

Building a retinal mosaic: cell-fate decision in the fly eye

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Across the animal kingdom, color discrimination is achieved by comparing the outputs of photoreceptor cells (PRs) that have different spectral sensitivities. Much remains to be understood about how the pattern of these different PRs is generated and maintained. The *Drosophila* eye has long provided a beautiful system for understanding various aspects of retinal-cell differentiation. Recent progress in this field is revealing that a highly ordered series of events, involving cell-cell communication, localized signaling and stochastic choices, creates a complex mosaic of PRs that is reminiscent of the human retina. Notably, several of the factors used in generating the retinal mosaic of the fruitfly have corresponding functions in vertebrates that are likely to have similar roles.

Despite the broad range of eye structures across the animal kingdom, all visual systems use similar cellular mechanisms to respond to environmental cues. For example, all animals use related opsin proteins in their photoreceptor cells (PRs) to capture photons (for review, see Ref. [1]). In addition, the eyes of most animals can be used to perform two distinct visual tasks: they not only form images of the surrounding environment, but also detect the 'quality' of the visual stimulus, such as color or light polarization, through the use of specialized subclasses of PRs. These PR subclasses have important morphological and molecular differences, as well as characteristic distribution patterns through the retina, that maximize the amount of information extracted from the environment. Humans, for example, use rod PRs ('rods') for detecting objects under conditions of low light, and cone PRs ('cones') for color discrimination.

To carry out these purposes most efficiently, the different subclasses of cones (called S, M and L, indicating their maximal sensitivity to short, medium or long wavelengths, respectively) are highly concentrated in the center of the retina, termed the fovea. Notably, their distribution there seems to be random, resulting in a cone mosaic that can be visualized *in vivo* (Figure 1a). This enables the fovea to function as the color and high-acuity center for the eye. Rods, by contrast, are concentrated towards the periphery of the eye and specialize in the detection of shape and motion under conditions of low light.

Even species as distantly related as *Drosophila* share important similarities with humans in the organization of

their retina. For example, specialized groups of PRs are used to discriminate between colors (in analogy to cones), whereas other PRs have been optimized for detecting shapes and motion (in analogy to rods). Despite the marked differences in retinal organization, both fly and human color PR subtypes show a similar random distribution through the retina (Figure 1a–c). In addition, another group of fly PRs is highly concentrated in one part of the retina, thereby forming a specialized eye region conceptually similar to the fovea. The retinal mosaic of the fruitfly therefore represents an attractive model system for studying both stochastic and localized specification events that occur during retinal patterning.

Emerging data indicate that retinal patterning includes a series of highly coordinated and organized processes. Several recent studies in the fly eye have begun to identify many of the factors involved and, notably, similar patterning events occur in the vertebrate retina, which are sometimes regulated by orthologous factors. These data further imply that the vertebrate single lens eye and the insect compound eye use similar strategies to achieve their function and to control the development of the retina. Rather than reflecting common ancestry of the visual systems, this might indicate that convergent mechanisms are used to control opsin expression in different PR subtypes and could provide insight into how the complexity of the retina is created and maintained.

Here, we provide a brief overview of the eye system in *Drosophila* before reviewing the recent progress made towards a better understanding of retinal patterning in *Drosophila*.

The *Drosophila* compound eye

The *Drosophila* eye consists of about 800 stereotypical unit eyes (ommatidia), which each contain eight light-sensing PRs (termed R1–R8) as well as accessory cells involved in forming the lens or in shielding PRs from light coming from other ommatidia (for review, see Ref. [2]). According to their morphology, axonal projections and expression of opsin, adult fly PRs can be grouped into two functional categories. The outer PRs (R1–R6) are the fly equivalent of the vertebrate rods and are involved in motion detection and image formation. Computation of their outputs begins in the first optic lobe, the lamina, where the outer PRs project their axons. Outer PRs capture photons with high efficiency, owing to both their expression of the broad spectrum rhodopsin Rh1 and the large diameter of their light-gathering membranes

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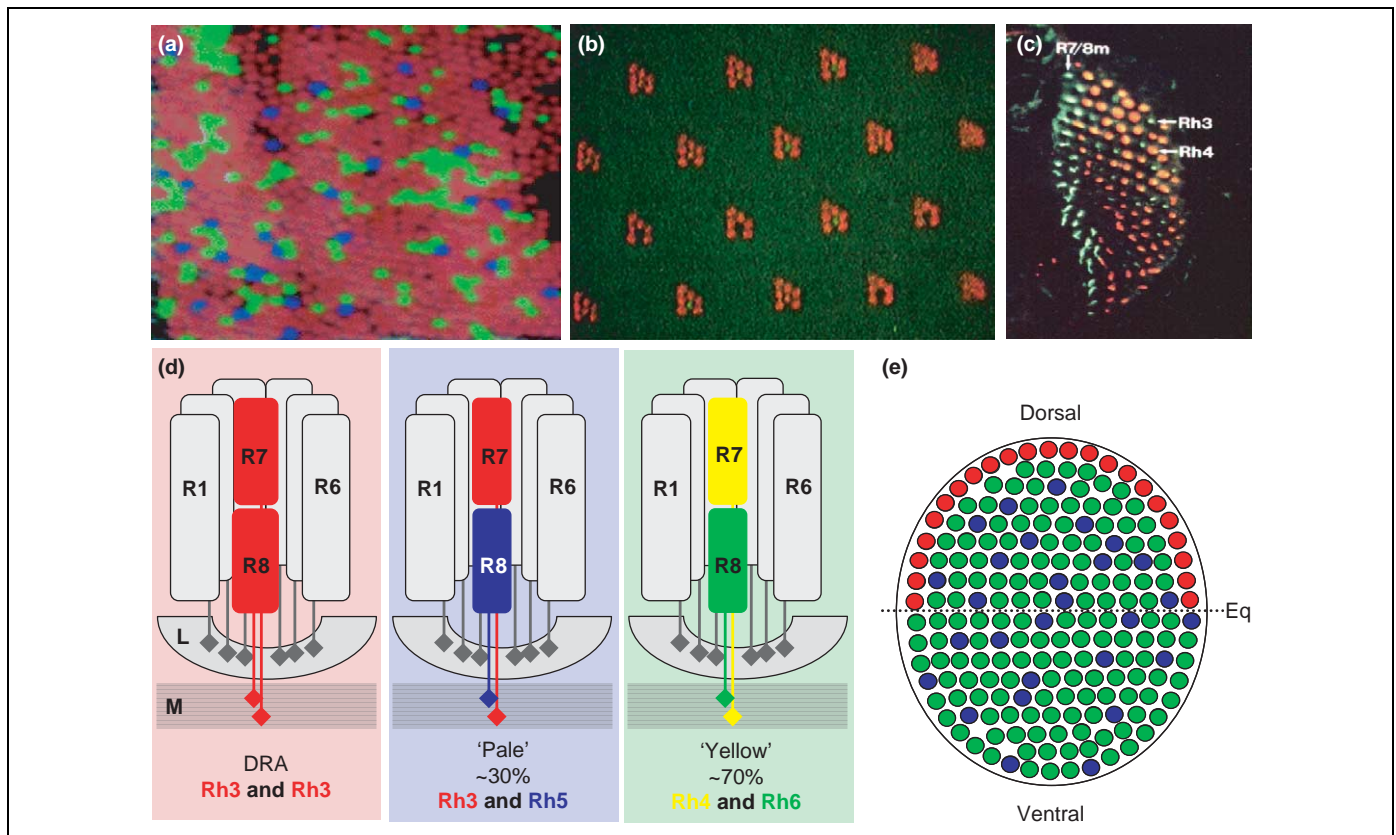


Figure 1. Retinal mosaics in humans and flies. **(a)** Pseudocolor image of the trichromatic cone mosaic from a living human retina. Blue, green and red colors represent the S, M and L cones, respectively. Reprinted, with permission, from Ref. [44] (<http://www.nature.com/>). **(b)** Visualization of the ommatidial mosaic in the housefly, *Musca domestica*, using epifluorescence and water immersion microscopy. Reprinted, with permission, from Ref. [6]. © (1981) American Association for the Advancement of Science (<http://www.sciencemag.org/>). **(c)** Visualization of the ommatidial mosaic in *Drosophila* using fluorescent antibodies against the opsin proteins Rh3 (green) and Rh4 (red). Dorsal to the left [R7/R8m: inner photoreceptor cells (PRs) of the 'dorsal rim area' (DRA)]. Reprinted, with permission, from Ref. [45]. © 1992 by the Society for Neuroscience. **(d)** On the basis of opsin expression and rhabdomere morphology of the inner photoreceptor cells (R7 and R8), three ommatidial subtypes can be distinguished. In ommatidia in the DRA, R7 and R8, which both express the ultraviolet (UV)-specific opsin Rh3, always show a strongly enlarged rhabdomere diameter (left). So-called 'pale' (p) ommatidia always express Rh3 in R7 cells and Rh5 in R8 cells (middle), whereas 'yellow' (y) ommatidia always express Rh4 in R7 cells and Rh6 in R8 cells (right). **(e)** DRA ommatidia are always found in 1–2 rows at the dorsal periphery of the adult retina, whereas p (~30%) and y (~70%) ommatidia are distributed randomly through the retina. Abbreviations: eq, equator; L, lamina; M, medulla.

(rhabdomeres), which extend from the basal to the apical side of the retina [3] (Figure 1d). The six outer PRs are organized in a chiral trapezoid (Figure 1b,d).

The center of each ommatidial trapezoid is occupied by the two inner PRs (R7 and R8). The rhabdomeres of these two PRs have significantly smaller diameters than those of the outer PRs and span only half of the retina. Because the R7 rhabdomere is located distally on top of R8, the inner PRs are in the same path of light, providing the ideal configuration to compare their outputs. This is absolutely required for the two functions of inner PRs: color vision and detecting the vector of polarized light. This comparison and the neuronal processing for both tasks begin in the second optic lobe, the medulla, where inner PRs project (for review, see Refs [4,5]).

The *Drosophila* retinal mosaic

Although the general external morphology of the fly eye does not indicate heterogeneity among ommatidia, elegant studies by Franceschini *et al.* [6] in the early 1980s revealed that at least two separate classes of ommatidia were interspersed randomly in the fly retina: when observed by fluoroscopy, the inner PRs seemed either pale (p) or yellow (y), with 30% being p

and the remaining 70% being y. On the basis of their different spectral sensitivities, these p and y ommatidia were proposed to contribute to the discrimination of different wavelengths.

Almost 25 years later, the cloning of the *rhodopsin* (*rh*) genes from *Drosophila* has provided a molecular basis for these two subtypes: p ommatidia always contain the ultraviolet (UV)-sensitive opsin, Rh3, in R7 and the blue-sensitive opsin, Rh5, in R8. A different UV-sensitive opsin, Rh4, is found in the R7 of y ommatidia, which always express the green-sensitive opsin, Rh6, in R8 [7–9]. Therefore, expression of the *rh* genes is always coupled between R7 and R8, leading to the characteristic pattern of p and y ommatidia (for review, see Ref. [10]). It is thought that these differences in opsin expression are crucial for the fly's ability to discriminate between colors: p ommatidia discriminate among shorter wavelengths (UV to blue), whereas y ommatidia are specialized in the perception of longer wavelengths, reaching into the green part of the spectrum. Notably, like p and y ommatidia, human cones, which express blue-, red- or green-specific opsins, are distributed stochastically in the fovea, but there is no coupling of opsin expression between different cells (for review, see Ref. [11]).

In addition to these two randomly distributed classes of color-sensitive ommatidia, a third ommatidial subset is always found in one or two rows at the dorsal rim of the fly eye, which is called the 'dorsal rim area' (DRA). The inner PRs in these ommatidia are monochromatic because they express Rh3 in both R7 and R8 [12] (Figure 1c). They have an enlarged rhabdomere diameter and specialized rhabdomeric microvilli, making them strongly sensitive to polarization. It is thought that DRA ommatidia are used to improve navigation by measuring the oscillation plane of polarized sky light (for review, see Refs [13,14]). DRA ommatidia are always highly localized, whereas p and y ommatidia are distributed randomly through the retina, enabling the fly to detect optimally the various qualities of light. Together, these three ommatidial subtypes form the complex ommatidial mosaic of the fruitfly (Figure 1e).

Below we describe the recent progress made towards a better understanding of retinal patterning in *Drosophila*. First, we discuss the genes required for establishing the basic PR cell types that constitute a functional adult ommatidium [the six outer PRs (R1–R6) and the two inner PRs (R7 and R8)]. Subsequently, we focus on the genetic pathways that further subdivide the ommatidia into three different subtypes to create the retinal mosaic (p, y and DRA). We conclude by presenting a model of how the fly has adapted its retina by integrating two fundamentally different strategies of ommatidial specification.

Building an adult ommatidium

During the third instar larval stage, the eight *Drosophila* PRs of each ommatidium (R1–R8) are selected from an undifferentiated pool of cells (for review, see Ref. [15]). Through a process that is now fairly well understood, interplay of the Notch, epidermal growth factor receptor (EGFR) and Sevenless signaling pathways at the 'morphogenetic furrow' leads to the sequential recruitment of PRs into evenly spaced clusters. R8 is the first PR to be determined. This 'founder cell' then recruits all six outer PRs in a pair-wise fashion (first R2 and R5, then R3 and R4, and finally R1 and R6). R7 is the last PR to be recruited [16,17]. On the basis of their order of recruitment and the combination of transcription factors that they express, the eight larval PRs therefore represent at least five different types of cell (R8, R2 and R5, R3 and R4, R1 and R6, and R7; Figure 2a, left). It should be noted that the larval R3 and R4 cells can be also viewed as individual cell types because they respond differently to the positional information that establishes the chirality of the ommatidium in a process called 'planar polarity' (for review, see Ref. [18]).

During the next 4 days of pupal development, the PRs undergo marked morphological changes as the rhabdomeres form and the expression of opsin is induced. At the end, only three functional classes of PRs can be distinguished in the adult ommatidium: the outer PRs (R1–R6), R7 and R8 (Figure 2a, right). By this stage, the outer PRs seem identical. They all express the same opsin and their morphology, as well as their axon projection pattern, enables the fly to use them as a separate visual system for shape and motion vision. The two inner PRs, which, notably, were the first (R8) and the last (R7) PRs to be

recruited into the ommatidium, are now grouped together to form the second visual system. These PRs have become morphologically similar, they project to slightly different layers at the same position in the medulla and they work together in the same optical path. Therefore, the maturation from larval to adult ommatidia requires extensive reorganization and respecification of PRs to group them into different functional categories.

Distinguishing inner from outer PRs

An important first step towards understanding ommatidial maturation came with the description of the role of the *spalt* gene complex, which encodes two homologous zinc-finger transcription factors [19]. The *spalt* genes are specifically expressed in R7 and R8 (Figure 2b, top left), and loss of *spalt* leads to a loss of inner PR characteristics. Instead, R7 and R8 appear morphologically as outer PRs: they gain outer PR markers (e.g. rhabdomere morphology and Rh1 expression; Figure 2b, top right) and lose inner PR characteristics (e.g. loss of Rh3, Rh4, Rh5 or Rh6 expression). Because the axonal projections to the medulla of these transformed inner PRs are maintained [20], however, it seems that inner PRs are initially properly specified in the larval disc in *spalt* mutants, but then lose their later identity and instead differentiate terminally into outer PRs, suggesting that two programs exist for neural determination and for PR differentiation.

Spalt is therefore necessary to distinguish differentiating inner PRs from an otherwise outer PR-like 'ground state' towards which all PRs tend to develop (Figure 2b, top right). This is particularly interesting because it might provide a simple explanation of how the originally very divergent outer PRs, R1–R6, adopt their uniform 'ground state' cell fate simply by being denied further differentiation signals such as *spalt* expression. The presence of the two distinct genetic programs, for specification and for differentiation, might also illustrate the dual function of *Drosophila* PRs: first they are specified as neurons that must find their appropriate target in the optic lobes, and then they further differentiate as light-sensing cells. In vertebrates, these two functions are actually performed by two different cell types (PRs and retinal ganglion cells).

Distinguishing between R7 and R8 cell fates

Both inner PRs require *spalt* to adopt their appropriate cell fate. Nevertheless, R7 and R8 represent different PRs, both morphologically (position within the retina) and molecularly (expressing different *rh* genes); thus, other factors must be required to distinguish further between these two inner PR cell fates. By screening for factors that bind to conserved sequences in the *Drosophila* opsin promoters, the gene *prospero* has been recently shown to be necessary for distinguishing the R7 cell fate from the R8 cell fate [21]. *Prospero* is a homeodomain transcription factor that is known to be important for both peripheral nervous system development and asymmetric cell division [22].

In the adult eye, *prospero* is expressed specifically in R7 in response to the Notch, EGFR and Sevenless signaling pathways that are responsible for R7 specification [23] (Figure 2b, bottom left). *Prospero* binds to conserved sequences in the R8-associated opsin promoters (*rh5* and

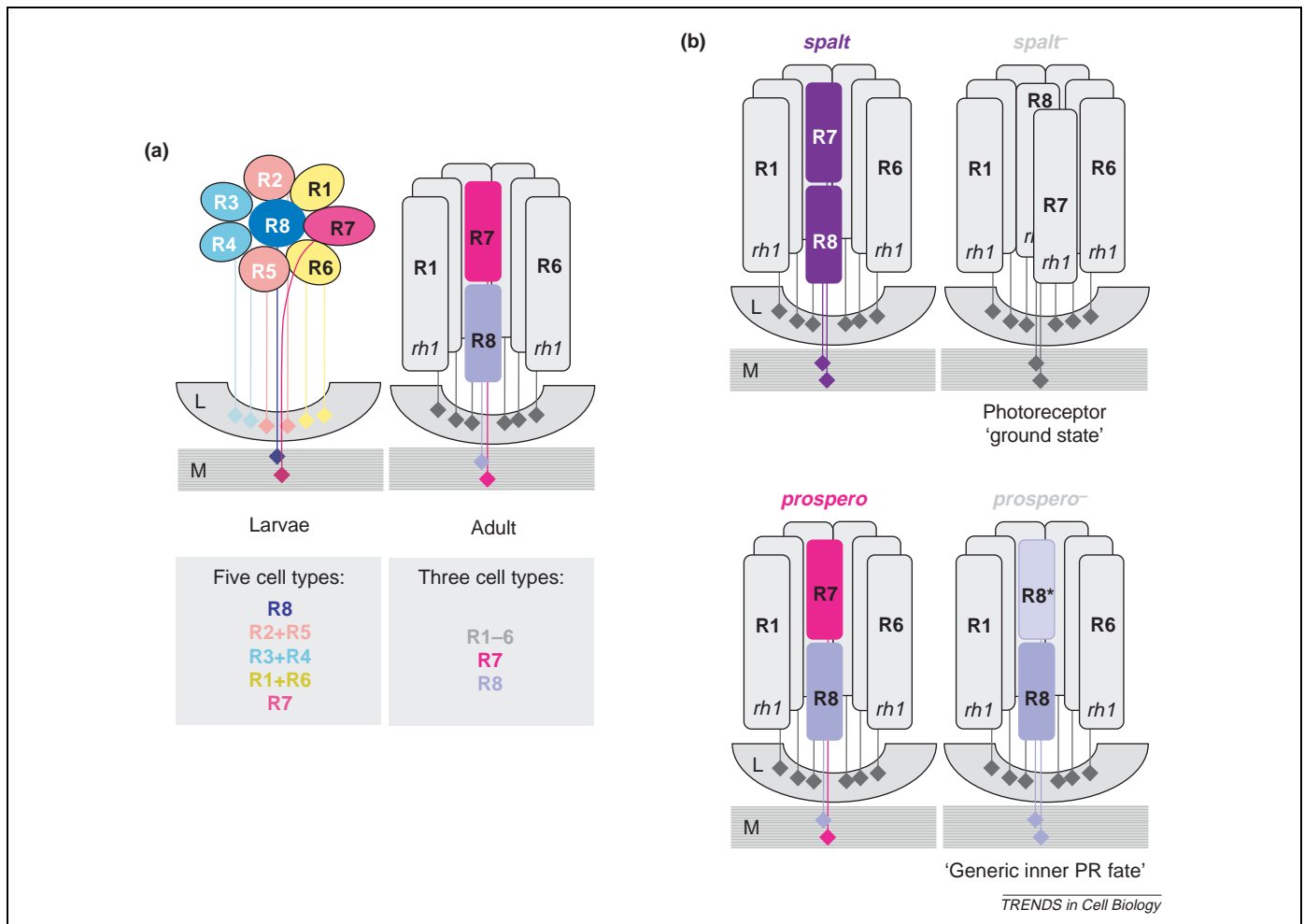


Figure 2. Photoreceptor cell (PR)-fate decisions during ommatidial maturation. **(a)** During larval life (left), eight PRs are specified successively in each ommatidium, either individually or pairwise, resulting in at least five types of cell (in order of birth): R8, R2+5, R3+4, R1+6 and R7. These PRs then project to the appropriate layer in the optic lobe. In adult ommatidia (right), only three functional cell types can be identified: the outer PRs (R1–R6) are morphologically identical and express the same opsin gene *rh1* (*ninaE*), whereas the inner PRs (R7 and R8) are located centrally on top of each other. **(b)** The transcription factor Spalt is expressed in both inner PRs (R7 and R8; top left). In adult *spalt* mutant retinas (*spalt*⁻), all eight PRs become indistinguishable both morphologically and molecularly (expressing *rh1*), suggesting that *spalt* is necessary to distinguish R7 and R8 from an otherwise outer-PR-like ‘ground state’ (top right). Another transcription factor, Prospero, is expressed specifically in R7 cells (bottom left). Loss of *prospero* in the retina (bottom right) results in R7 cells losing their typical adult characteristics (opsin expression and nuclear position; not shown). Instead, these cells now resemble R8 cells (R8*). This suggests that *prospero* is necessary to distinguish the fate of R7 cells from an R8-like ‘generic inner PR fate’, in particular by repressing opsin expression. Abbreviations: L, lamina; M, medulla.

rh6), thereby repressing them in R7. Indeed, loss of *prospero* in adult R7 cells leads to a derepression of *rh5* and *rh6*, creating a second R8-like cell (R8*) per ommatidium (Figure 2b, bottom right), whereas activating Prospero in R8 leads to loss of Rh5 and Rh6 expression. Loss of *prospero* also results in repression of the proper *rh* genes in R7, which most probably occurs to avoid the coexpression of opsin molecules – a situation that is generally not observed in sensory receptors (for review, see Ref. [24]).

These observations suggest that *prospero* can act on a generic R8-like inner PR fate, which requires *spalt*, to push cells towards an R7 fate and away from an R8 fate. Although R7 cells misexpress R8-associated *rh* genes in *prospero* mutants, however, they do not gain all of the R8 markers and their rhabdomeres are still positioned correctly in the distal part of the retina. This observation can be interpreted as a reversion of R7 back to a generic inner PR fate, which favors the expression of R8-associated *rh* genes, but suggests that other genes are

necessary to push the generic inner PR fate fully towards the R8 cell type.

Localized specification: the DRA ommatidia

The stereotypical *Drosophila* ommatidia are further divided into three subtypes, the p, y and DRA ommatidia, to create a retinal mosaic. Regulatory genes that specify the ommatidia localized in the DRA and the stochastically distributed p-type ommatidia have been identified only recently (Figure 3a,b). Furthermore, the responsible signaling pathways could be characterized in detail by using classical *Drosophila* genetics (Figure 4a,b).

DRA ommatidia form in one or two rows along the head cuticle in the dorsal half of the eye. The morphogen *wingless* (*wg*) is expressed in the head cuticle all around the eye (Figure 4a). Overactivation of the Wg pathway in all PRs leads ommatidia in the dorsal half of the eye to adopt the DRA fate, suggesting that ommatidia located in this half of the eye are competent to respond to the Wg signal [25,26]. The genes of the *Drosophila Iroquois*

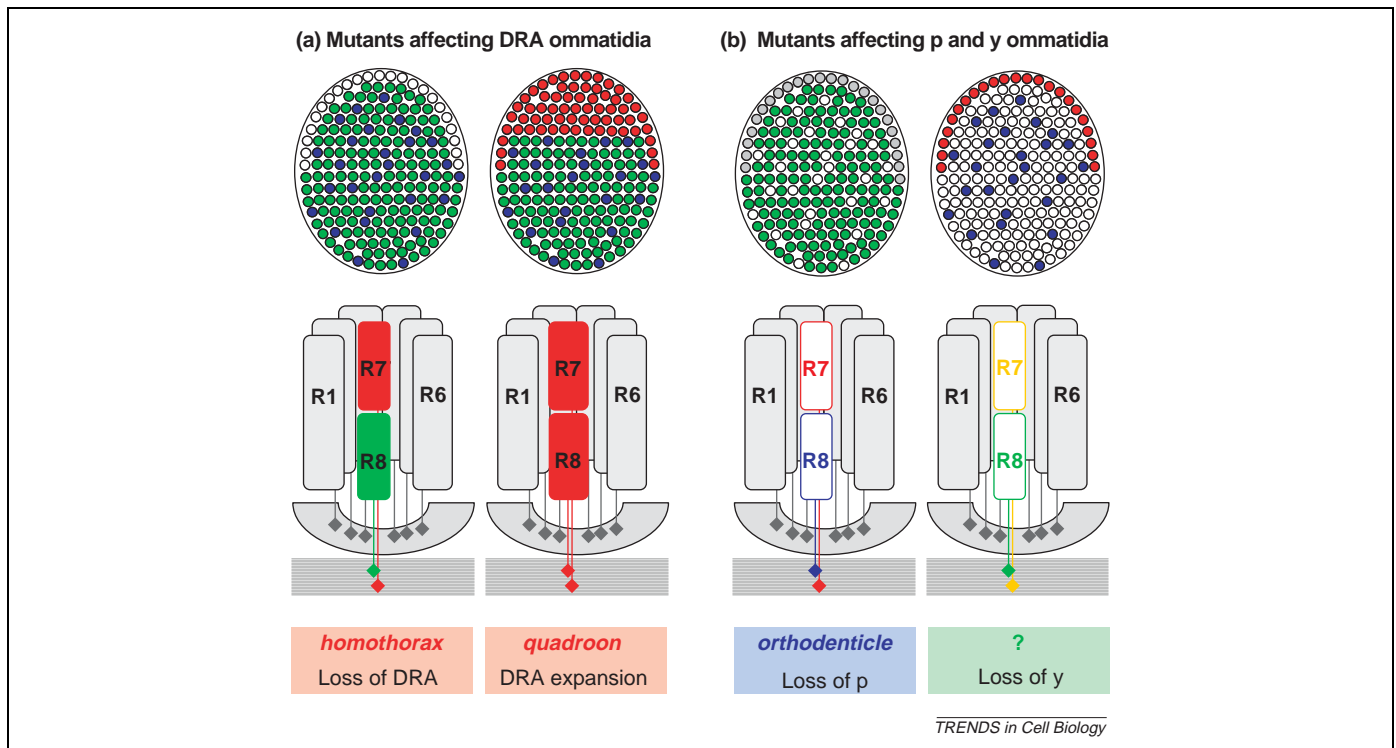


Figure 3. Mutations affecting specification of ommatidial subtypes. **(a)** Mutations affecting the dorsal rim area (DRA) ommatidia. Loss of the gene *homothorax* (*hth*), which encodes a homeodomain transcription factor, leads to the loss of the DRA ommatidia (top left). *hth* is expressed specifically in R7 and R8 cells in the DRA (not shown) and is also sufficient to induce DRA development. Loss of *hth* leads to a loss of both DRA morphology and typical DRA-type expression of opsin in R7 and R8 (bottom left). The DRA is expanded in *omb^{quadroon}* mutants of the *optomotor-blind* (*omb*) locus (top right). An unusually high number of ommatidia in the dorsal half of the eye adopts the DRA fate, expressing DRA-type Rh3 in R7 and R8 (bottom right). **(b)** Mutants affecting specification of pale (p) and yellow (y) ommatidia. Eye-specific mutants of the gene *orthodenticle* (*otd*), which encodes a K₅₀ homeodomain transcription factor, show a specific loss of p ommatidia (top left). Both p opsins Rh3 and Rh5 are lost (bottom left). Notably, y ommatidia do not increase in number, consistent with the observation that *otd* directly binds to and activates the *rh3* and *rh5* promoters. DRA ommatidia (top left, shown in gray) are indirectly affected because they usually express the R7 opsin Rh3. Mutants manifesting the specific loss of y ommatidia (right) have not yet been identified. Such mutants would enable the stochastic choice between p and y ommatidia to be studied in greater detail.

(*IRO-C*) complex are specifically expressed in the dorsal half of the developing eye [27] (Figure 4a) and are therefore good candidates for providing this positional information. Indeed, overexpression of any of the three *IRO-C* genes in all PRs leads to the induction of DRA ommatidia around the whole eye margin [25,26]. A combination of the Wg and *IRO-C* signals therefore seems to be sufficient to restrict specification of DRA ommatidia to the right place in the eye.

The downstream effector of the Wg and *IRO-C* pathways has recently been identified: the gene *homothorax* (*hth*) is necessary and sufficient to mediate the DRA fate in response to these signals [25,26]. *hth* encodes a homeodomain transcription factor that is a cofactor for *Hox* genes and that is essential for the correct development of *Drosophila* appendages [28]. In the adult eye, Hth is specifically expressed in R7 and R8 cells of the DRA. Loss of *hth* leads to a loss of the characteristic morphology of the DRA polarization sensors (a decrease in the rhabdomere diameter of inner PRs) and to a loss of the typical DRA-type expression of Rh3 in both R7 and R8 (compare Figures 1e and 3a).

Ommatidia of the DRA lacking Hth function manifest an unusual coupling of Rh3 in R7 and Rh6 in R8 (Figure 3a, bottom left). These ommatidia can, therefore, be viewed as having reverted to unusual color-sensitive PRs that are observed in a small proportion (~7%) of naturally occurring ommatidia in wild type [29]. Ectopic

expression of Hth leads to the transformation of all ommatidia into the DRA fate, in which all R7 and R8 cells express Rh3 and show an increase in rhabdomere diameter [25], providing clear evidence that Hth is a key regulator that can induce DRA cell-fate changes.

Another mutant that affects the specification of DRA ommatidia provides further insight into the regulatory network involving Wg and *IRO-C* [26]. The Quadroon phenotype is caused by to a gain-of-function mutation in the *optomotor-blind* (*omb*) locus [30]. *omb* encodes a T-box transcription factor that is important both in the development of *Drosophila* appendages and in the establishment of planar polarity in the abdomen and acts downstream of Wg [31–33]. *omb^{quadroon}* flies show a marked expansion of DRA ommatidia that is very reminiscent of overactivation of the Wg pathway, although the DRA does not extend through the whole dorsal eye [26] (Figure 3a, top right). *omb* might, therefore, be important in transducing the effect of Wg onto the DRA ommatidia. The DRA ommatidia therefore provide another powerful model system with which to study the Wg/Wnt signal transduction pathway in greater detail [26].

Two simple mutant backgrounds that affect the initial cell-fate specification of individual inner PRs have been used to build a mechanistic model of the localized specification strategy used in the DRA. For example, in ommatidia lacking R7 cells (*sevenless* mutants), R8 cells always choose the DRA fate correctly (i.e. they have

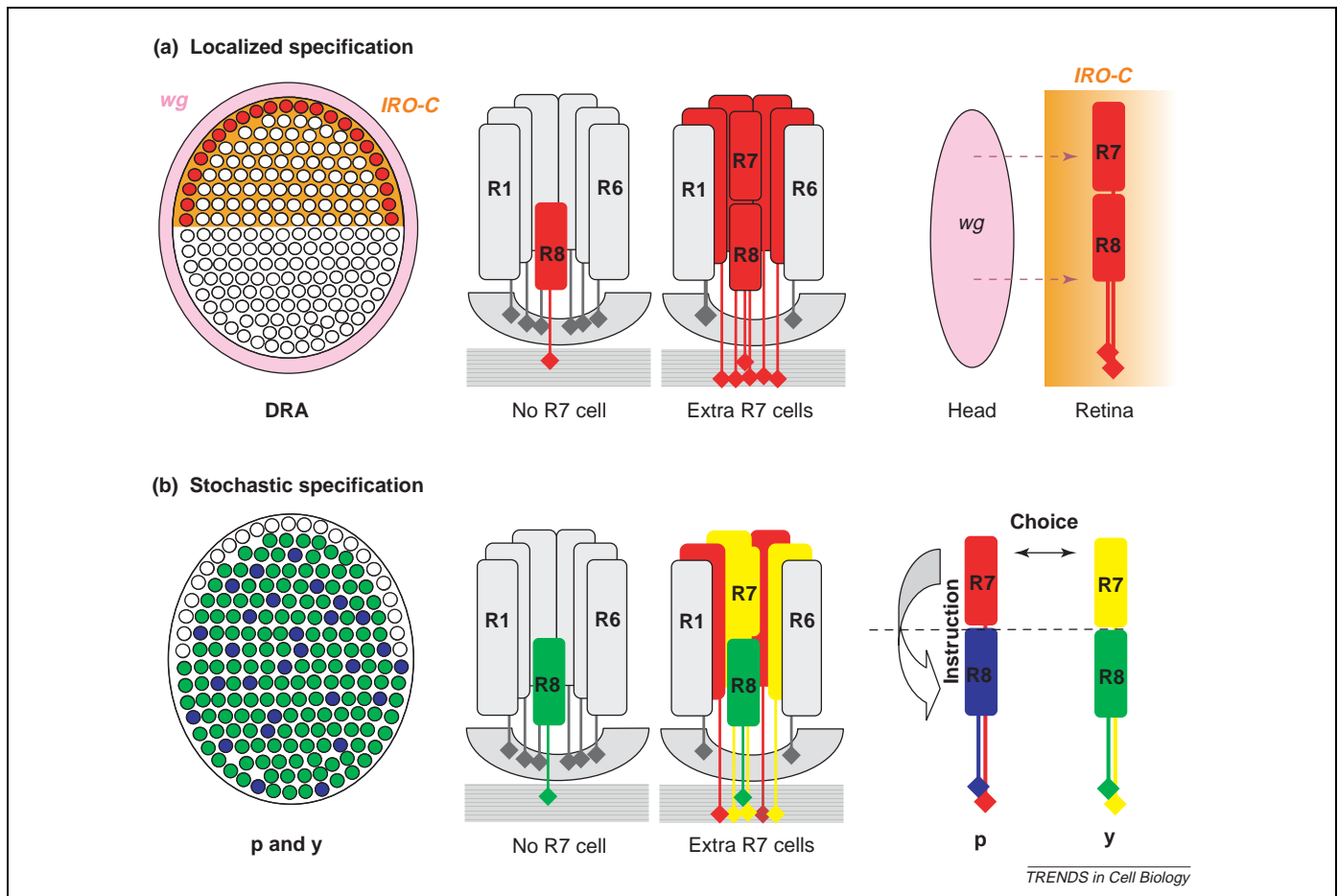


Figure 4. Two different strategies to specify ommatidial subtypes. **(a)** Localized specification. Dorsal rim area (DRA) ommatidia (red) are specified along the dorsal head cuticle (left). The morphogen *wingless* (*wg*; pink) is expressed in the head cuticle all around the eye and can induce DRA ommatidia. Furthermore, the genes of the *Iroquois* complex (*IRO-C*; orange), expressed specifically in the dorsal eye, provide important positional information for DRA specification. Different mutant backgrounds can be used to elucidate the mechanism of DRA specification (middle). In *sevenless* mutants, which lack all R7 cells, and in mutants in which several R7 cells form per ommatidium (*seven-up*⁻ or Ras-activation mutants), inner photoreceptor cells (PRs) always choose the DRA fate when in close proximity to the dorsal head cuticle. Therefore, R7 and R8 seem to choose the DRA fate cell-autonomously when under the influence of a short-range signal such as *wg* (right). **(b)** Stochastic specification. Pale (p) and yellow (y) ommatidia are distributed stochastically but unevenly (left) throughout the retina (p and y are shown in blue and green, respectively). In the absence of R7 cells (*sevenless* mutants), R8 cells always express Rh6 ('default state'), whereas supernumerary R7 cells (*seven-up*⁻ or Ras-activation mutants) choose randomly between Rh3 and Rh4 expression (middle). A two-step model of p or y specification is shown on the right: first, R7 cells choose randomly between p and y fates (choice); second, R7 cells impose their subset choice onto the underlying R8 cells (instruction). In the absence of the second step, R8 cells are caught in their 'default state' (*sevenless* mutants).

enlarged rhabdomeres and express Rh3) but only if they are located in close proximity to the dorsal head cuticle [25] (Figure 4a). Reciprocally, extra R7 cells, induced by different genetic manipulations (e.g. a mutation in *seven-up* or constitutive activation of the Ras pathway) always acquire the DRA fate but only at the dorsal rim (Figure 4a).

It therefore seems that both inner PRs can choose to acquire the DRA fate autonomously when they are located close enough to the dorsal head cuticle. These observations fit very well with the localized specification model involving *Wg* or a signal downstream of *Wg*, emanating from the developing dorsal head (Figure 4a, right). Recent data indeed suggest that *Wg* might not directly induce the DRA fate in R7 and R8, but rather that it requires a second, diffusible downstream factor [26].

Stochastic specification: p and y ommatidia

Outside the DRA, pale (p) and yellow (y) ommatidia are distributed stochastically through the retina in a ratio of 30:70. This distribution is in stark contrast to the highly localized position of the DRA. How apparent randomness

occurs in a fixed ratio is an area of interest to many investigators. The gene *orthodenticle* (*otd*) has been recently shown to be indispensable for the correct specification of the p subtype [34]. From *Drosophila* to mice, *otd* has a highly conserved role in specifying the anterior part of the early embryo [35,36]. Loss of *otd* in the eye leads to the specific loss of Rh3 and Rh5 expression (Figure 3a, bottom left), as well as to an expansion of Rh6 expression into outer PRs (not shown for simplicity), suggesting that *Otd* acts as both an activator of *rh3* and *rh5* and a repressor of *rh6*.

otd encodes a 'K50' homeodomain transcription factor that carries a lysine at position 50 of the DNA recognition helix, resulting in highly specific DNA binding that is distinct from that of most other homeodomain proteins. It can bind specifically to conserved, so-called 'K50 recognition sequences', present only in the *rh3*, *rh5* and *rh6* promoters, and can mediate activation of *rh3* and *rh5* and repression of *rh6* *in vivo*. This late role in PR differentiation is highly reminiscent of the role of one of the four homologs of *Otd* in vertebrates, the cone-rod-specific

homeobox transcription factor (Crx). Mutations in Crx have been identified as the cause of some forms of cone-rod dystrophy, in which opsin gene expression in PRs is strongly affected [37,38]. The function of Otd/Crx in eye development is thus highly conserved.

Because Otd is expressed in all adult PRs [37,39], specific cofactors must contribute to restrict its activity to R7 and R8 for the activation of *rh3* or *rh5*, and other factors might cooperate in outer PRs for the repression of *rh6*. Loss of *otd* results in the apparent loss of the whole p subset of ommatidia by abolishing transcription of both *rh3* and *rh5*. However, expression of the y opsins *rh4* and *rh6* does not expand into the mutant p ommatidia, suggesting that initially the p and y ommatidia are correctly specified in *otd* mutants. Ommatidial subtype specification and opsin expression therefore seem to be two processes that can be dissociated. Neither the factors and pathways specifying ommatidial subtypes upstream of Otd nor the factors specifying the y subset are currently known (Figure 3b, right).

To explain the distribution of M and L cones in humans, an elegant model involving the stochastic choice of the L or M opsin promoter by a single upstream locus control region has been proposed [11]; however, it is still not clear how stochastic choices are made between other PR cell fates in humans or in flies. In particular, although R7 and R8 seem to choose to become DRA PRs in a cell-autonomous manner, R7 and R8 cells of the p and y ommatidia clearly depend on each other for the decision. The same mutant backgrounds described above can be used to build a mechanistic model of the stochastic specification strategy used by the fly to establish the p and y ommatidia.

It seems that outside the DRA, PRs are under the influence of fundamentally different instructive signals (Figure 4b): in the absence of R7 cells (*sevenless* mutants), R8 cells always express Rh6, the opsin associated with y ommatidia [29]. Therefore, it has been proposed that Rh6 represents the 'ground state opsin' that is expressed in R8 (Figure 4b,c) and that a signal from R7 cells that have chosen to express Rh3, the opsin associated with p ommatidia, is necessary for R8 to acquire the p fate and express Rh5. Moreover, Chou *et al.* [29] have generated adult ommatidia lacking R8 cells and found that both Rh3 and Rh4 were expressed randomly in the R7 cells of these retinas, suggesting that R8 is not necessary for the stochastic choice to occur in R7 cells.

In support of this model, in genetic backgrounds that lead to extra R7 cells within one ommatidium, the extra R7 cells choose randomly between the p and y fates [29] (Figure 4b). Thus, the model of the stochastic specification of p and y ommatidia that can be drawn from these experiments is very different from the localized strategy described above for the DRA: the stringent coupling of R7 and R8 opsins seems to be achieved in two steps. First, the stochastic, but biased choice between p and y fates seems to be made in R7 cells, and this decision is then imposed on the underlying R8 cells (Figure 4b, right). The result is the fly color vision system: a mosaic of two ommatidial subsets in which the R8 cells that show highest spectral sensitivity

in the blue (p) or green (y) part of the spectrum compare their inputs with UV-sensitive R7 cells.

Retinal patterning combines different specification strategies

In many species, the retinal mosaic is the result of a nearly perfect adaptation to the challenges that the environment imposes on an animal. For example, one necessity is the regional specification of some parts of the retina: patches of retinal tissue that show an optimal exposure to an important stimulus develop a heightened sensitivity to it. Examples of such localized specifications are the fovea in some vertebrates that are used when the subject fixes on an object, and the DRA ommatidia in *Drosophila* that face the sky to interpret reflected polarized light.

Specification of such parts of the retina requires both the use of short-range patterning mechanisms, for example the Wg/Wnt morphogens, and genes that provide positional information in the retina (such as the *Drosophila* *IRO-C* genes). But, because the animal also depends on additional visual stimuli and must therefore 'multitask', it is probably more effective to limit the expanse of such highly specialized retinal regions. This is obvious, for example, in the very limited surface area of the DRA in most insects.

Both humans and *Drosophila* use an approach to distribute PRs with different spectral sensitivities stochastically because only a comparison between their inputs will enable effective discrimination between colors. Interestingly, although the distribution is stochastic, there are biases in the proportion of the different subtypes: for example, there are fewer S cones in the human fovea and fewer p ommatidia in the fly mosaic, presumably to accommodate their function of absorbing shorter wavelengths.

Concluding remarks

The retinal mosaic of *Drosophila*, similar to that of vertebrates, reflects a set of complex events that lead to the specification of different types of PR in each adult ommatidium (inner versus outer PRs, R7 versus R8) and to three subtypes of ommatidia in the retina (p versus y versus DRA). The data discussed here already point towards a model in which the fate of a given PR becomes increasingly restricted by a series of consecutive cell-fate decisions accomplished through the recruitment of transcription factors. Further genetic experiments, as well as the precise time course of expression of the genes involved in this process, will facilitate a detailed description of their epistatic relationship.

For example, both *pros* and *hth* expression are lost in a retina lacking *spalt* function, suggesting that establishment of the inner PR fate by *spalt* is necessary for all further ommatidial specification steps to occur [10,25,40]. The vertebrate retina similarly seems to use related strategies. Although the initial recruitment of retinal cells follows a different strategy in which multipotent progenitor cells are restricted in their fate by both intrinsic and extrinsic events, it is clear that a cascade of transcription factors can sequentially refine the fate of cells from a multipotent fate to a highly differentiated

state. The example of rods cells best illustrates this: rod cells are distinguished from the 'ancestral' cone fate by the gene *Nrl* [41], thereby showing a similar function to that of *spalt*, which distinguishes inner (cone-like) from outer (rod-like) PRs in *Drosophila*. As mentioned above, Crx in mammals also has a role similar to that of Otd in controlling *rh* gene expression in a subset of PRs. Finally, the regionalization of the fly retina by Hth can be compared to the function of the thyroid hormone receptor TR2 β , which might receive a diffusible signal (thyroid hormone rather than Wg), to affect the distribution of S and M cone subclasses along the dorso-ventral axis of the retina in mice [42].

Many animals generate their retinal mosaic by combining localized and stochastic specification strategies, as has been shown in some detail for *Drosophila*. Nevertheless, it should be also noted that some species form particularly stereotypical PR mosaics by purposely avoiding stochastic or localized mechanisms. The retina of most fishes, for example, is organized as a very regular lattice of the different types of cone. Furthermore, salmonid fish have the ability to considerably reorganize their retinal mosaic with developmental time. It has been shown that sexually mature salmonids regenerate their previously lost UV-sensitive cones and therefore their ability to detect polarized light (for review, see Ref. [43]). This represents a striking example of the metamorphosis of a retinal mosaic as the animal enters a new developmental phase: to reproduce, the animals must leave the ocean and migrate back to their place of birth, a task for which they have to regain an increased ability to navigate.

It seems that the instructive and permissive cell-fate choices underlying the localized versus the biased stochastic choice between DRA, p and y ommatidial fates in *Drosophila* represent fundamental neurobiological problems. A more complete understanding of the fly retinal mosaic might, therefore, enable us to get a clearer view on how our own retina helps us to enjoy our environment with all its shapes and colors.

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