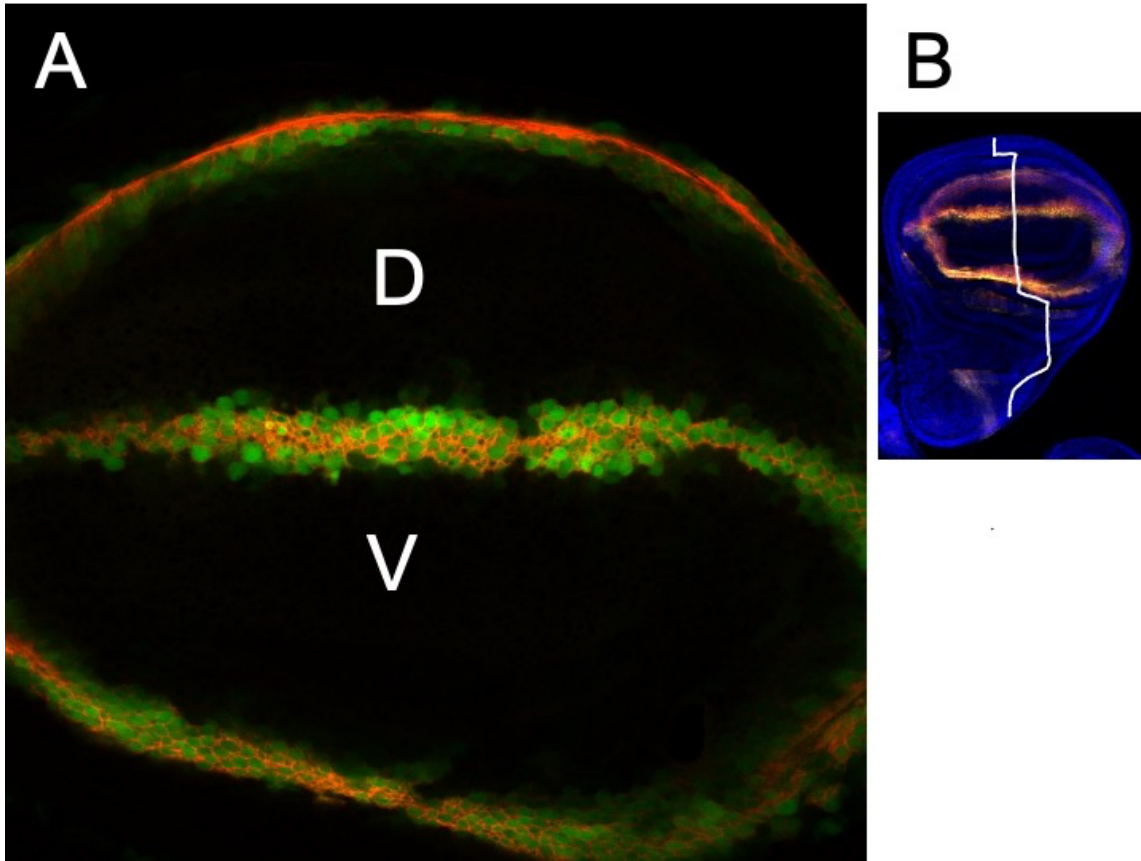
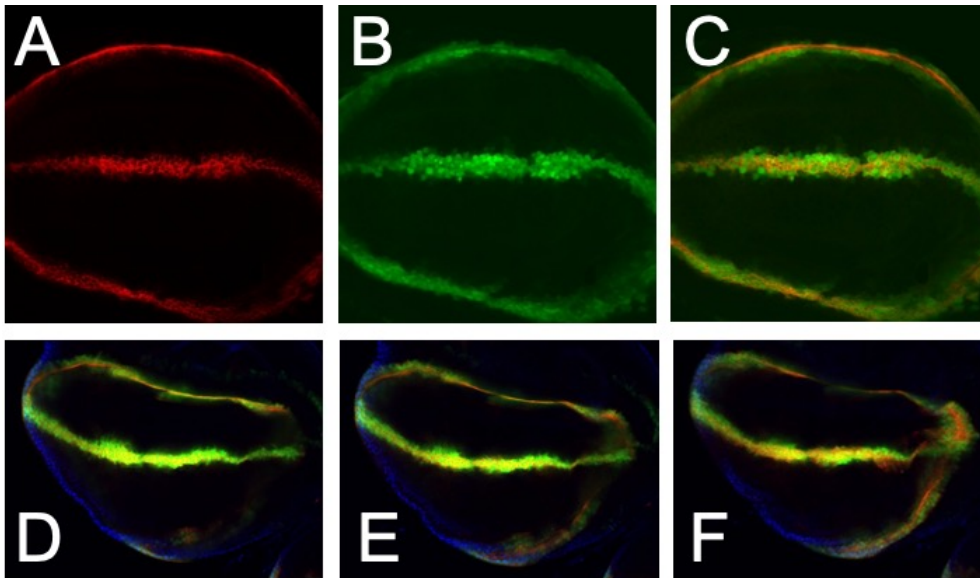


## Chapter 12. Thin-film diffusion gradients, polarised flux and active vesicle trafficking.

While intracellular gradients have well-established functions during oogenesis, the contribution of passive diffusion to extracellular signal transmission is less certain. In imaginal discs, morphogen diffusion may be restricted within the thin fluid film between the peripodial membrane and the disc epithelium. In addition both Wg and Hh are lipidated, such that retention by the extra-cellular matrix may limit their diffusion<sup>1</sup>. Notably, a myristolated fluor remains associated with the Ap epithelial surface, when driven by *wg-Gal4* along the D/V wing margin, with limited lateral diffusion (Figs. 17, 18).



**Fig. 17. Pr ring and marginal loop of Wg-Gal4 expression.** A Pr ring separates the presumptive wing blade from the surrounding notum, pleura and hinge, while a D/V loop delineates the wing margin. L3 imaginal disc, confocal images. **A.** The *wg-Gal4* transgene drives expression of GFP fluor (green) carrying a nuclear localisation peptide, the Tom fluor (orange/red), carries an N-terminal secretion peptide and a myristoyl tag, in *wg-Gal4; UAS-GFP<sup>NLS</sup> UAS-Tom<sup>myr</sup>*. Secreted Tom<sup>myr</sup> remains associated with the Ap epithelial surface, with restricted lateral diffusion; while the GFP<sup>NLS</sup> fluor localises to the nuclei of the underlying epithelium (disc viewed through Ba surface). Myr-Tom transgene of Potter and Luo, 2010 (FlyBase communication). **B.** Confocal image of twin membrane-anchored fluors, *GFP-mCD8-mCherry* (yellow/red), carrying an N-terminal secretion peptide, in L3 *wg-Gal4; UAS-GFP<sup>NLS</sup> UAS-myr-Tom* wing disc. DAPI (blue) signal shows nuclei at the edges of the imaginal disc. White line indicates the position of the A/P compartment boundary. D. Gubb and J-M. Reichhart, unpublished.



**Fig.18. Limited diffusion of Tom<sup>myr</sup> fluor.** Single A, B and combined C channels of Fig. 17. D, E, F; Z-series (Ba, medial and Ap) focal plane, *wg-Gal4; UAS-GFP<sup>NLS</sup> UAS-myr-Tom* L3 wing disc. GFP<sup>NLS</sup> (green), Tom<sup>myr</sup> (orange/red), DAPI (blue).

Notably, membrane-tethered Wg can rescue the *wg* null mutant phenotype<sup>2</sup>, consistent with Wg being endocytosed on membrane-bound vesicles, followed by active trafficking in the epithelial plane. The extracellular matrix proteoglycans Dally (Division abnormally delayed) and Dally-like (Dly) modify the transmission and uptake of Wg, Hh and Dpp. In turn, Dally and Dly are regulated by the Ft/Ds/Fj cassette via the Hippo pathway<sup>3 4</sup>. Thus, lipidation may affect morphogen partitioning between membrane-bound vesicles, matrix proteoglycans and Lipophorin-associated lipid particles<sup>5</sup>. In particular, Dally stabilises the retention of Dpp on the collagen matrix<sup>6 7 8 9</sup>. In combination, these trans-membrane/extracellular matrix interactions may regulate morphogen activity, with localised uptake, degradation and transmission in the epithelial plane.

In this context, Dpp is trafficked away from the A/P boundary of the wing disc, through baso-lateral cell interfaces, in association with MVBs<sup>10 11 12 13</sup>. The Dpp gradient is restricted to the A compartment, although *dpp-Gal4* driven expression of an N-terminal Dpp-GFP fragment protein gives a fluorescent gradient within the disc lumen, to either side of the A/P boundary<sup>14</sup>. Under these conditions, uptake of Dpp by the disc epithelium is dependent on the Dynamin motor Shibire (Shi); while the extracellular Dpp gradient remains independent of *shi* function<sup>6 15 16 17</sup>. Thus, the absence of a P gradient of Dpp is consistent with its rapid uptake and degradation. By contrast, newly translated Wg is transported as an inactive cargo component, with signal peptide removal during exocytosis, followed by matrix retention and endocytotic recycling<sup>18 5</sup>. Recycled Wg may trigger cytoskeletal remodelling in the cells from which it has been secreted and their adjacent neighbours; with Wg-tagged MVBs translocated predominantly through baso-lateral cellular interfaces. Meanwhile, Hh is exported through the basal surface of the wing disc, with differential Ap/Ba and baso-lateral trafficking mediated via iHog and Boi<sup>19</sup>, see below Chapter 13. By implication, altered Ap/Ba trafficking may select alternative developmental pathways to either side of the A/P (AMS), or the D/V (segmental), boundary. Notably, the Toll and Ttk transmembrane receptors require to be taken up in membrane-bound vesicles before signal transduction, which does not take place from the epithelial surface<sup>20 21</sup>.

### Summary:

**Imaginal disc growth is controlled via active trafficking of morphogens through epithelial surfaces, or the lateral interfaces between adjacent cells. The diffusion of lipidated morphogens is limited by their adsorption onto extracellular matrix components, and thin-film diffusion within the convoluted lumen of imaginal discs. Morphogens captured on membrane-anchored receptors may be endocytosed and trafficked in the epithelial plane. Thus, morphogen flux is limited by restricted extracellular diffusion,**

**the export/import of MVBs and lipid particles, and subsequent polarised partitioning between lateral cell boundaries in the epithelial plane. These processes are all dependent on cytoskeletal remodelling at membrane interfaces, modulated by the active responses of signal-receiving cells.**

## References:

1. Reichsman, F., Smith, L. & Cumberledge, S. Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction. *J. Cell Biol.* **135**, 819–827 (1996).
2. Alexandre, C., Baena-Lopez, A. & Vincent, J. P. Patterning and growth control by membrane-tethered Wingless. *Nature* **505**, 180–185 (2014).
3. Belenkaya, T. Y. *et al.* The retromer complex influences Wnt secretion by recycling Wntless from endosomes to the trans-Golgi network. *Dev. Cell* **14**, 120–131.
4. Han, C., Yan, D., Belenkaya, T. Y. & Lin, X. Drosophila glypicans Dally and Dally-like shape the extracellular Wingless morphogen gradient in the wing disc. *Development* **132**, 667–679 (2005).
5. Marois, E., Mahmoud, A. & Eaton, S. The endocytic pathway and formation of the Wingless morphogen gradient. *Development* **133**, 307–317 (2006).
6. Belenkaya, T. Y. *et al.* Drosophila Dpp Morphogen Movement Is Independent of Dynamin-Mediated Endocytosis but Regulated by the Glypican Members of Heparan Sulfate Proteoglycans. *Cell* **119**, 231–244 (2004).
7. Akiyama, T. *et al.* Dally regulates Dpp morphogen gradient formation by stabilizing Dpp on the Cell Surface. *Dev. Biol.* **313**, 408–419 (2008).
8. Sawala, A., Sutcliffe, C. & Ashe, H. L. Multistep molecular mechanism for Bone morphogenetic protein extracellular transport in the Drosophila embryo. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 11222–11227 (2012).
9. Bunt, S. *et al.* Hemocyte-secreted type IV collagen enhances BMP signaling to guide renal tubule morphogenesis in Drosophila. *Dev. Cell* **19**, 296–306 (2010).
10. Strigini, M. & Cohen, S. M. Wingless gradient formation in the Drosophila wing. *Curr. Biol.* **10**, 293–300 (2000).
11. Valentine, Hogan Justin, & Collier Simon. The Drosophila Chmp1 protein determines wing cell fate through regulation of epidermal growth factor receptor signaling. *Dev. Dyn.* **243**, 977–987 (2014).
12. Tjota, M. *et al.* Annexin B9 binds to  $\beta$ -H-spectrin and is required for multivesicular body function in Drosophila. *J. Cell Sci.* **124**, 2914–2926 (2011).
13. Harmansa, S., Hamaratoglu, F., Affolter, M. & Caussinus, E. Dpp spreading is required for medial but not for lateral wing disc growth. *Nature* **527**, 317 (2015).
14. Entchev, E. V., Schwabedissen, A. & González-Gaitán, M. Gradient formation of the TGF- $\beta$  homolog Dpp. *Cell* **103**, 981–992 (2000).
15. Gibson, M. C. & Schubiger, G. Peripodial cells regulate proliferation and patterning of Drosophila imaginal discs. *Cell* **103**, 343–350 (2000).
16. Gibson, M. C., Lehman, D. A. & Schubiger, Gerold, G. lumenal transmission of Decapentaplegic in Drosophila imaginal discs. *Dev. Cell* **3**, 451–460 (2002).
17. Kwon, Y. *et al.* The Hippo signaling pathway interactome. *Science* **342**, 737–740 (2013).
18. Lecourtois, M., Alexandre, C., Dubois, L. & Vincent, J. P. Wingless capture by Frizzled and Frizzled2 in Drosophila embryos. *Dev. Biol.* **235**, 467–475 (2001).
19. Callejo, A. *et al.* Feature Article: From the cover: Dispatched mediates Hedgehog basolateral release to form the long-range morphogenetic gradient in the Drosophila wing disk epithelium. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 12591–12598 (2011).
20. Belenkaya, T. Y. *et al.* Drosophila Dpp Morphogen Movement is independent of dynamin-mediated endocytosis but regulated by the glypican members of heparan sulfate proteoglycans. *Cell* **119**, 231–244 (2004).
21. Delotto, R. Shedding light on Toll signaling through live imaging. *Fly (Austin)* **5**, 141–146 (2011).