Abstract: An In Vivo RNA-interference Screen to Identify Novel Planar Polarity Genes in Drosophila

Fat (Ft) and Dachsous (Ds) are atypical cadherins known to be involved in the establishment of planar polarity in the developing Drosophila wing (Brittle et al, 2012). Using mass spectrometry, Kwon et al. 2013 identified proteins found to interact with Ft and Ds (Kwon et al. 2013), leading to a list of proteins possibly involved in planar polarity signaling. For this project, an in vivo RNA-interference (RNAi) screen was performed to identify whether any of these ‘interactors’ had a specific effect on the polarisation or levels of Ft or Ds in the epithelium.

The RNAi pathway was used to specifically down-regulate 19 of the identified genes using two independent RNAi fly stocks for each gene. Each RNAi line was crossed with a Patched (Ptc) Gal4, and an MS1096 (whole wing) Gal4 driver stock. Crosses were incubated at 25°C.

The resulting phenotypes of progeny of the Ptc Gal4 crosses were analysed through dissecting, fixing and staining third instar larval wings discs with antibodies against Ft, Dachs (D) and Armadillo (Arm), a cell adhesion molecule found at cell junctions. Confocal microscopy was used to reveal differences in polarisation of Ft and Ds proteins in the Ptc domain of the wing disc when compared to neighbouring wild-type tissue.

Adult wings from the progeny of the MS1096 crosses were dissected and analysed under a microscope, and trichome (hair) orientation was compared to wild-type wings exhibiting distally pointing hairs on the epithelium surface.

Of the 19 genes analysed, 2 showed interesting phenotypes when down-regulated. One gene showed an increase in Ft in the Ptc domain in the wing disc and the adult wing exhibited an extra vein. Another gene produced crumpled wings. Down-regulating the other genes produced negative results with no obvious differences in the polarisation of Ft or D observed.

To produce reliable results, crosses will need to be repeated and possibly examined at higher or lower temperatures to increase or reduce knock down. If the resulting crosses confirm the phenotypes, further investigation, such as looking at a gene knockout or protein over-expression, would be required to investigate whether the identified genes are required for the establishment and maintenance of planar polarisation in the wild type adult wing.