Dispatches

Eye Development: Random Precision in Color Vision

In insect and vertebrate eyes, different types of color-detecting photoreceptors are randomly distributed throughout the retina. A recent study has provided important new insights into the developmental mechanisms that generate the retinal mosaic required for color vision.

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> *It is not quantity which counts [with colors], but choice and organization*
>
> (Henri Matisse)

To collect information about the outside world, eyes have evolved in different animal species as highly specialized sensory structures which, although anatomically quite diverse, show common organizational features. The visual system not only allows the perception of shape and motion, but also can often discriminate colors. Light of different wavelength is detected by a set of Opsin type receptor molecules with distinct spectral properties. They are expressed in specific photoreceptor types following the 'one-receptor-one-neuron' rule characteristic for most sensory neurons [1,2]. Interestingly, within the human and the fly retina, the different color detectors are distributed in a random fashion, but so far the developmental mechanisms underlying stochastic pattern formation have been elusive. Using the *Drosophila* eye as a model, the Desplan lab [3] has now been able to link the stochastic pattern of color-sensitive photoreceptors to the expression of a single transcription factor.

The *Drosophila* compound eye has been an extremely useful model system for understanding the logic of pattern formation [4,5]. Here 750–800 single eye units called ommatidia are assembled into an almost crystalline-like arrangement. This ordered pattern continues in the organization of the eight photoreceptor cells (R cells) inside each ommatidium. On the basis of their different spectral sensitivities, the eight R cells can be grouped into two functional classes. Six monochromatic outer photoreceptor cells (R1–R6), the functional homologues of vertebrate rods, express only one type of rhodopsin (Rh1) and are involved in motion detection [6]. In contrast, two inner photoreceptor cells, R7 and R8, express one out of several rhodopsins with distinct spectral sensitivities (Rh3–Rh6), allowing them to function in color discrimination, much as cone cells do in vertebrate eyes [7].

In the fly retina, two invariable R7/R8 combinations define the corresponding two color-sensitive ommatidia classes (Figure 1A). In 'pale' ommatidia, sensitive to shorter wavelengths, an R7 cell expressing Rh3 always matches with an R8 cell expressing Rh5; and in 'yellow' ommatidia, specialized in the perception of longer wavelengths, an Rh4-positive R7 cell is housed together with an Rh6-positive R8 cell. The distribution of pale and yellow ommatidial subtypes does not follow the morphologically homogeneous retinal pattern, but seems to be random, resulting in a functional mosaic inside the retina [8]. Although the distribution is stochastic, however, the ratio of photoreceptor cell types is well defined, with 30% pale and 70% yellow ommatidia (Figure 1A). How is a random but biased mosaic pattern generated in an otherwise regularly organized sensory field?

The process of R cell differentiation in *Drosophila* extends over a period of five days and can be divided into an early specification period, in which the different R cell subtypes first acquire their neuronal ground states, followed by their terminal differentiation into functional photoreceptors with class-specific rhodopsin expression [9]. Important insights into the mechanisms underlying photoreceptor maturation came from the analysis of mutations that disrupt the strict pairing of Rh3/Rh5 and Rh4/Rh6 in the pale and yellow ommatidia, respectively [10]. When all R7 cells are missing, as in the *sevenless* mutant, the number of R8 cells is unaffected, but they express exclusively Rh6, indicating that the decision between a yellow or pale fate is initiated when the R7 cell in each ommatidium makes a choice of whether to express Rh3 or Rh4 (Figure 1B,C). An Rh3 decision is then communicated to the adjacent R8 cell, leading to the induction of Rh5 expression. Although the signal from R7 to R8 is still obscure, we now have a satisfying understanding of what triggers the initial choice of R7 between the Rh3 or Rh4 status.

Genetic studies identified the *spineless* gene, which encodes a bHLH-PAS transcription factor, as the critical determinant of both the initial yellow R7 fate choice and the stochastic distribution of the two types of color-sensitive ommatidia [3]. In *spineless* mutants, R cells develop normally through the first phase of cell type specification, leading to a regularly patterned and normal sized compound eye. Visualizing *rhodopsin* expression, however, revealed the presence of only the pale ommatidia subtype with a wild-type Rh3/Rh5 pairing. Subsequent clonal analysis demonstrated a strictly cell-autonomous function of spineless in the yellow R7 cell for the induction of Rh4 expression.

The notion that *spineless* expression triggers Rh4 expression received further support from gain-of-function experiments: *spineless* expression in all developing photoreceptor

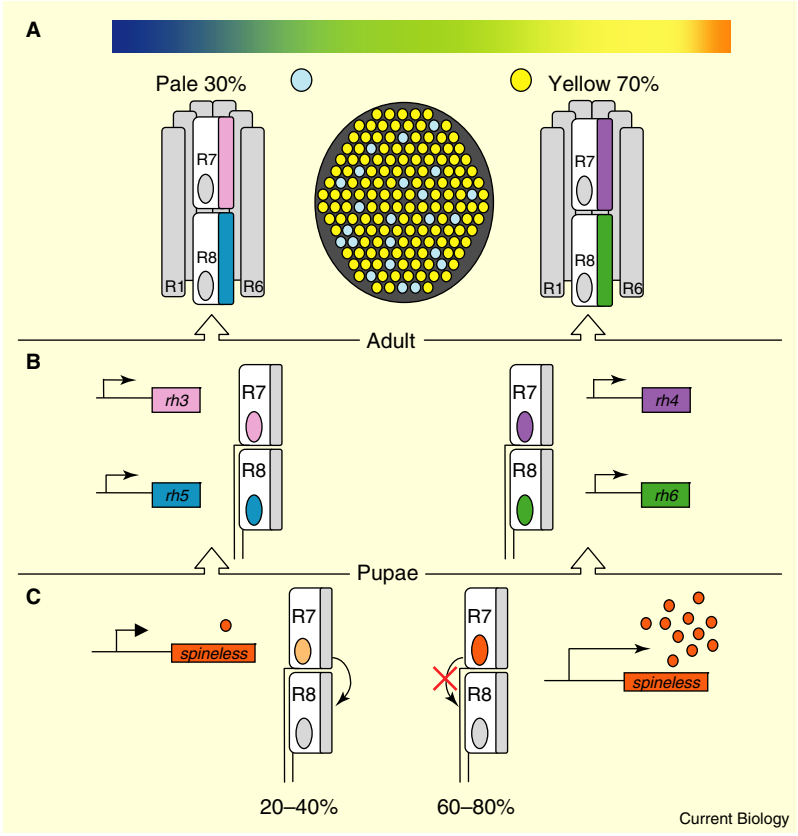

Figure 1. Organization and development of *Drosophila* color-sensitive photoreceptors.

(A) In the adult retina, each ommatidium contains 6 outer (R1–R6) and 2 inner (R7 and R8) photoreceptor cells. Specific combinations of rhodopsin expression define pale (Rh3/Rh5) and yellow (Rh4/Rh6) ommatidia, which are randomly distributed throughout the retina in a 3:7 ratio. (B,C) Different stages of R7/R8 differentiation in late (B) and mid (C) pupal development. Different levels of *spineless* expression lead to a cell-autonomous Rh3/Rh4 fate choice in R7, followed by an induced or default R8 differentiation.

neurons resulted in an efficient ectopic Rh4 activation. In R8, the endogenous, cell type specific rhodopsin expression was maintained leading to a violation of the 'one-receptor-one-neuron' rule; in R7 cells, however, *spineless* induced a perfect switch from Rh3 to Rh4 terminal differentiation status. Furthermore, expression of *spineless* even in late differentiating pale R7 cells, using a *rhodopsin* promoter construct, was able to trigger the switch from Rh3 to Rh4 expression. The induced Rh5 expression in the neighboring R8 cell cannot be reverted, however, resulting in untypical ommatidia with Rh4/Rh5 inner receptor cells pairing. From these results, Wernet *et al.* [3] concluded that a single transcription factor, Spineless, is both necessary and sufficient to initiate the assembly of the yellow type ommatidium, first specifying Rh4 expression in R7, which then prevents the induction of the R8 cell to express the Rh5 protein (Figure 1C).

Spineless also has a direct role in establishing the stochastic distribution of the color-sensitive ommatidia in the retina [3]. Expression of *spineless* RNA occurs in only a randomly distributed 60–80% of R7 cells, which presumably will later differentiate into yellow type R7 photoreceptors (Figure 1C). Furthermore, the level of expression varies greatly among the Spineless-positive R7 cells, suggesting a direct link between a Spineless threshold and Rhodopsin expression choice. Temporally enhanced *spineless* expression leads to a clear increase in the number of yellow at the expense of pale ommatidia. In contrast, this ratio decreases following experimental reduction of *spineless* expression, either by inactivating one gene copy or by adding transgenes carrying the *spineless* eye enhancer, which compete for endogenous activating factors. These data thus support a two-step model combining cell-autonomous mechanisms and cell–cell communication to establish cell fate and distribution of the two color-sensitive ommatidia types.

The cell autonomous function of Spineless implies the existence of R7-specific or combinatorial acting upstream factors acting on a 1.6 kilobase enhancer fragment of the *spineless* regulatory region. Neither the activating factors nor the final mechanisms that set the Spineless threshold for inducing Rh4 expression are known. It is possible that, once the fate choice is made, further stabilization is needed, as it has been reported for the Rh5/6 decision in the R8 cell [11]. At the same time, Spineless, which is expressed only in a short pulse during eye development, likely activates downstream targets that control the expression of Rh4 sensory receptor, but concomitantly leads to the exclusion of Rh3 receptor co-expression. *In vivo*, *spineless* and *rhodopsin* expression are temporally separated by one day, but even when *spineless* expression is forced during the time of rhodopsin expression, it can reprogram terminal fate [3]. Additionally, Spineless or its downstream targets antagonize the generation of an inductive signal, yet to be identified, emanating from the R7 cell. Interestingly, like Spineless, other members of the bHLH/PAS transcription factor family often control the initial choice in various cell fate decisions [12]. As bHLH/PAS transcription factors commonly act as heterodimers, the identification of Spineless interaction partners will be very informative regarding the mechanism through which Spineless leads to a selective activation of distinct differentiation programs.

Finally, sensory receptor specificity in the peripheral nervous system has to be matched with precise synaptic connections

in the central brain to allow color discrimination. In *Drosophila*, the integration of sensory information coming from randomly distributed receptor neurons into the visual system's topographic organization has not been analyzed. Although most of the cellular components and their projection patterns in the fly visual system have been described [13], our understanding of how the brain 'sees' the colorful world is still in its beginnings.

References

1. Mombaerts, P. (2004). Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. Curr. Opin. Neurobiol. *14*, 31–36.
2. Mazzoni, E.O., Desplan, C., and Celik, A. (2004). 'One receptor' rules in sensory neurons. Dev. Neurosci. *26*, 388–395.
3. Wernet, M.F., Mazzoni, E.O., Celik, A., Duncan, D.M., Duncan, I., and Desplan, C. (2006). Stochastic spineless expression creates the retinal mosaic for colour vision. Nature *440*, 174–180.
4. Voas, M.G., and Rebay, I. (2004). Signal integration during development: insights from the Drosophila eye. Dev. Dyn. *229*, 162–175.
5. Reifegerste, R., and Moses, K. (1999). Genetics of epithelial polarity and pattern in the Drosophila retina. Bioessays *21*, 275–285.
6. Heisenberg, M., and Buchner, E. (1977). The role of retinula cell types in visual behavior of Drosophiila melanogaster. J. Comp. Physiol. *117*, 127–162.
7. Chou, W.H., Hall, K.J., Wilson, D.B., Wideman, C.L., Townson, S.M., Chadwell, L.V., and Britt, S.G. (1996). Identification of a novel *Drosophila* opsin reveals specific patterning of the R7 and R8 photoreceptor cells. Neuron *17*, 1101–1115.
8. Franceschini, N., Kirschfeld, K., and Minke, B. (1981). Fluorescence of photoreceptor cells observed *in vivo*. Science *213*, 1264–1267.
9. Mollereau, B., Dominguez, M., Webel, R., Colley, N.J., Keung, B., de Celis, J.F., and Desplan, C. (2001). Two-step process for photoreceptor formation in *Drosophila*. Nature *412*, 911–913.
10. Chou, W.H., Huber, A., Bentrop, J., Schulz, S., Schwab, K., Chadwell, L.V., Paulsen, R., and Britt, S.G. (1999). Patterning of the R7 and R8 photoreceptor cells of *Drosophila*: evidence for induced and default cell-fate specification. Development *126*, 607–616.
11. Mikeladze-Dvali, T., Wernet, M.F., Pistillo, D., Mazzoni, E.O., Teleman, A.A., Chen, Y.W., Cohen, S., and Desplan, C. (2005). The growth regulators warts/lats and melted interact in a bistable loop to specify opposite fates in *Drosophila* R8 photoreceptors. Cell *122*, 775–787.
12. Crews, S.T. (1998). Control of cell lineage-specific development and transcription by bHLH-PAS proteins. Genes Dev. *12*, 607–620.
13. Fischbach, K.F., and Dittrich, A.P. (1989). The optic lobe of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structures. Cell Tissue Res. *258*, 441–475.

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DOI: 10.1016/j.cub.2006.04.012

Bacterial Cell Biology: Managing Magnetosomes

Sensing of magnetic fields by living organisms — magnetosensing — is best understood in magnetotactic bacteria. Recently work has provided new insight into the biogenesis of bacterial magnetosomes, and links these organelles to a newly recognized prokaryotic cytoskeletal filament which organizes magnetosomes into a sensory structure capable of aligning cells with the geomagnetic field.

Craig Stephens

Several centuries ago, humans learned to construct navigational compasses that could sense the earth's magnetic field [1]. By that time, the living world was millions of years ahead of us in geomagnetic sensing technology. While the significance and mechanisms of magnetosensing in animals, such as migratory birds, fish and insects, that execute remarkable global navigational feats have been debated for years, the biological compass mechanism we know the most about at the cellular level is found in magnetotactic bacteria. These aquatic microbes are thought to use their internal magnets for the relatively mundane task of pointing themselves downward, toward their preferred homes in oxygen-depleted sediments [2]. We will discuss here recent insights into how 'magnetosomes', the membrane-enclosed magnetite crystals central to bacterial magnetosensing, are produced and organized [3,4]. Magnetosome-like structures have been observed in many animals, and the work discussed here may provide insight into the development, function and evolution of magnetosomes in eukaryotes.

Experimental work on bacterial magnetotaxis began over 30 years ago, when Richard Blakemore made the curious observation that a population of motile bacteria from salt marsh mud responded dramatically to magnetic manipulation [2]. Since Blakemore's initial discovery, magnetotactic bacteria have been found in freshwater and marine sediments around the world [5]. Most magnetotactic bacteria seen in the Northern hemisphere are north-seeking, and most in the Southern hemisphere are south-seeking [6,7]. Why is this? Blakemore hypothesized that, because of the significant vertical component of the geomagnetic field at latitudes away from the equator, alignment of a bacterial cell with the geomagnetic field would facilitate downward migration by north-seeking bacteria in the northern hemisphere (and conversely in the south) [2]. Since the magnetotactic bacteria isolated so far prefer anaerobic or microaerobic conditions, if they find themselves in an O_2-rich environment, such as the water column above the sediment, following the geomagnetic field downward — and supplementing magnetotaxis with O_2 and/or redox sensing and taxis — should help them to find more anoxic sediments [2,8].

The cell biology of magnetotaxis is under active investigation [9,10]. Magnetosomes contain crystalline particles of magnetite (Fe_3O_4) or greigite (Fe_3S_4). The individual crystals are generally 35–100 nm in size, and constitute a permanent single magnetic domain [11,12]. To generate a sufficiently large