

Some assembly required: building the fly eye for motion detection and colour discrimination

Rhian F. Walther and
Franck Pichaud
(University College
London, UK)

Among the many eyes that have evolved on Earth, the insect compound eye is the most abundant. Its crystal-like lattice structure is a feat of engineering that has evolved over millions of years, and is exquisitely adapted to detect moving objects and discriminate colours. This enables many behaviours, including foraging for food, finding a mate and avoiding predators. Our understanding of how the compound eye is built and works has been greatly expanded by studying the humble fruit fly, *Drosophila melanogaster*. The simple outward appearance of the fly eye belies a host of sophisticated features. Through the precise arrangement of photosensitive cells in the retina and their connections to the brain, the fly eye packs an astonishing amount of hardware into a very tiny volume. In this primer, we introduce the molecular pathways that underpin the building and inner workings of the fly eye.

The design and architecture of the compound eye of insects is very different from our own camera-type eye, yet both types of eyes are capable of building an accurate representation of the outside world. The compound eye consists of an array of approximately 750 basic visual units, or facets, each of which is capped by a lens and contains a complement of cells capable of detecting different wavelengths of light. Together, these visual units assemble into a dome-shaped hexagonal lattice that allows insects to detect moving objects and discriminate colours (Figure 1a).

Pioneered by Thomas H. Morgan in the early 20th century, the genetically amenable fruit fly *Drosophila melanogaster* became a model of choice to elucidate the molecular pathways that underpin animal cell and tissue biology. This is because it is inexpensive to maintain, has a short life cycle of 2 weeks from egg to adult and has the added advantage of having less gene redundancy than other popular model organisms such as mice and fish. In particular, the fly eye has been and remains a very popular model to study how cells work together to assemble into a functional organ.

Building the compound eye

The basic visual unit of the compound eye is called the ommatidium. Each ommatidium consists of a strictly

defined set of cells that work together to (i) collect light and (ii) detect and transform it into an electric impulse that is sent to the brain, where it is processed to detect motion and discriminate colour. Within each ommatidium (Figure 1b), the task of collecting light is performed by the corneal lens. It focusses light onto eight photoreceptors, which point in different directions in space and potentially can be activated by a single photon of light. Surrounding the photoreceptors are the pigment cells, which function as optical insulators that prevent the incoming photons from straying into adjacent units. This insulation function is achieved through pigment granules present in these cells, which also give the fly eye its red colour.

Eye specification

Generating an eye, be it in a fly or a human, first requires the growth of an eye primordium. Remarkably, the same family of molecular regulators controls the formation of eye tissue in flies and humans. Perhaps the most famous of these regulators is the Eyeless protein. This molecule, discovered in flies in the lab of Dr Walter Gehring in the 1990s, has the amazing ability to induce ectopic eyes when expressed in the fly leg or antenna. The Eyeless molecule also exists in humans, where it is called Pax6. Like in flies, it determines where the eye forms and is required for its development. The finding that the same gene is involved

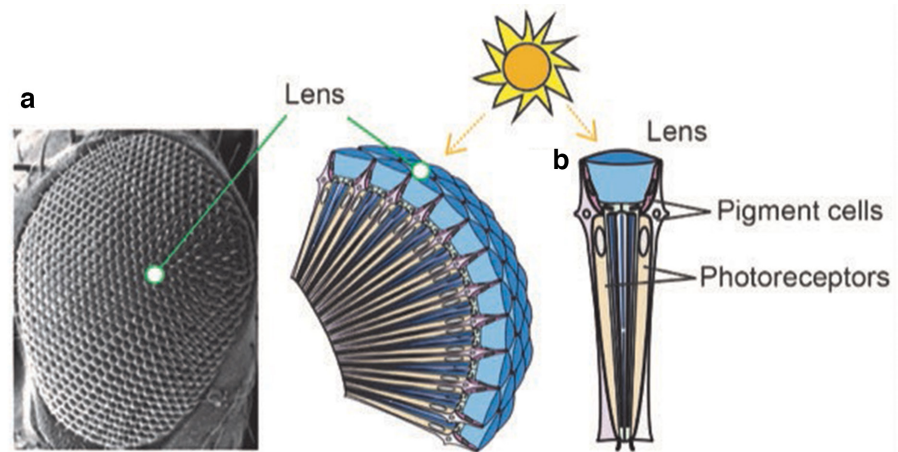


Figure 1. The compound eye of the fruit fly *Drosophila melanogaster*. (a) A scanning electron micrograph of the compound eye shows how the individual unit eyes, or facets (also called ommatidia), are precisely arranged in a hexagonally packed array. In the fruit fly, the compound eye contains approximately 750 facets/ommatidia arranged in a dome-like structure. (b) Each ommatidium consists of a facet lens, eight light-sensitive photoreceptors and a complement of pigment cells that optically insulate each unit eye.

in eye development in humans and flies suggested that despite having gone through millions of years of independent evolution, the insect and human eye might share a common origin. In this suggestion, *Eyeless/Pax6* could have controlled the expression of a light-sensitive pigment in a cell. Then, with time, *Pax6* could have gradually acquired new roles to eventually control many aspects of the genetic and molecular blueprint that makes up an entire eye (Figure 2). Researchers wondered whether they could reveal this ancestral function by asking if the human *Pax6* molecule could substitute for *Eyeless* in flies. The result was striking. When expressed in the fly, human *Pax6* could induce the formation of insect eyes! This greatly supported the model that both types of eyes share a common ancestor where *Pax6* likely played a key role in enabling light detection. However, the scientific process is to continuously question theories and models, even those that are well established. The evolution of the eye is no exception, and the model of a common ancestor to all animal eyes is still hotly debated. Recent evidence suggests, for instance, that instead, eyes might have arisen many times independently. In this scenario, *Eyeless* and *Pax6* would have been independently recruited to initiate the earliest events in eye development in flies and vertebrates, respectively.

Patterning the eye

As the eye primordium is specified through *Pax6/ Eyeless*, it must grow to produce a large pool of retinal cells that will then become lens cells, pigment cells or photoreceptors in the adult eye (Figure 3a). Waddington's 1962 treatise '*New Patterns in Genetics and Development*' includes a first account of how these

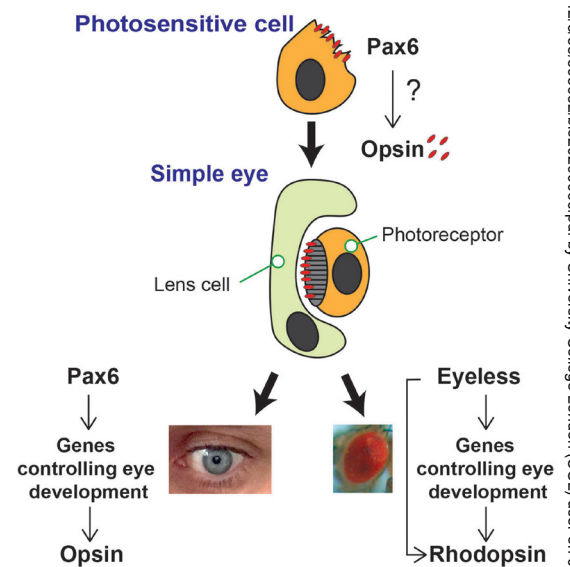


Figure 2. *Eyeless/Pax6* in early fly and human eye development. In a hypothetical ancestral situation, *Pax6* controls the expression of an opsin in simple photosensitive cells. As this situation evolves and primitive eyes arise, a photosensitive cell is now coupled with a second type of cell whose function is to form a lens to increase light-gathering efficiency. This simple arrangement is then expanded upon through time, leading to more complex types of eyes, including the human camera-type eye and the compound eye of insects. In both cases, the gene *PAX6* (or its homologue, *Eyeless*, in flies) plays a key role early in the specification of the eye primordium. In both evolutionary lineages, other functions for *Pax6/ Eyeless* in eye development are gradually acquired through time.

cells, and the ommatidium as a whole, are generated. Following this seminal work, the advent of molecular biology opened the door for researchers to study how genes and protein networks control the assembly of such a precise optical apparatus.

Building the compound eye of the fly begins with a wave of differentiation that sweeps across the eye primordium to lay down columns of ommatidia in its wake. This wave is called the morphogenetic furrow (Figure 3b). Propagation of this wave relies on two diffusible molecules, Hedgehog and Decapentaplegic, which can activate each other's expression. Hedgehog is produced by the newly induced ommatidia and diffuses towards the anterior side of the eye primordium where it activates Decapentaplegic, which in turn starts ommatidia assembly, including the expression of Hedgehog. In this manner, the Hedgehog and Decapentaplegic system powers the anterior movement of the morphogenetic furrow. In the wake of the morphogenetic furrow, ommatidium assembly starts with one founder cell, R8, which initiates recruitment of all the remaining cell types of the unit through a reiterative process. This involves precise cell interaction and communication via the secretion of signalling molecules that bind to receptors found on the surface of cells.

The making of a photoreceptor

As retinal cells are recruited to the ommatidium, they must next get into shape so that they can carry out their specific function. Like many of the cells that make up our organs, photoreceptors are highly polarized cells that have distinct top (apical) and bottom (basal) poles. At their apical pole, these cells develop a specialized light-gathering structure

called the rhabdomere that houses the molecules that detect light, the rhodopsins (Figure 4). The rhabdomere is an enormously amplified membrane that consists of tightly packed finger-like projections. Each projection contains a filamentous structure called F-actin. Actin is both widely conserved and abundant in cells, and is a structural component that gives cells their shape. By amplifying the amount of cell membrane at their apical pole, photoreceptors can pack in large amounts of rhodopsin, which maximizes light detection.

Remarkably, while human photoreceptors are very different in their organization and morphology when compared to insect photoreceptors, they share common molecular regulators. For example, the Crumbs molecule is required to build the rhabdomere and the membrane that supports it in flies. In human photoreceptors, this same molecule is required to build the inner segment, which supports the outer segment that houses the visual pigments. Mutations in Crumbs cause severe human eye diseases, including retinitis pigmentosa 12 and Leber congenital amaurosis disorder. Both of these diseases cause blindness in humans. In fact, due to the similar requirement for Crumbs in these different photoreceptors, some of these human mutations could recently be studied in flies, where genes can be manipulated easily and drugs can be trialled on a large scale.

The molecular biology of light detection

Integral to all visual systems are the rhodopsins. This large family of molecules consists of a protein moiety,

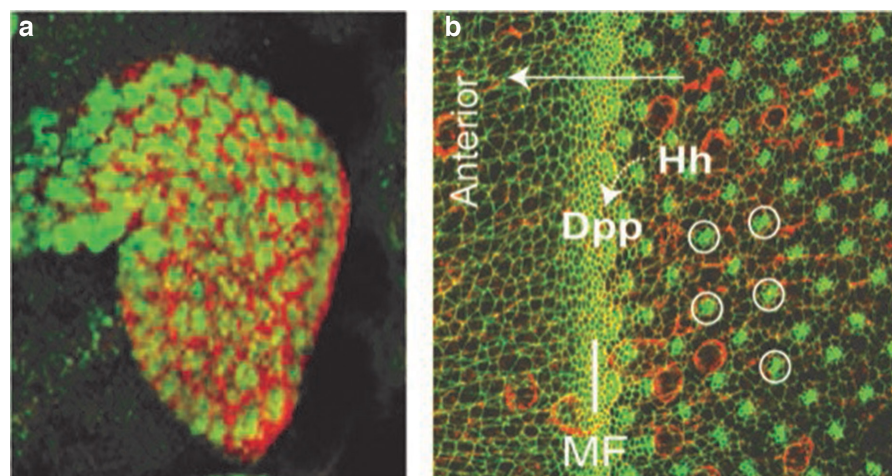


Figure 3. *Pattern formation in the fly eye.* Within the fly larvae, the eye primordium is a small bag of cells with little discernible structure (a). Through development, this primordium grows and becomes highly patterned. As eye development proceeds (b), a wave called the morphogenetic furrow (MF) sweeps across the eye primordium. As cells emerge from the MF, assembly of the ommatidia begins. Two key molecules in this process are Hedgehog (Hh) and Decapentaplegic (Dpp), which diffuse anteriorly to induce movement of the MF. Columns of ommatidia emerge in the wake of the MF, some of which are circled in the figure.

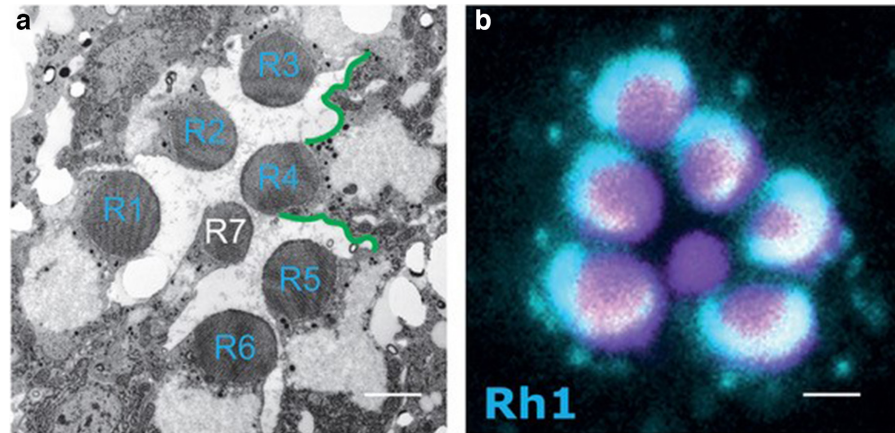


Figure 4. *Individual photoreceptors in the fly eye.* (a) Transmission electron micrograph of a cross-section through one ommatidium. The 'outer' photoreceptors R1–R6 are arranged in a trapezoid-like structure surrounding the 'inner' photoreceptors R7 and R8. R7 sits on top of R8, which therefore is below the plane of this cross-section and not visible in this image. The rhabdomeres, which detect light, are visible as round, dark structures at the apical end of each photoreceptor. One photoreceptor is outlined in green, as an example. (b) Confocal micrograph through one ommatidium. Filamentous actin has been stained in purple, while the opsin protein Rhodopsin 1 (Rh1) has been stained in blue. Note that the blue staining is absent from the inner R7 photoreceptor, which instead expresses either Rh3 or Rh4. Image reused with permission from Pichaud, 2018 | <https://doi.org/10.3389/fncel.2018.00090>

called an opsin, and a light-sensitive, retinol-derived pigment molecule that changes shape when struck by a photon. This shape change is relayed within the cells through a molecular route that transforms light detection into a nerve impulse. Five types of rhodopsin are expressed in fly photoreceptors, and each photoreceptor predominantly expresses one type of rhodopsin, resulting in distinct subpopulations of photoreceptor cells. By having these subpopulations, the fly eye is able to perform distinct visual tasks, including motion detection and colour discrimination.

Rhodopsin 1 and motion detection

The task of motion detection is the job of photoreceptors R1–R6, all of which express Rhodopsin 1. While a full description of motion detection is beyond the scope of this primer, one feature that we would like to introduce is neural superposition.

To better understand the fly eye, it helps to first compare it to other insect eyes. Insects that are active during the day, including bees and grasshoppers, have an apposition eye with a fused rhabdom that forms a single light-gathering unit (Figure 5). Any photon that enters the lens within the inter-facet angle will be focussed onto the rhabdom to potentially generate a nerve impulse. Photons that enter the lens outside of this angle are lost. In bright light, where there are plenty of photons, this loss is tolerable. Each ommatidium sees overlapping regions in space, and this information is transmitted to the brain following the same spatial map that is found in the retina.

Neural superposition has evolved alongside the open rhabdom to improve fly vision, even under low-light conditions (Figure 5). With the open rhabdom, each rhabdomere has its own optical axis, which leads to an increased spatial resolution. Alongside this, a given point in space is seen by six photoreceptors that belong to six different ommatidia – the R1 of one ommatidia, the R2 of an adjacent ommatidia and so on through to R6. This increases the sampling of each point in space, thus increasing sensitivity under low-light conditions. As an object moves across the insect visual field, it will activate photoreceptors across neighbouring ommatidia in a spatial and temporal sequence. As these photoreceptors fire a nerve impulse, this spatial and temporal sequence will be interpreted in the brain. This triggers the 'jump and fly' response escape behaviour (which makes flies so hard to swat!).

Neural superposition describes how photoreceptors are wired to the fly brain. Information from photoreceptors that simultaneously see the same point in space is pooled into one neural cartridge. How nerve fibres from photoreceptors that belong to different ommatidia converge onto one cartridge in the brain involves adhesion molecules, but the exact recognition code is not fully understood. Like for the fly eye, wiring the human brain requires nerve cells to establish precise contacts with each other. Elucidating the genetic and molecular basis of wiring the fly visual system has greatly helped to reveal some of the rules and mechanisms that underpin brain development. Solving how neural superposition is established will likely have important repercussions for our understanding of how a brain is built, including in humans.

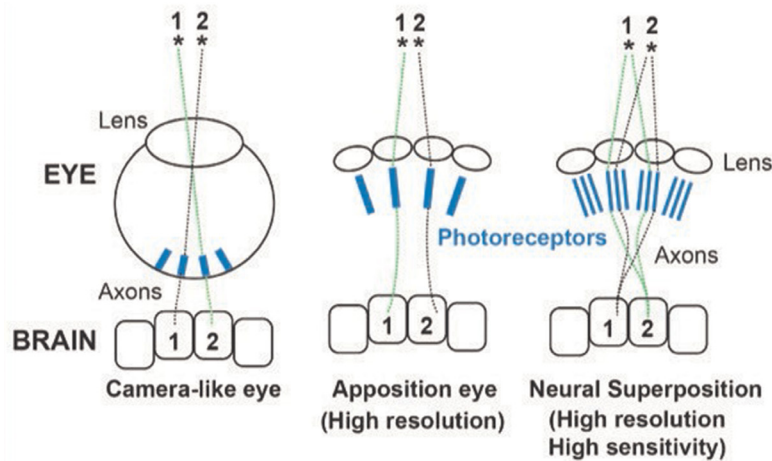


Figure 5. *Neural superposition.* A representation of the human camera-type eye. Light enters through one lens, and an inverted image is projected onto the retinal field. In the insect apposition eye, each ommatidium surveys discrete regions of space that overlap only slightly. Here, the photoreceptor axons of each ommatidium form one bundle that connects to one unit in the brain. In the neural superposition eye of dipteran flies, a given region of space is surveyed by photoreceptors from six different ommatidia. This requires a rewiring of the photoreceptors to the brain. Here, the axons of photoreceptors coming from different ommatidia must connect onto the same neural cartridge in the brain. Redrawn from Agi (2014) 10.3109/01677063.2014.922557.

Rhodopsin 3–6 and colour discrimination

Colour discrimination is the task of the R7 and R8 photoreceptors. These two ‘inner’ photoreceptors share their optical axis, and thus, they see the same point in space. Each inner photoreceptor preferentially expresses one of the rhodopsin molecules Rh3 through to Rh6 (Figure 6). Within most R7/R8 pairs, there are only two possible combinations of rhodopsin expression. R7 can express Rh3 or Rh4, while R8 can express either Rh5 or Rh6. When R7 expresses Rh3, its companion R8 always expresses Rh5. Alternatively, when R7 expresses Rh4, its paired R8 expresses Rh6 (Figure 6). Rh3 or Rh4 rhodopsins expressed in R7 have similar sensitivities to UV light. However, R8s are different in that Rh5 is more sensitive to blue light while Rh6 detects green light.

By comparing the level of UV light detected by the R7 photoreceptors and the level of either blue or green light detected by the R8 photoreceptors, the fly brain is able to discriminate blue from green. As for the motion detection system, precise wiring of R7/R8 to the brain is key for the colour discrimination system. Each R7/R8 pair connects to different regions within one neural cartridge. Thus, the 750 R7/R8 pairs of the retina project onto 750 neural cartridges found in the brain to form a retinotopic map of the outside world. Communication between cartridges is enabled as neural cells make connections between cartridges. The precise computation that the fly brain needs to perform to interpret the colour information provided by the R7/R8 cell is not fully understood, but is a fascinating field of study, with implication for human health and diseases.

Concluding remarks

In this short primer, we have only scratched the surface of the features of the fly eye. By looking beyond fruit flies, we see some interesting variations. For example, in predators such as dragonflies, the colour discrimination

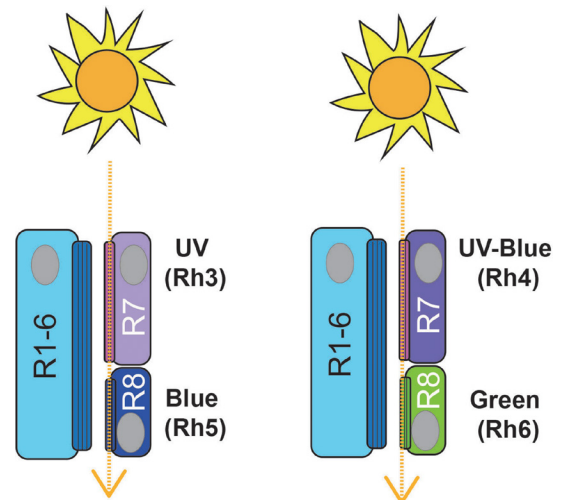


Figure 6. *Colour discrimination in the fly eye.* Colour discrimination in flies relies on the expression of different types of rhodopsin molecules in the inner photoreceptors R7 and R8. The different sensitivities of each rhodopsin molecule to different wavelengths of light, coupled with the wiring of the R7 and R8 photoreceptors to distinct areas of the brain, enables colour discrimination.

system has been partially repurposed in part of the eye. Here, the R7 cell has been recruited to serve alongside R1 through to R6 for improved motion detection, in order to allow better detection of prey. A similar example is that of the 'love spot' that is found in the retina of the male house fly. Here again, motion detection has been prioritized over colour vision, but specifically in male flies to help them pursue females for courtship. How

this sexual dimorphism is achieved is not yet known. By looking at fruit fly and other insect eyes, a picture is emerging of how it is not just the acquisition of new visual features that improves insect vision. Their ability to adapt and tailor molecular pathways and visual features to their specific needs has helped insects to see the world more clearly, in ways that best suit their ecological niches. ■

Further reading

- Halder, G., Callerts, P. and Gehring W.J. (1995) Induction of ectopic eyes by targeted expression of the eyeless gene in *Drosophila*. *Science*. **267**, 1788–1792 DOI: 10.1126/science.7892602
- Schwab, I. (2018) The evolution of eyes: major steps. The Keeler lecture 2017: Centenary of Keeler Ltd. *Eye*. **32**, 302–313. <https://doi.org/10.1038/eye.2017.226>
- Waddington, C. H.: *New Patterns in Genetics and Development*. Columbia University Press, New York und London 1962. XIV + 271 Seiten. Preis geb.
- Wolff, T. and Ready, D.F. (1991) The beginning of pattern formation in the *Drosophila* compound eye: the morphogenetic furrow and the second mitotic wave. *Development*. **113**, 841–850.
- Casares, F. and Almudi, I. (2016) Fast and furious 800. The retinal determination gene network in *Drosophila*. In: *Organogenetic Gene Networks* (Castelli-Gair Hombría, J. and Bovolenta, P. eds) pp. 95–125, Cham, Springer.
- Montell, C. (2012) *Drosophila* visual transduction. *Trends Neurosci.* **35**, 356–363. DOI:10.1016/j.tins.2012.03.004
- Pichaud, F. (2018) PAR-complex and crumbs function during photoreceptor morphogenesis and retinal degeneration. *Front. Cell. Neurosci.* DOI: 10.3389/fncel.2018.00090
- Sanes, J.R. and Zipursky, S.L. (2010) Design principles of insect and vertebrate visual systems. *Neuron*. **66**, 15–36. DOI: 10.1016/j.neuron.2010.01.018
- Agi, E., Langen, M., Altschuler, S.J., et al. (2014) The evolution and development of neural superposition. *J. Neurogenet.* **28**, 216–232. DOI: 10.3109/01677063.2014.922557
- Meinertzhagen, I.A. (2018) The world of the identified or digital neuron. *J. Neurogenet.* **32**, 149–154. DOI: 10.1080/01677063.2018.1474214
- Viets, K., Eldred, K.,C. and Johnston, R.J. Jr (2016) Mechanisms of photoreceptor patterning in vertebrates and invertebrates. *Trends Genet.* **32**, 638–659. 10.1016/j.tig.2016.07.004
- Perry, M.,W. and Desplan, C. (2016) Love Spots. *Curr Biol.* **26**, R484–R485. DOI: 10.1016/j.cub.2016.02.020



Dr Rhian F. Walther is a cell and developmental biologist based in the Pichaud lab at the Medical Research Council Laboratory for Molecular Cell Biology, University College London. Dr Walther obtained a PhD in Biochemistry at the University of Ottawa in Canada. She then joined UCL through a post-doctoral fellowship from the Terry Fox Foundation. Since joining the Pichaud lab, she has made seminal discoveries on the mechanisms of cell shape and polarity changes during organogenesis, using the developing fly eye as a model system. Email: r.walther@ucl.ac.uk



Franck Pichaud is a Professor of Cell and Developmental Biology at University College London in the UK. His research seeks to understand how cells work together to form organs. His lab uses the genetically amenable fly eye as the primary model system to study this important problem in health and disease. Work in the Pichaud lab is funded by the MRC, Wellcome Trust and Biotechnology and Biological Sciences Research Council. Email: f.pichaud@ucl.ac.uk