

# Epithelial Biology Laboratory

Understanding Tissue  
Growth & Form  
in *Drosophila*



## Overview

We use *Drosophila* as a model organism to investigate both normal tissue development and tumour formation. We focus on the epithelial tissues of the fly, particularly on the developing wing and eye.

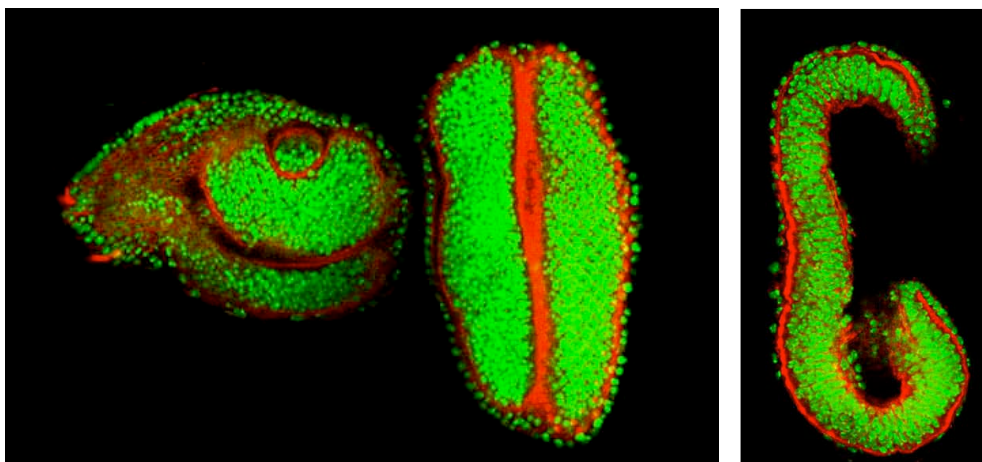
We are interested in two major questions:

- What determines the architecture of an epithelium?
- How is the growth of an epithelial tissue controlled?

These two questions lie at the heart of both developmental biology and cancer research.

## Epithelial morphogenesis

The morphology, or form, of a tissue depends upon the shapes of its constituent cells and how they are connected to one another. In epithelial tissues, cells are polarised along an apical-basal axis and are connected via adherens junctions to form a sheet of cells. The adult *Drosophila* wing and eye develop from single layered epithelial sacs, known as 'imaginal discs' after 'imago' – spanish for 'adult' – and after their disc-like shape.



top view of eye-antennal disc

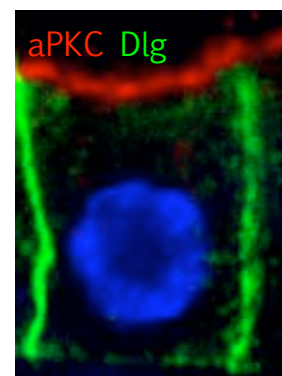
cross section

We wish to learn how epithelial cells establish and maintain their polarity, adhesion and shape. Understanding these processes is of direct relevance to cancer, because most human tumours are epithelial in origin and loss of epithelial morphology is a key step in tumour progression. For example, once tumour cells escape the epithelium, they can invade local tissues – a prerequisite for progression to metastasis.

### Epithelial polarity

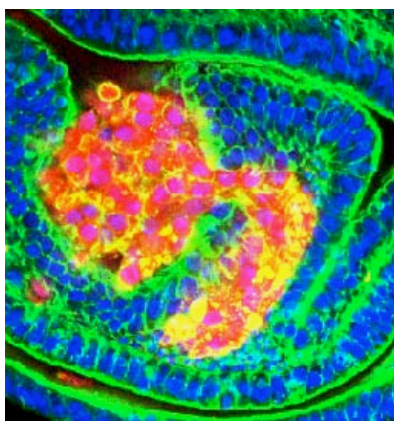
How cells become polarised is a fundamental problem in biology. Epithelial cells exhibit apical-basal polarity. The plasma membrane of epithelial cells is divided into distinct apical and baso-lateral domains, each with particular characteristics. How these cells distinguish apical from basal is not understood. However, genetic screens in *Drosophila* have identified key molecules that are essential for polarity.

Three proteins: Lgl, Dlg and Scrib were discovered in these screens and found to be localised to the baso-lateral membrane. When any one of these factors are absent, the cell is unable to maintain a distinct baso-lateral membrane domain and markers of the apical membrane (such as aPKC, Bazooka, PATJ) spread into the previously baso-lateral regions. Eventually, all of the plasma membrane becomes apical in nature and the adherens junctions can no longer be positioned correctly. Consequently, the cell rounds up and leaves the epithelium – just like tumour cells initiating local invasion.



Interestingly, recent analysis of the expression of Lgl during the progression of human colorectal cancer has found that this factor is strongly downregulated during the adenoma – carcinoma transition, when tumour cells lose their epithelial morphology. Thus, results from the fly can lead the way for pathological studies.

### Membrane trafficking & polarity

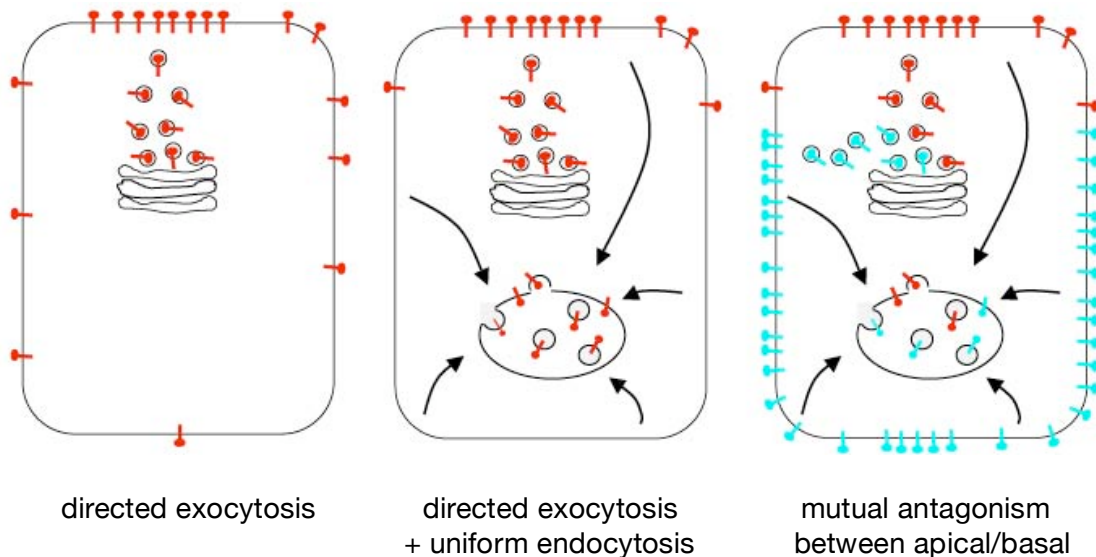


We have recently discovered that endocytic trafficking is essential to maintain epithelial polarity in *Drosophila*. Mutants in *vps25*, a gene encoding a component of the ESCRT-II complex, block endocytic trafficking of membrane proteins into multivesicular bodies (MVBs). The *vps25* mutants lose their polarity, just like mutants of Lgl, Dlg and Scrib.

An example of a *vps25* mutant clone is shown on the left. The mutant cells (red) have lost their polarity, but are surrounded by normal epithelia.

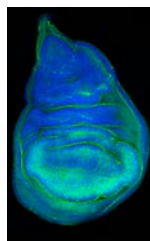
Our results with *vps25* point to a model in which membrane trafficking is central in organising epithelial polarity. They suggest that apical and baso-lateral markers are localised by continuous exocytic trafficking to their correct locations, coupled with continuous endocytic downregulation from the plasma membrane as a whole. A diagram of this model is shown below.

*A model for generation of polarity by membrane trafficking*

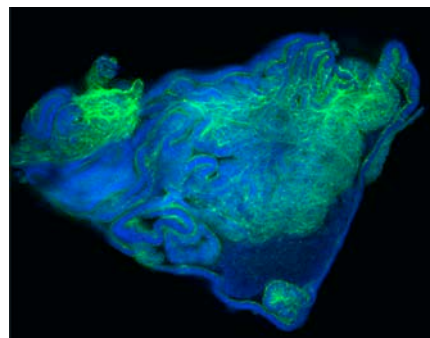


### Epithelial polarity & proliferation control

The polarity genes *Dlg* (*Discs large*) and *Lgl* (*Lethal giant larvae*) were first discovered not for their polarity phenotypes, but for the fact that they act as tumour suppressors in imaginal discs. The mutant discs fail to arrest proliferation at the normal timepoint and continue to grow – reaching very large sizes. The same phenotype is seen with other polarity mutants, such as *scrib* and *vps25*. Why these mutant discs fail to stop proliferating is not understood, but suggests that loss of epithelial polarity may contribute to overproliferation of tumour cells, in addition to promoting local invasion and metastasis. This may explain why tumour growth becomes so much more aggressive at the adenoma-carcinoma transition, which is marked by loss of epithelial polarity.



wing disc



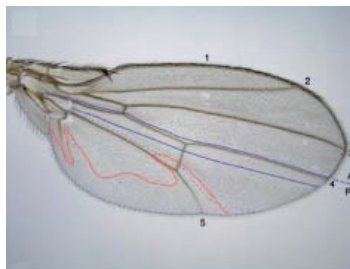
*vps25* wing disc tumour

## Tissue growth

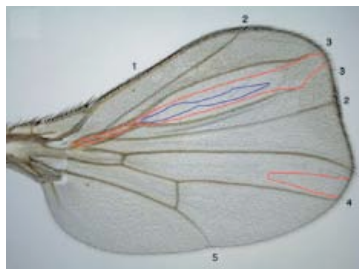
How the size of tissues is determined is still to be discovered. It is obvious that the size of any tissue in a particular dimension is simply the product of the number of cells and the size of each cell. However, the mechanisms that determine cell number and size are not yet understood. What is clear is that each tissue must have a unique developmental program that produces tissues of particular proportions. In addition, it is well known that animals adjust the growth of all body tissues in response to good nutrition or starvation. Recently, genetic screens in *Drosophila* have managed to uncover several key regulators mediating the developmental and nutritional control of tissue growth.

### Developmental controls

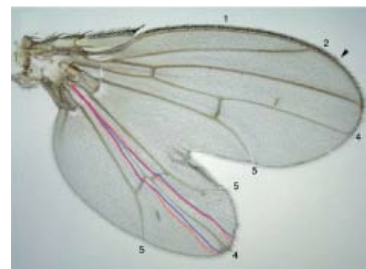
*Drosophila* tissues are divided into compartments and the boundaries between compartments act as organising centres that control growth of the tissue. For example, the boundary between anterior and posterior compartments in the developing wing is the source of a TGF-beta signaling molecule called Decapentaplegic (Dpp) that forms a gradient across the tissue and acts as a morphogen to control wing size along the anterior-posterior axis. Dpp is known to control wing size by controlling cell number. Since cell number is determined simply by the rate of cell proliferation minus the rate of cell death during development, Dpp must regulate cell proliferation and survival. How it achieves this is not yet understood.



wild-type wing



ectopic Dpp in anterior



ectopic Dpp in posterior

(from Zecca et al. *Development* 1995)

### Nutritional controls

In response to nutritional status, Insulin-like signaling molecules are released from the brain and act as hormones to control growth of the body as a whole. The insulin signaling pathway is able to modulate organ size without altering developmentally programmed proportions. To achieve this, the insulin pathway stimulates cell growth, but has only moderate effects on cell survival or the cell cycle. When cells grow faster but do not divide faster, cell size is increased. Thus, the insulin pathway modulates organ size mainly by altering cell size.

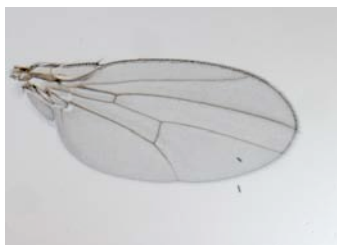
### Interactions between developmental and nutritional controls

How does the Insulin pathway alter tissue size when the growth of tissues is already subject to strict developmental control? When increased insulin signaling causes more rapid tissue growth, developmental signals such as Dpp are able to respond. The gradient of Dpp along the anterior-posterior axis of the wing has been shown to scale to the size of the compartments over which it spreads. Thus, while Dpp clearly controls the rate of tissue growth, the rate of tissue growth also influences the spread of the Dpp morphogen gradient. This mechanism is needed for the integration of Developmental and Nutritional controls on tissue growth.



### New players: The Hippo pathway & *bantam* microRNA

Genetic screens for new regulators of tissue growth has uncovered a new signaling pathway, called the Hippo pathway, that controls cell number by coordinately regulating both cell proliferation and survival. In addition, a microRNA named *bantam* was discovered that also coordinately promotes cell proliferation and survival. We found that the Hippo pathway regulates the expression of *bantam* to control tissue growth. However, it is still not fully understood how *bantam* and other target genes of the Hippo pathway drive cell proliferation and survival.



wild-type wing



hippo pathway overactive



hippo pathway off

We are currently investigating the developmental role of the Hippo pathway, and whether it may be regulated by other developmental signals, such as the Dpp morphogen. In addition we are also exploring how the Hippo pathway and *bantam* control cell proliferation and survival.

### Relevance to cancer

All of the growth control pathways identified in *Drosophila* have critical roles in human cancer. For example, intercellular signaling pathways such as Dpp/TGF-beta, Wnt, Hedgehog, EGFR-Ras and Notch were largely discovered in *Drosophila* and later found to have key roles in human cancer. Similarly, the Insulin pathway includes the PTEN tumour suppressor and the Hippo pathway includes Merlin, the product of the NF2 tumour suppressor gene. Thus, understanding tissue growth control in *Drosophila* is likely to be of direct relevance to understanding human cancer.

**Tumour – microenvironment interactions**

Tumours arise as the clonal progeny of a single mutant cell. Surveillance mechanisms exist to catch clones of cells that lose their epithelial morphology and begin to metastasise. In such clones, a signalling pathway (the JNK pathway) becomes active and triggers apoptosis, preventing tumour formation. How this pathway is activated is not known, but seems to depend on the presence of normal neighbouring cells. When a cell with defective morphology is surrounded by wild-type cells, the defective cell is killed. But when the entire tissue is composed of mutant cells, the JNK pathway is not activated and cells can manage to survive. Thus, normal epithelial cells appear to monitor their neighbours' behaviour and to eliminate those that behave abnormally. This ability of cells to kill their abnormal neighbours has been called 'cell competition'.

This phenomenon may partly explain why resistance to apoptosis is such a common, and possibly essential, feature of tumour formation. Invading and metastasising cells would be eliminated by surveillance mechanisms (like cell competition) unless apoptosis is blocked.

**Summary**

Because of its powerful genetics, the *Drosophila* model system has made many important contributions to our knowledge of how cell behaviour is controlled during normal development. The time is now ripe to use this system to explore how cell behaviour is re-directed during the development of tumours.