Identifying novel regulators of tissue shape and size

Planar polarity is the orientation of cells within the plane of an epithelial sheet perpendicular to the basal-apical axis. The Fat (Ft)/Dachsous (Ds) pathway, which is involved in Hippo signalling, plays a key role in this architectural regulation and works alongside the core protein pathway, however the mechanisms of the former pathway are comparatively less understood. Currently, it is known that in *Drosophila* Ft and Ds are atypical cadherins associating with Dachs, a crucial downstream component of Fat signalling. A Hippo signalling pathway interactome revealed by Kwon *et al* 2013 identified other proteins that may be involved in the Ft/Ds pathway as novel regulators of tissue formation, therefore a genetic screen was performed to elucidate whether these played a genuine role.

Using RNA interference (RNAi), a subset of the genes with functions in endocytosis, vesicle trafficking and protein binding, were knocked down in *Drosophila*. The gene subset encompassed lines from TRiP and VDRC sources. The males were crossed with virgin females of MS1096-Gal4 and ptc-gal4 driver lines to produce progeny that permitted transgene expression in whole adult wings and the ptc domain of wing imaginal discs respectively. To examine the effects of modification on protein localisation of Ft and Dachs, ptc-gal4 crosses were incubated at 29°C, wing discs dissected from 3rd instar larvae and then stained with primary antibodies against Ft, Dachs and Armadillo. Finally, the discs were examined by confocal imaging. The investigation of overall effect on tissue shape and size was conducted by incubating the MS1096-Gal4 crosses at 29°C, then dissecting and imaging wings from the male adult progeny. For both tissue types the wings were compared to wild type specimens to determine any significant findings.

Of the genes screened, 9 lines demonstrated a phenotype. A number of genes caused lethal phenotypes as the larvae failed to pupate or eclose. Flies were cultured at lower temperatures to enable development, which extended mortality temporarily, however the mutations still proved to be lethal. Failure of the larvae to eclose could infer an effect on general wing development. Confocal imaging of wings discs of one particular gene showed cells in the ptc domain to have some increase in Ft staining and a more subtle effect on Dachs staining. The adult wing of this line also showed a subtle phenotype characterised by a slightly narrowed wing with untidy trichome arrangement, suggesting the protein, which has a role in intracellular transportation, could have a role in the Ft/Ds pathway. The two additional lines for this gene were lethal and without phenotype respectively. Examining genetic mutants, if available could advance investigations by confirming the presence of similar phenotypes to those discovered in the RNAi lines.