challenge in developmental biology is to understand how cells coordinate developmental behaviors with that of their neighbours. Cells often employ secreted signaling molecules that act at a distance on target cells. The proteins of the Hedgehog (Hh) family are such powerful signaling molecules, acting as short range and long range morphogens to control vertebrate and invertebrate development. Hhs are unusual signaling molecules, however, because all are dually lipid-modified and yet get released as large morphogen complexes from producing cells. Moreover, all Hh family members bind to heparan sulfate (HS) glycosaminoglycan chains expressed on Heparan Sulfate Proteoglycans (HSPGs). Genetic evidence in *Drosophila* indicated that the HSPGs dally and dally-like are required for Hh signaling, and optical imaging revealed that HS binding is a prerequisite for Hh multimerization on producing cells. However, the manners in which HSPGs affect Hh dispersal and gradient formation are not yet understood. One reason is that HSPGs are tremendously diverse and simultaneously interact with multiple growth factors and morphogens. This makes interpretation of HS-deficient mutant phenotypes difficult. The objective of this project was thus to study the *in vivo* relevance of direct Hh/HS interactions by the expression of mutated, HS-binding-deficient morphogen clusters in *Drosophila melanogaster*.

2. Methods used: Initially, we expressed wild-type Hh and 21 mutant proteins lacking one or more amino acids predicted to participate in HS-binding in *Drosophila* S2 cells. Unimpaired Hh multimerization of all proteins was confirmed by size exclusion chromatography. HS-binding of wild-type Hh and the various Hh mutants was compared by heparin- and HS-affinity chromatography. Three constructs encoding Hh impaired in HS-binding were used to generate transgenic flies.

3. Results: Contrary to our expectations, we found that HS-binding Cardin-Weintraub (CW) functions are not conserved between vertebrate and invertebrate Hh family members. In contrast to CW-dependent vertebrate Sonic hedgehog (Shh) binding to HS, site-directed mutagenesis of corresponding CW-residues R93, R95 and R97 into alanines left HS- and heparin binding of the

mutated fly ortholog largely intact. Subsequent inactivation of seven highly conserved basic residues revealed that *Drosophila* Hh amino acids R238 and R239 contribute to HS-binding. We thus generated transgenic flies expressing 1) wild-type Hh, 2) Hh lacking CW-residues R93/R95/R97, 3) Hh lacking R238/R239 and 4) a combination of the two latter mutants under engrailed-UAS control in the normal Hh pattern. The ability of these constructs to rescue Hh-related developmental phenotypes in mutants deficient in endogenous Hh function ($Hh^{ts/AC}$) is currently being investigated.