Development 140, 493-503 (2013) doi:10.1242/dev.079095

Establishing and maintaining gene expression patterns: insights from sensory receptor patterning

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Summary

In visual and olfactory sensory systems with high discriminatory power, each sensory neuron typically expresses one, or very few, sensory receptor genes, excluding all others. Recent studies have provided insights into the mechanisms that generate and maintain sensory receptor expression patterns. Here, we review how this is achieved in the fly retina and compare it with the mechanisms controlling sensory receptor expression patterns in the mouse retina and in the mouse and fly olfactory systems.

Key words: Hippo pathway, Cell identity maintenance, Olfactory receptor, Opsin, Photoreceptor, Sensory

Introduction

Multicellular organisms are able to perceive and discriminate a broad range of environmental stimuli within a number of sensory modalities. To achieve this, the visual and olfactory systems deploy large numbers of sensory receptors (SRs). For example, five Rhodopsin genes are differentially expressed in the fly retina (Rister and Desplan, 2011), while over 1200 olfactory receptor genes are expressed in the nose of the mouse (Buck and Axel, 1991). In sensory systems of high discriminatory power, each sensory neuron generally expresses only one or very few SR gene(s), excluding all others (Mazzoni et al., 2004; Mombaerts, 2004; Serizawa et al., 2004). Importantly, the choice of expressing a particular SR determines the identity and response spectrum of the sensory neuron. Thus, each sensory neuron faces two regulatory challenges during its terminal differentiation: it first has to make an unambiguous choice of SR expression, and it must then maintain this decision throughout its lifespan. Failure in either case would compromise the ability of the sensory system to discriminate between stimuli. In this Primer (see Box, Development: the big picture), we summarize our current understanding of the mechanisms governing SR patterning in the fly retina, one of the best-understood systems for SR gene choice. We then compare these regulatory mechanisms with those used in the mouse retina and in the fly and mouse olfactory systems.

The *Drosophila* eye as a model system for sensory neuron differentiation

The compound eye of *Drosophila melanogaster* consists of ~800 unit eyes called ommatidia (Fig. 1A), each of which contains ~20 cells, including the cornea, support and pigment cells, as well as eight photoreceptor cells (PRs; R1-R8) (Fig. 1B,C) (Hardie, 1985; Fischbach and Dittrich, 1989). The specification of PRs and the assembly of ommatidia is initiated during the late larval stages of development (Tsachaki and Sprecher, 2012). During pupal development, PRs differentiate into distinct subtypes and undergo considerable morphological changes that result in the formation of specialized subcellular compartments called rhabdomeres (Fig. 1B-E). The rhabdomeres are rod-like structures, which contain Rhodopsins (Rhs), light-sensitive G protein-coupled receptors (GPCRs) that define the spectral sensitivity of the PR (Fig. 1D,E). Absorption of photons by Rhs activates the G protein $G\alpha_q$ and triggers the phototransduction machinery, which transforms Rh activity into electrical signals (Hardie, 2001; Montell, 2012).

Five Rh proteins – Rh1 (NinaE – FlyBase), Rh3, Rh4, Rh5 and Rh6 – with different spectral sensitivities are expressed in two classes of PRs in the *Drosophila* retina (Rister and Desplan, 2011). The 'outer' PRs (R1-R6) span the length of the ommatidium and express Rh1, which has a broad sensitivity to blue-green wavelengths (Fig. 1B-D; Fig. 2A) (O'Tousa et al., 1985; Zuker et al., 1985). These outer PRs are required for dim light vision, similar to vertebrate rods, and mediate motion vision as well as orientation behavior (Heisenberg and Buchner, 1977; Yamaguchi et al., 2008). In the center of each ommatidium are the rhabdomeres of two types of 'inner' PRs: R7 and R8 (Fig. 1B-E). They are positioned above one another (R8 is proximal to R7), thus sharing the same light path. For each R7/R8 pair, the choice of Rhs expressed in each PR is coupled and thereby defines two main subtypes of ommatidia (Fig. 2A): 'pale' (p) ommatidia contain UV-sensitive Rh3 in R7 and blue-sensitive Rh5 in R8 (Fig. 1E), whereas 'yellow' (y) ommatidia express an Rh that is sensitive to longer UV wavelengths (Rh4) in R7 and the green-sensitive Rh6 in R8. These two subtypes of ommatidia (p and y) are stochastically distributed in a roughly 30:70 ratio throughout most of the retina (Fig. 2B,C) and are involved in wavelength discrimination and color vision (Menne and Spatz, 1977; Gao et al., 2008; Yamaguchi et al., 2010).

Two additional subtypes of ommatidia are present specifically in the dorsal region of the eye. At the dorsal margin of the eye, the so-called dorsal rim area (DRA) ommatidia contain R7 and R8 PRs with enlarged rhabdomeres that both express Rh3 and mediate polarization vision (Wernet et al., 2003; Wernet et al., 2012). Furthermore, in the dorsal third of the eye y ommatidia are modified (located above the dotted line in Fig. 2B): they contain R7s that co-express Rh3 and Rh4, whereas R8s express only Rh6. It has been suggested that these 'dorsal y' ommatidia enhance UV detection, which is used for navigation (Mazzoni et al., 2008; Stavenga and Arikawa, 2008).

These well-defined Rh expression patterns and the wealth of genetic tools available in *Drosophila melanogaster* allow a detailed

Development: the big picture

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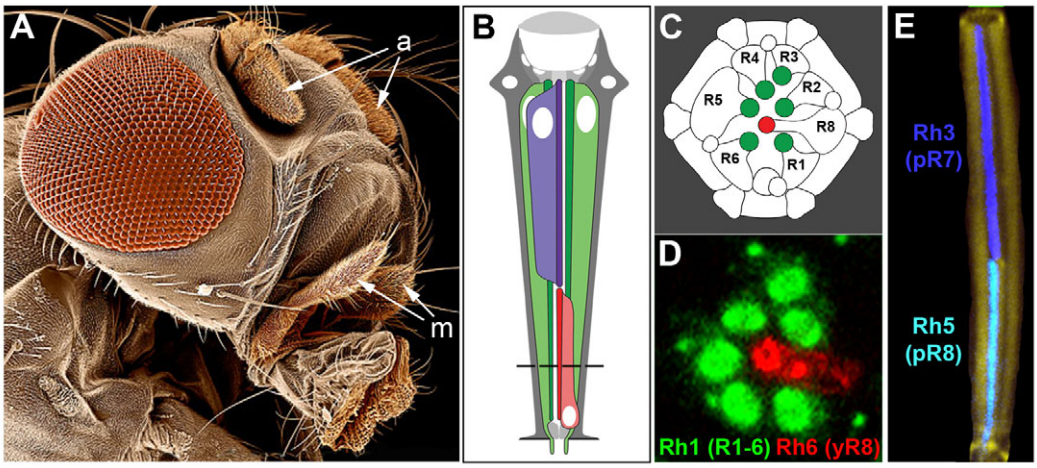

Fig. 1. Structure of the *Drosophila* eye. (**A**) Scanning electron microscope image of a *Drosophila melanogaster* head. The compound eye (pseudocolored in red) is composed of ~800 unit eyes, the ommatidia, each covered by its own lens (visible on the surface of the eye) that focuses light on the photoreceptors below. The third segments of the antennae (a) and the maxillary palps (m) are covered with sensilla, which are hair-like structures that house the olfactory receptor neurons. (**B**) Schematic side view of an ommatidium (unit eye). Under the lens (top) reside eight photoreceptor neurons (PRs R1-R8; only four are shown, colored). Each PR has a rod-like, subcellular specialization, the rhabdomere (darker colors), loaded with Rhodopsin proteins, which detect photons. Rhabdomeres of 'outer' PRs (green, only two of six are shown) span the entire length of the ommatidium. The rhabdomeres of the 'inner' PRs are located above each other, with R7 (violet) distal to R8 (red), such that they share a common light path and face the same point in space. (**C**) Schematic of a section through an ommatidium at the level indicated by the dashed line in B. Rhabdomeres (colored circles) belonging to the 'outer' PRs (R1-R6, green) form a trapezoid configuration, whereas the rhabdomere of the 'inner' PRs (R8 in this section, in red) is in the center of the trapezoid. (**D**) Image corresponding to the schematic in C showing expression of Rh1 (green) in R1-R6 PRs and Rh6 (red) in R8. (**E**) A longitudinal view of rhabdomeres within a single ommatidium. The 'inner' rhabdomeres contain Rh3 (blue) and Rh5 (turquoise) belonging to R7 and R8 PRs, respectively. The 'outer' rhabdomeres (brown) were visualized using an actin counterstain. The image shown in A was obtained with permission from Clouds Hill Imaging. Schematics shown in B and C were adapted with permission (Cagan and Ready, 1989).

examination of two general questions in sensory receptor patterning: (1) What are the terminal cell-fate decisions that generate PR subtypes and identities, as defined by the expression of particular Rhs? (2) How are the functional identities of these subtypes maintained throughout the life of the fly?

Establishing Rhodopsin expression in the *Drosophila* eye: early cell fates are integrated with stochastic and positional inputs

A number of studies have shown that specification events in the late larval and early pupal stages of development establish R1-R8 PR identities that lead to the expression of distinct combinations of transcription factors (TFs). These TFs play a part in controlling terminal PR differentiation by restricting the Rh genes that each PR can express (reviewed by Rister and Desplan, 2011). As summarized above, the outer PRs R1-R6 exclusively express Rh1, whereas R7s can express Rh3 and/or Rh4, and R8s can express either Rh5 or Rh6 (Mollereau et al., 2001; Cook et al., 2003; Tahayato et al., 2003; Johnston et al., 2011). Positive regulation of Rh expression is largely mediated by TFs that are expressed in all PRs. The homeodomain TF Otd (Oc – FlyBase) activates the expression of *Rh3* and *Rh5* (Tahayato et al., 2003), whereas the

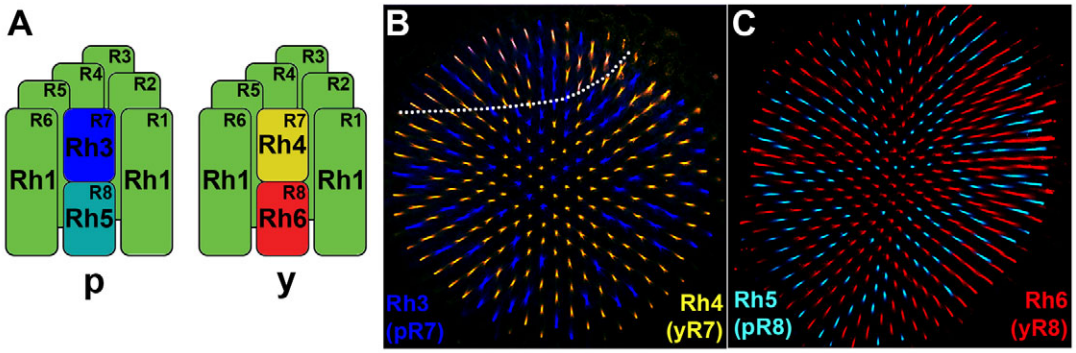

Fig. 2. Subtypes of ommatidia in the *Drosophila* retina. (**A**) Two main subtypes of ommatidia are distinguished in the adult retina by coupled Rhodopsin (Rh) expression in the inner photoreceptors (PRs): in the p subtype, pR7 expresses Rh3 and pR8 expresses Rh5; in the y subtype, yR7 expresses Rh4 and yR8 expresses Rh6. All outer PRs express Rh1. (**B**,**C**) The two ommatidial subtypes are stochastically distributed in a roughly 30:70 ratio (p:y). A section at the R7 level (B) shows p and y R7s expressing Rh3 (blue) and Rh4 (yellow), respectively. The 'dorsal y' R7s (white, located above the dotted line) co-express Rh3 and Rh4. A section at the R8 level (C) shows mutually exclusive expression of Rh5 (turquoise) and Rh6 (red) in p and y R8s, respectively.

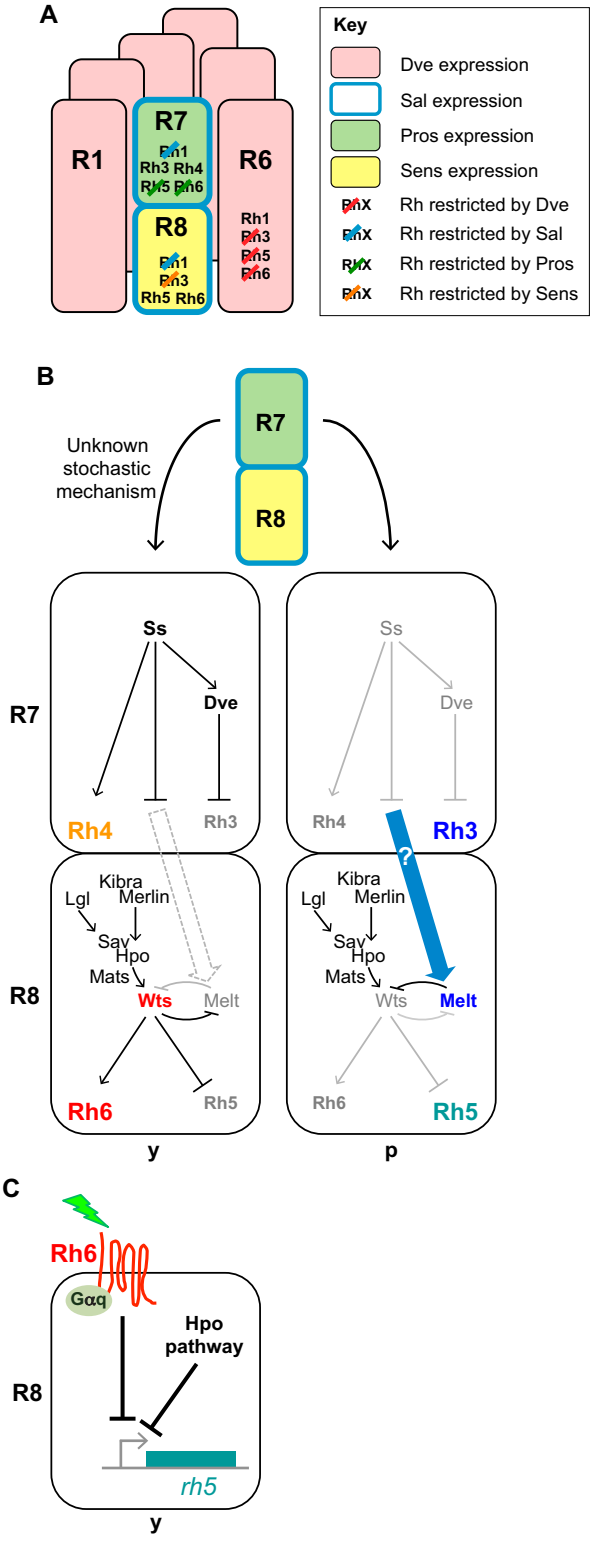

Fig. 3. The control of Rhodopsin expression patterns in *Drosophila*. (**A**) Transcriptional repressors restrict Rhodopsins to specific photoreceptor (PR) types. Defective proventriculus (Dve), which is expressed in outer PRs (R1-R6), represses *Rh3*, *Rh5* and *Rh6* to allow exclusive expression of *Rh1* in outer PRs. Spalt (Sal) is expressed in the inner PRs (R7 and R8), where it represses *Rh1*. Finally, Prospero (Pros) prevents expression of R8 opsins (*Rh5* and *Rh6*) in R7s, whereas Senseless (Sens) prevents expression of *Rh3* in R8. These mechanisms thereby restrict Rh3 expression to R7, whereas R8 can only express Rh5 and Rh6. (**B**) The two main PR subtypes (p and y) are specified by a stochastic decision made independently in each R7. Stochastic Spineless (Ss) expression in ~70% of R7s (left) induces Rh4 and Dve expression and determines yR7 fate. Dve, in turn, represses *Rh3*, allowing exclusive *Rh4* expression in yR7. The other 30% of R7s (right) lack Ss and therefore acquire p identity and exclusively express *Rh3*. The coordination of p and y identities in R7 and R8 in each ommatidium is achieved by an unknown signal (blue arrow, right) sent by pR7. Ss represses this signal in yR7 (left), and in the underlying R8 the constitutively active Hippo (Hpo) tumor suppressor pathway specifies y identity by default. Warts (Wts), the nexus of the Hpo pathway, represses *melted* (*melt*) and *Rh5*, and promotes *Rh6* expression. The pR7 signal instructs the underlying R8 to become pR8 (right), by inducing *melted* expression. Melted represses Warts expression, thereby disrupting Hpo pathway activity and allowing exclusive expression of Rh5. (**C**) The exclusion of Rh5 in yR8s requires two mechanisms: continued Hpo pathway activity (as shown in B) and negative feedback from Rh6 itself. This feedback also involves $G\alpha_q$, but not the other components of the canonical phototransduction pathway.

homeodomain TF Pph13 activates *Rh6* expression (Mishra et al., 2010). Repressive mechanisms then play a key role in restricting Rh expression to specific PR subtypes (Fig. 3A). The homeodomain TF Defective proventriculus (Dve) (Johnston et al., 2011) is strongly expressed in outer PRs, where it represses the expression of the R7 and R8 Rh genes (*Rh3*, *Rh5* and *Rh6*), most likely by binding to their promoters (Fortini and Rubin, 1990; Tahayato et al., 2003; Johnston et al., 2011), leading to exclusive expression of *Rh1*, presumably mediated by Eyeless (also known as Pax6) (Sheng et al., 1997). The zinc finger TF Spalt (Sal; Salm/Salr – FlyBase) is expressed in R7s and R8s (Mollereau et al., 2001) and prevents expression of *dve* and *Rh1* in inner PRs. The homeodomain TF Prospero (Pros) is expressed in R7s and represses the R8 Rh genes *Rh5* and *Rh6* probably by binding to their promoters (Cook et al., 2003), whereas the zinc finger TF Senseless (Sens) expressed in R8s represses the R7 Rh gene *Rh3* (Xie et al., 2007). These combinatorial mechanisms restrict Rh3 expression to R7s, whereas Rh5 and Rh6 can only be expressed in R8s.

The TF network described above provides the context within which the p and y subtypes are established through a binary stochastic decision made independently by each R7 (Fig. 3B). Stochastic expression of the PAS domain-containing basic helix-loop-helix (bHLH) TF Spineless (Ss) in ~70% of R7s induces yR7 fate and expression of *Rh4* (Wernet et al., 2006). Ss also induces *dve* in yR7s to repress expression of *Rh3*, thus yielding exclusive expression of *Rh4* in yR7s. The remaining 30% of R7s, which lack Ss, acquire p identity and exclusively express *Rh3*. The resulting Rh3-Rh4 pattern in R7s is random (Fig. 2B) and differs from individual to individual and between the two retinas in the same animal (Bell et al., 2007), resembling the pattern of human long and medium-wavelength sensitive cone PRs (Roorda and Williams, 1999).

The two additional ommatidial subtypes in the dorsal eye region are generated by positional cues. First, TFs of the dorsally expressed Iroquois Complex (IroC) oppose repression of *Rh3* by Dve in ‘dorsal y’ ommatidia and thereby induce co-expression of *Rh3* and *Rh4* (Fig. 2B) (Mazzoni et al., 2008; Johnston et al., 2011). Second, DRA ommatidia are determined by the expression of the homeodomain TF Homothorax (Hth) in R7 and R8 PRs at the dorsal margin of the eye (Wernet et al., 2003). Hth is necessary and sufficient to induce enlarged rhabdomeres and expression of

Rh3 in both R7 and R8. Expression of Hth appears to be induced by the intersection of two positional cues: the head capsule cells that surround the eye signal to the narrow band of DRA ommatidia via secreted Wingless, whereas the dorsally expressed IroC TFs direct Hth expression to the dorsal half of the eye (Tomlinson, 2003; Wernet et al., 2003).

Coordinating and consolidating mutually exclusive Rh expression patterns

A coordinating mechanism ensures that both R7 and R8 of a given ommatidium adapt the same p or y subtype identity (Fig. 3B); pR7s send a signal (yet to be characterized) that instructs the underlying R8s to become pR8s (Chou et al., 1996; Papatsenko et al., 1997; Chou et al., 1999). In the absence of the R7 signal, the constitutively active Hippo tumor suppressor pathway (see Box 1) specifies y identity in R8 and Rh6 expression by default. This Hippo pathway signaling is mediated by a 'core complex' composed of the Ser/Thr kinase Hippo, the scaffolding protein Salvador, the co-factor Mats and the Ser/Thr kinase Warts (Jukam and Desplan, 2011). Warts, the nexus of the pathway, is engaged in a double negative-feedback loop with Melted (Fig. 3B), a PH-domain protein implicated as a growth regulator in the Insulin/TOR pathway (Teleman et al., 2005). Warts is expressed in yR8s, where its activity represses expression of *melted* and *Rh5*, and promotes *Rh6* expression. In pR8s that have received the signal from pR7s, Melted is upregulated and represses *warts* expression, thereby interrupting the Hippo pathway (Jukam and Desplan, 2011). This triggers pR8 fate and exclusive Rh5 expression (Mikeladze-Dvali et al., 2005).

This post-mitotic role of the conserved Hippo pathway in terminal PR differentiation differs from its role in growth. For growth control, Warts activity is biochemically regulated by the upstream Hippo pathway and is tuned for optimal levels of activity. In the context of Rh regulation, however, the addition of reciprocal transcriptional repression with Melted assures a binary ('ON' or 'OFF') configuration and thereby consolidates a robust and unambiguous cell identity decision (Mikeladze-Dvali et al., 2005; Jukam and Desplan, 2011).

Maintaining photoreceptor subtype identity

The choice for expression of a particular Rh determines the spectral sensitivity of a PR. Because the connections with postsynaptic neurons are hard-wired (Sanes and Zipursky, 2010), each PR must maintain its choice for a particular Rh (as well as exclusion of other Rhs) to avoid misinterpretation of sensory information in the brain. In line with this hypothesis, mutually exclusive expression of Rh5 and Rh6 is maintained throughout the life of the animal (Vasiliauskas et al., 2011).

Two mechanisms have recently been identified that mediate the maintenance of PR subtype identity (Fig. 3C). First, Hippo pathway activity is continuously required in yR8s to maintain exclusion of Rh5 in adult flies (Jukam and Desplan, 2011); conditional inactivation of the upstream Hippo regulators Merlin or Lethal giant larvae in adult flies leads to de-repression of *Rh5* in yR8s. In addition, a second mechanism involves feedback from Rh6 itself to maintain exclusion of Rh5 (Vasiliauskas et al., 2011). In *Rh6* mutants, *Rh5* is gradually de-repressed in all yR8s as the fly ages. This exclusion pathway branches early from the phototransduction cascade, as only $G\alpha_q$, but not other canonical core components, are involved in repression of *Rh5*. Keeping wild-type flies in complete darkness causes partial de-repression of *Rh5* in yR8s, suggesting that this exclusion mechanism is activity dependent (Vasiliauskas et al., 2011). Therefore, in addition to detecting photons, Rh6 also contributes to maintenance of the functional identity of the PR through transcriptional repression of an alternative Rh gene (*Rh5*; Fig. 3C).

Surprisingly, de-repression of *Rh5* in the absence of Rh6 activity occurs without switching of cell fates (from yR8 to pR8) (Vasiliauskas et al., 2011), as the mutually regulated factors Warts and Melted are maintained in their original subtypes. As a result, Warts and Rh5 are co-expressed in yR8s. This implies that the early specification mechanisms (Warts or Melted expression) can be separated from the later-acting maintenance mechanisms (Rh6 feedback).

Box 1. The role of the Hippo pathway in growth

The precise regulation of growth and tissue size is a fundamental problem during organ development and regeneration. First discovered in *Drosophila*, the highly conserved Hippo signaling pathway plays a major role in growth control for both invertebrates and vertebrates (Pan, 2010; Halder and Johnson, 2011; Zhao et al., 2011; Staley and Irvine, 2012). At its core is a kinase cascade that involves the Ser/Thr kinases Hippo and Warts, the scaffold adapter protein Salvador and the Warts co-factor Mats. These four proteins were identified as tumor suppressors in genetic mosaic screens, as their loss-of-function phenotypes were massive overgrowth and decreased apoptosis (which serves to eliminate excessive cells). The Hippo pathway limits organ size by Warts-mediated phosphorylation, which prevents nuclear localization of the transcriptional co-activator Yorkie. Yorkie interacts with different transcription factors to activate target genes that promote proliferation and negatively regulate apoptosis. Inputs from multiple upstream branches, including cell polarity regulators and cell adhesion complexes, are integrated for the regulation of Hippo signaling.

Parallels with other sensory systems

Drosophila photoreceptors represent a simple model for a number of features that sensory systems might employ: (1) establishment of distinct sensory neuron subtypes defined by SR expression; (2) establishment of exclusive SR expression or, in a few cases, expression of a specific combination of SRs; (3) stochastic distribution and intermingling of neurons of different subtypes; (4) coupling of SR expression between different neurons; and (5) maintenance of the chosen SR expression throughout the life of the sensory neuron. Next, we highlight what is known about the mechanisms that underlie some of these features in the rodent retina, as well as in the fly and mouse olfactory systems.

Establishing opsin expression patterns in the mouse retina

Recently, significant progress has been made in understanding the mechanisms that regulate opsin expression in the rodent retina (Swaroop et al., 2010). Similar to the outer/inner PR distinction in *Drosophila*, vertebrates also have two main classes of PRs: rods and cones. Rods mediate dim light vision and express rod opsin. Cones, by contrast, detect light at higher intensities and are required for wavelength discrimination. Mouse and rat cones can express two opsins, the short wavelength-sensitive S opsin and the medium/long-wavelength sensitive M opsin, and, as such, can be classified as S cones and M cones, respectively. In rats, the retinal cone mosaic consists of 10% S cones and 90% M cones (Szél et al., 1994; Glaschke et al., 2011). In mice, however, the pattern is more complex (Fig. 4A,B) (Röhlich et al., 1994; Applebury et al., 2000; Haverkamp et al., 2005). M opsin is distributed in a

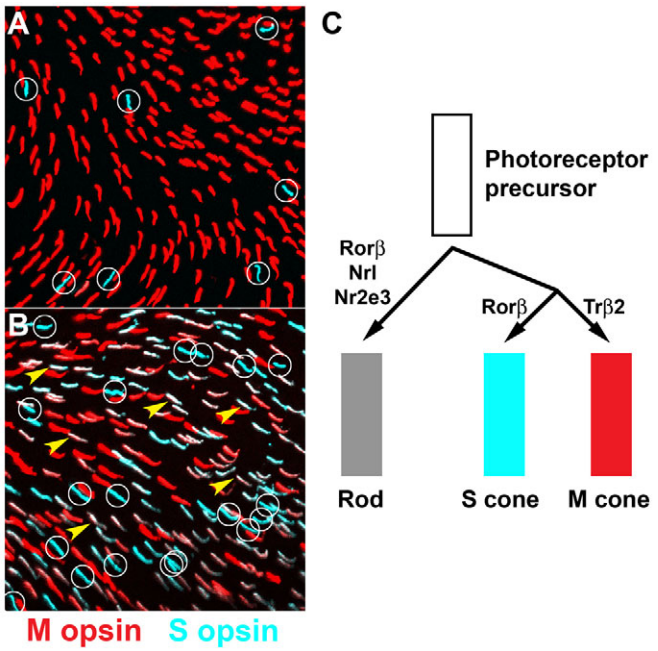

Fig. 4. Sensory receptor expression in the mouse retina. (**A**,**B**) Expression of S opsin (turquoise) and M opsin (red) in cones of the mouse retina (Haverkamp et al., 2005). In the dorsal retina (A), M opsin-expressing cones dominate, and only a few cones express S opsin (circled). In the ventral retina (B), cones that exclusively express S opsin are numerous (circled), but many co-express variable levels of M opsin (yellow arrowheads). (**C**) Cell-fate decisions in the mouse retina that lead to either rod or cone fate. Rorβ, Nrl and Nr2e3 promote rod fate and repress cone fate. In the absence of Nrl, two subtypes of cones are distinguished by expression of either Rorβ (S cone) orTrβ2 (M cone). Images in A and B were adapted with permission (Haverkamp et al., 2005).

dorsoventral gradient, whereas S opsin levels are highest in the ventral retina. Thus, in the dorsal region of the retina, the majority of cones are true 'M' cones that exclusively express high levels of M opsin, and only a minority of cones (3-5%) appears to express S opsin exclusively. As a result, the dorsal eye region exhibits distinct S and M identities, an arrangement that is found throughout the retina in many dichromatic mammals and therefore appears to correspond to the ancestral opsin configuration of the mammalian retina (Haverkamp et al., 2005). In the ventral part, only a minority of cones are genuine S cones, whereas the vast majority co-expresses high levels of S opsin with variable levels of M opsin (Applebury et al., 2000; Haverkamp et al., 2005; Nikonov et al., 2006). Thus, the ventral eye region in the mouse appears to be similar to the dorsal fly retina, which tolerates Rh co-expression in specialized 'dorsal yR7' PRs. The functional significance of opsin co-expression in cones, which is not common in mammals (Neitz and Neitz, 2001), is unclear.

Several regulators of rod, S cone and M cone identity are known (Fig. 4C). Rod fate is promoted by the neural retina leucine zipper TF Nrl, which, together with the nuclear receptor Nr2e3, also represses cone fate (Swaroop et al., 2010). In cones, S opsin expression appears to be the default state and requires RORβ (Srinivas et al., 2006). M opsin is induced by expression of the thyroid hormone nuclear receptor TRβ2 (THRB), which potentially acts in a heterodimer with the retinoid receptor RXRγ (Ng et al., 2001; Harpavat and Cepko, 2003; Roberts et al., 2006; Applebury et al., 2007). In TRβ2 mutants, M opsin is absent and all cones adopt S identity (Ng et al., 2001; Applebury et al., 2007). TRβ2 therefore not only promotes M identity, but also represses S opsin. However, it is not clear why this repressive mechanism is insufficient to prevent co-expression of S and M opsins in the ventral eye region. A recent study suggests that bone morphogenetic protein (BMP) signaling through COUP-TF nuclear receptors is involved in S and M opsin repression in the two eye regions (Satoh et al., 2009), but the mechanisms underlying this repression event are not completely understood.

Hence, in both fly and mouse retina, early cell fate decisions lead to PR types, and a network of TFs subsequently restricts opsin expression to PR subtypes. However, whereas there appears to be a clear separation between S and M identities in the dorsal mouse retina, similar to the p and y subtypes in the main region of the fly eye, this is not the case for most of the ventral part of the retina in mice. Moreover, the factors used for patterning in the two systems differ significantly, as exemplified by the predominant use of nuclear receptors in the mouse retina (Forrest and Swaroop, 2012).

Maintaining photoreceptor identity in the mouse retina

Once established, the S and M opsin expression patterns have to be maintained in the adult retina and this raises the issue of whether there are conceptual similarities to the mechanisms used in fly PRs. For instance, are the regulators that establish the M/S opsin expression pattern also involved in their maintenance, and do opsins themselves participate in this process?

As mentioned above, TRβ2 promotes M opsin expression and represses S opsin. Pharmacological suppression of its ligand thyroid hormone (TH) in adult mice leads to activation of S opsin and a concomitant reduction of M opsin in all cones (Glaschke et al., 2011). Similarly, this treatment also induces the expression of S opsin in M cones in rats, which usually do not exhibit co-expression (see above). Furthermore, the abnormal dominance of S opsin in athyroid *Pax8* mutant mice can be restored to a wild-type S/M pattern by TH treatment (Glaschke et al., 2010; Glaschke et al., 2011). These data show that continuous TH signaling is required for maintenance of M identity in mature cones.

Is an exclusion mechanism involving the expressed SR also present in the mouse retina? Mice in which the gene encoding S opsin (*Opn1sw*) has been knocked out have increased expression of M opsin in the ventral retina (Daniele et al., 2011), potentially indicating a repressive effect of S opsin on M opsin. However, as many cones already co-express S and M opsins in wild-type mice, the observed change in M levels might not be due to *de novo* transcription of the M opsin gene, but rather due to enhanced translation in the absence of potential competition with S opsin mRNA (Daniele et al., 2011). Therefore, it is not yet clear whether opsin cross-repression occurs in the mouse retina. The intriguing observation of increased M levels in an S opsin mutant needs to be investigated further using transcriptional reporters of M and S opsin expression. Furthermore, it would be interesting to test whether a complementary S opsin upregulation occurs in the dorsal eye region in M opsin mutants.

Establishing and maintaining olfactory receptor expression in *Drosophila*

In the fly, the olfactory receptor neurons (ORNs) are found on two pairs of organs located on the head: the third segments of the two antennae and the two maxillary palps (Fig. 1A). Similar to the fly visual system, most ORNs express a single olfactory receptor (OR) gene (Fuss and Ray, 2009) and OR expression is a key feature that

defines ORN classes, just as Rh expression defines PR subtypes. However, the OR number is significantly greater, as the *Drosophila* olfactory receptor repertoire consists of 62 members of the Or family (Clyne et al., 1997; Clyne et al., 1999b; Vosshall et al., 1999; Gao et al., 2000; Vosshall et al., 2000), three members of the gustatory receptor (Gr) family and ~15 members of the antennal ionotropic receptor (Ir) gene family (Jones et al., 2007; Benton et al., 2009; Silbering et al., 2011), which unlike opsins or mouse ORs are evolutionarily unrelated to the GPCR superfamily. The ORNs that co-express more than one OR express specific OR combinations that are established via two strategies: co-expressed OR genes can share common regulatory motifs, generally because these ORs are closely related, or they can be encoded by the same locus, which produces alternatively spliced mRNAs or mRNAs that carry two tandem open reading frames (Dobritsa et al., 2003; Goldman et al., 2005; Ray et al., 2007). Moreover, unlike photoreceptors, most ORNs that express OR gene family members also express a common co-receptor, Orco (also known as Or83b) (Larsson et al., 2004). Another feature of the fly olfactory system is that all ORNs with the same OR project axons to the same target: one of the ~50 glomeruli, which are subdivisions of the antennal lobes in the brain. The glomeruli in the fly are morphologically distinct and are arranged in a stereotypical manner that allows their identification by the position they occupy within the antennal lobe. The link between OR expression and target selection makes it essential for ORNs to maintain the expression of the chosen OR throughout the life of the SRN in order to prevent sensory confusion.

The dendrites of ORNs are housed in sensilla, hair-like structures on the surface of the antennae and maxillary palps (Fig. 1A). Each sensillum contains dendrites of one to four ORNs (Fig. 5A). Initially, the existence of different physiological classes of sensilla was discovered by recording their responses to odorants (Clyne et al., 1997; de Bruyne et al., 1999; de Bruyne et al., 2001; Yao et al., 2005). Subsequently, through the functional mapping of OR responses (Hallem et al., 2004), receptor expression studies and identification of the target glomeruli (Suh et al., 2004; Couto et al., 2005; Fishilevich and Vosshall, 2005; Goldman et al., 2005; Benton et al., 2009; Silbering et al., 2011) it was recognized that each sensillum contains a specific combination of ORN classes resulting in a relatively small number (<30) of sensillar subtypes. Thus, for example, each ab4 sensillum contains a stereotypical pair of one Or7a-expressing and one Or56a-expressing ORNs, which project to DL5 and DA2 glomeruli, respectively. No other sensillar subtype contains ORNs expressing either of these ORs or projects to the same glomeruli. Sensilla of different subtypes are intermingled and distributed in overlapping zones in antennae and maxillary palps (Fig. 5A). Thus, in summary, as in the fly retina, the *Drosophila* olfactory system establishes exclusive SR expression in most ORNs, coordinates OR expression within groups of two to four ORNs, generates a broad and intermingled distribution of sensillar subtypes and maintains OR expression for the life of the ORN.

What are the mechanisms that underlie these features of the *Drosophila* olfactory system? The emerging evidence suggests that OR expression is controlled by dedicated combinations of positively and negatively acting TFs (Clyne et al., 1999a; Ray et al., 2007; Ray et al., 2008; Tichy et al., 2008; Bai et al., 2009; Bai and Carlson, 2010; Jafari et al., 2012; Song et al., 2012). A small number of TFs has been identified that is required to specify expression of OR genes, but how the expression or activity of the TFs themselves is regulated resulting in the broad distribution and intermingled pattern of sensillar subtypes is less well understood. In contrast to the stochastic expression of the TF Ss in R7 PRs acting as the driving force, recent work suggests that ORNs achieve this distribution through pre-patterning by positional cues and subsequent cell migration (Song et al., 2012). First, the positional cues that pattern the developing antennal imaginal disc (the tissue that ultimately gives rise to the antennae and maxillary palps) direct establishment of intersecting TF expression domains. This leads to ORN precursors that express different combinations of TFs. Subsequently, the future ORNs disperse and intermingle through cell migration. Although some ORNs switch their TF status, no stochastic expression control needs to be evoked to explain the final sensillar distribution patterns (Song et al., 2012).

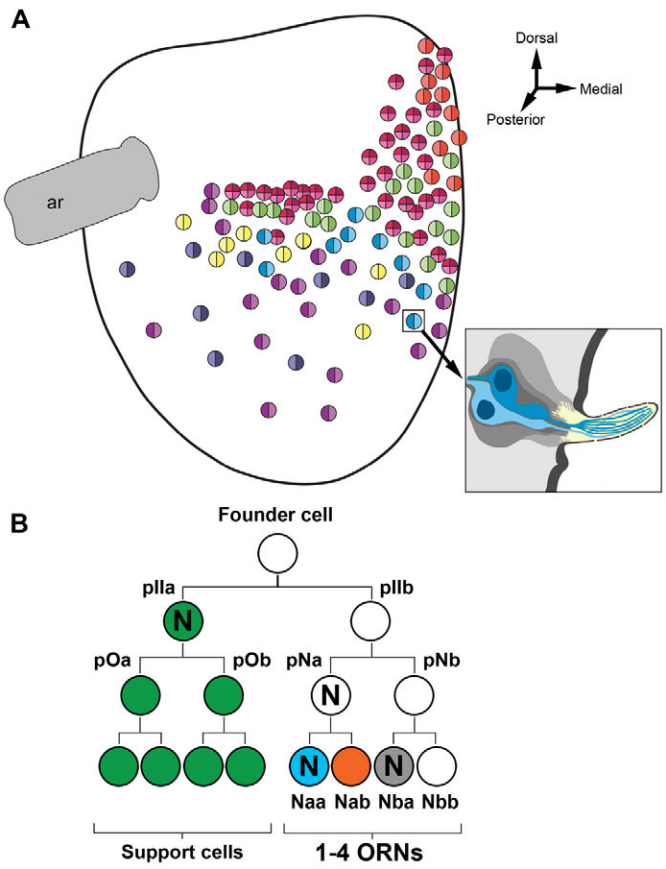

Fig. 5. Sensory receptor expression in the *Drosophila* antenna. (**A**) Schematic of the third antennal segment illustrating the distribution of seven classes of sensilla (represented by colored circles), which are intermingled, but also show zonal patterning (de Bruyne et al., 2001). Arrow points to a schematic of the side view of a two-neuron sensillum. The dendrites of the two olfactory receptor neurons (ORNs) (in two shades of blue) are encased in a hair-like external sensillum formed by support cells. ar, arista. (**B**) The cell lineage of a sensillum (Endo et al., 2012). A single founder cell undergoes three rounds of divisions producing support cells (green lineage) and neuronal cells. The diversity of ORNs in a sensillum arises from asymmetry of these divisions, whereby only one of the daughter cells activates the Notch signaling pathway (labeled with N). This directs the two daughters towards different fates and ultimately results in one to four neurons that express distinct olfactory receptors. Each sensillum of a particular class thus generates a stereotypical combination of ORN types. Images of antenna (A, left) (Bruyne et al., 2001) and sensillum (A, right) (Goldman et al., 2005) were adapted with permission.

The mechanism described above establishes sensillar subtypes. ORN diversity within a sensillum is generated through asymmetric cell divisions (Endo et al., 2007; Ray et al., 2007; Endo et al., 2012). Each sensillum is generated from a single founder cell, which undergoes three rounds of division to generate eight cells (Fig. 5B) (Endo et al., 2007; Endo et al., 2012); but see other publications for an alternative model (Ray and Rodrigues, 1995; Reddy et al., 1997; Sen et al., 2003). The first division segregates two lineages; one daughter cell gives rise to neurons and the second to support cells. Two additional asymmetric cell divisions in the neuronal lineage produce four cells, of which one to four become ORNs and the remainder have an unknown fate. At each division, the two daughter cells are directed towards distinct fates by activation, in only one cell, of the evolutionarily conserved Notch signaling pathway. As a result, each sensillum of a particular subtype acquires a specific set of up to four neurons of different classes, i.e. ORNs of a particular sensillum show 'coupled' OR expression.

The association between OR expression and the glomerular targeting underscores the importance of maintaining the expression of the chosen OR. In contrast to the repression of *Rh5* by Rh6 in yR8 PRs, no evidence for one OR repressing an alternative OR gene has been found in *Drosophila* (Ray et al., 2007) (but see below for mouse). However, as in yR8 PRs, specification mechanisms do play a role in maintenance: at least some of the TFs involved in specification of OR gene expression are also continuously required for their maintenance (Jafari et al., 2012). Recently, evidence was found for epigenetic control of establishment and maintenance of OR expression in post-mitotic ORNs, which sense CO_2 (Sim et al., 2012). Repressive histone (H3K9me2) marked chromatin is present in OR genes specifically in the *Drosophila* antenna, as previously seen for mammalian OR genes (see below). A multi-protein complex, Myb-MuvB (MMB)/dREAM, interacts with the histone modification machinery, and its positively and negatively acting subunits ensure that the CO_2 receptor genes *Gr21a* and *Gr63a* are expressed only in the CO_2-sensing ORN subtype (Sim et al., 2012).

Establishing and maintaining olfactory receptor expression in the mouse

The mouse OR repertoire with >1200 OR genes probably represents the largest known gene family in any genome (Buck, 2000; Fuss and Ray, 2009). Despite this abundance, OR expression is highly restricted in olfactory sensory neurons (OSNs), as each individual OSN appears to express only one allele of a single OR gene (Chess et al., 1994).

Most mouse OSNs that express a particular OR are restricted to broad overlapping zones (Fig. 6A) of the main olfactory epithelium (Fuss and Ray, 2009) and within each zone an OR is selected from a large subset of OR genes in an apparently random manner. This results in an intermingled distribution of OSNs expressing different ORs reminiscent of the ORN pattern in *Drosophila* (Fig. 6B) (Miyamichi et al., 2005). However, the dramatically increased numerical complexity in the mouse olfactory system makes it highly unlikely that expression of each OR gene is controlled by a specific and distinct combination of TFs, as has been proposed for the fly. The global mechanism underlying the stochastic OR choice remains obscure, but two *cis*-regulatory DNA regions, the H and P elements, have been identified that influence the probability of OR choice from a small subset of linked OR genes (Serizawa et al., 2003; Fuss et al., 2007; Nishizumi et al., 2007; Khan et al., 2011).

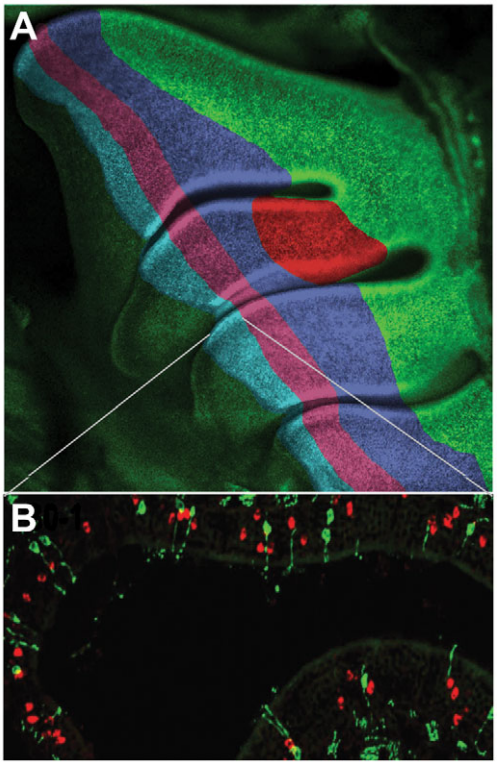

Fig. 6. Sensory receptor expression in mouse olfactory epithelium. (**A**) In the mouse olfactory epithelium, olfactory receptor (OR) genes are expressed in different zones (pseudocolored). (**B**) Within a zone, the choice made by each olfactory sensory neuron (OSN) to express a single OR gene appears to be random and leads to an intermingled distribution of OSNs expressing different ORs. Here, expression of MOR230-1 (known as Olfr1205) and the MOR28 (known as Olfr1205) transgene is shown in red and green, respectively. The images shown in A (Vassalli et al., 2002; Fuss and Ray, 2009) and B (Serizawa et al., 2004; Fuss and Ray, 2009) were adapted with permission.

Furthermore, epigenetic regulation at the chromatin and nuclear architecture level plays a crucial role in global repression of ORs (McClintock, 2010; Magklara et al., 2011; Clowney et al., 2012). Non-expressed OR loci carry histone modification marks, which are characteristic of transcriptionally repressed heterochromatin (Magklara et al., 2011). In addition, the repressed OR loci are clustered within the OSN nucleus into about five foci, whereas the transcriptionally active OR gene is located outside these foci (Clowney et al., 2012). Genetic disruption of this organization leads to expression of multiple ORs per OSN. The mechanism by which a particular OR is selected for expression under normal circumstances, however, remains obscure.

Once an OSN chooses to express a functional receptor protein, feedback from the OR prevents expression of other ORs in the same neuron (Reed, 2000; Feinstein et al., 2004; Lewcock and Reed, 2004; Serizawa et al., 2004; Shykind et al., 2004). This is not the case in *Drosophila* antenna, where such a mechanism was not found (Ray et al., 2007; Fuss and Ray, 2009), but is similar to the exclusion mediated by Rh6 in the *Drosophila* retina, although whether OR feedback is part of a choice or a maintenance mechanism (or both) has not been addressed. The OR feedback pathway does not involve the olfactory signaling cascade, as the associated G protein ($G_{\alpha olf}$) is not required to

repress alternative OR genes (Belluscio et al., 1998). Curiously, DNA sequences that mediate the repression of OR genes might be located within the coding regions of the OR genes themselves (Nguyen et al., 2007), contrasting with Rh gene repression in the fly retina, where a short *Rh5* promoter fragment that does not contain a coding sequence is controlled by the Rh6-feedback signal (Vasiliauskas et al., 2011). Another major difference between the two systems appears to be that repression through OR feedback is likely to be present across the entire olfactory epithelium, whereas it is active in only one specific subtype of PRs in the fly eye. In summary, control of OR expression in the mouse olfactory system is conceptually more similar to the patterning of *Drosophila* Rh genes than to the patterning of *Drosophila* OR genes, as it involves both random and deterministic processes, as well as SR feedback.

Conclusions

Sensory neurons use different mechanisms to choose SRs (Table 1). In the *Drosophila* retina, early cell fate specification leads to the expression of a network of TFs that restrict Rh expression. Much of this is achieved by shaping the expression of the repressor Dve (Johnston et al., 2011). *dve* is activated by Otd in all PRs, but Sal (expressed only in R7 and R8) prevents this activation in inner PRs. Furthermore, Ss overcomes this repression by Sal and activates Dve in the yR7 subset. This results in Dve expression in R1-R6 PRs, where it represses *Rh3*, *Rh5* and *Rh6* and allows exclusive *Rh1* expression, and in yR7s, where it represses *Rh3* to allow exclusive expression of *Rh4* (mediated by Ss). How Sal is restricted to inner PRs, how stochastic *ss* expression is established and how the input of transcriptional activators and repressors is integrated on the Rh gene promoters remain unanswered questions. In the *Drosophila* antenna, the combinatorial code of transcription factors also determines OR choice. However, there does not appear to be a significant role for a transcriptional stochastic component. Rather, the intermingled ORN pattern appears to be achieved through cell migration and 'mixing'.

In the mouse retina, the neural retina leucine zipper TF Nrl (Mears et al., 2001; Oh et al., 2007) plays a similar role as Sal in distinguishing PR classes (rod versus cone fate). Then, the S and M cone subtypes are specified by RORβ and TRβ2, respectively, together with positional cues that distribute the cone subtypes in an opposing dorsoventral pattern. Little is known about how these factors act in a subtype-specific manner and how the dorsoventral differences in M and S opsin expression are achieved.

Mouse visual and olfactory systems do not appear to group their sensory neurons into small functional units, which would require coordination of SR expression within the unit. By contrast, the *Drosophila* compound eye is composed of ommatidia in which inner photoreceptors coordinate the Rhs they express. This is probably required to correctly evaluate color information originating from a single point in space. This coordination is achieved by an R7-to-R8 signal, which remains to be identified. Fly olfactory receptor neurons housed in the same sensillum also coordinate OR expression, but this appears to be the consequence of the lineage relationship among them. Interestingly, it was recently shown that a sensillum acts as a peripheral processing unit of olfactory information. ORNs within a sensillum inhibit each other's neuronal activity when responding to different components present in an odorant mixture (Su et al., 2012). This lateral inhibition does not require synapses and appears to enhance contrast to improve the detection of a transiently applied odor. Thus, correctly matching OR expression by ORNs housed in the same sensillum probably has an important functional significance in fly olfaction.

After SR expression patterns are established, mechanisms are needed to maintain the differentiated state (Blau and Baltimore, 1991). This can be achieved either by continued activity of the mechanisms used for differentiation (Eade and Allan, 2009; Hobert, 2011; Eade et al., 2012), through involvement of epigenetic mechanisms (McClintock, 2010; Magklara et al., 2011; Clowney et al., 2012; Sim et al., 2012), or by additional maintenance mechanisms. Indeed, maintenance of repression of *Rh5* in yR8 PRs

Table 1. Comparison of known mechanisms involved in controlling sensory receptor (SR) expression in fly and mouse sensory receptor neurons

	Drosophila retina	Mouse retina	*Drosophila* olfactory system	Mouse olfactory system
Exclusive expression of SRs	Combinatorial TF code; double negative-feedback loop	Rare; mechanisms not clearly established	Combinatorial TF code; epigenetic mechanisms	SR feedback signal; epigenetic mechanisms
Co-expression of SRs	Positional cues directing TF expression	Mechanisms not clearly established	Transcriptional co-regulation; multiple SRs from same mRNA (alternative splicing or bicistronic mRNA)	Rarely observed
Intermingled distribution of sensory neuron subtypes	Stochastic expression of a TF (Spineless)	No known mechanisms	Cell migration	Stochastic choice via unknown mechanism; involvement of *cis*-regulatory elements
Coordination of SR expression between different sensory neurons	Unknown inter-photoreceptor signal	No evidence of coordination	Olfactory receptor neurons with coordinated SR expression are related by lineage	No evidence of coordination
Maintenance of SR expression pattern	Continuous requirement of early specification mechanisms; SR feedback signal	Continuous requirement of early specification mechanisms	Continuous requirement of early specification mechanisms; epigenetic mechanisms; no evidence for SR feedback signal	SR feedback signal; epigenetic mechanisms

TF, transcription factor.

requires continued activity of the Hippo pathway, which established the yR8 fate during PR differentiation. This is reminiscent of the continuous requirement of TH signaling for the maintenance of M identity in the mouse and rat retina and the continuous requirement of a small set of TFs to maintain OR expression in the *Drosophila* antenna. However, after PR differentiation, the terminal differentiation product Rh6 is also required in addition to the Hippo pathway to maintain *Rh5* repression. It is not yet clear whether opsin exclusion mechanisms are involved in maintenance of PR functional identity in the mouse retina, and strong evidence suggests that such a mechanism is not involved in the *Drosophila* antenna (Ray et al., 2007). In mouse OSNs, however, a similar feedback has been established as a major part of the mechanism yielding singular OR expression (Fuss and Ray, 2009). Interestingly, the distinction between choice and maintenance has not been clearly drawn for mouse OR expression, but this is likely to become clearer as more details become available about the relationship between the OR feedback and the epigenetic state and nuclear organization of OR loci. Consequently, a picture emerges of two types of strategies for maintaining the functional identity of sensory neurons: continuation of the differentiation pathways/TF networks and feedback from the sensory receptor expressed in these cells.

Differentiation and maintenance of sensory neuron subtypes can involve the same pathways and TFs, but their roles might change between the two cellular states, as exemplified by the insufficiency of the Hippo pathway to maintain yR8 identity. Recent work on a class of fly ventral nerve cord interneurons (Eade and Allan, 2009; Eade et al., 2012) illustrates how differentiation and maintenance networks can change. The same set of TFs that controls terminal differentiation of these interneurons also maintains their functional identity, but several cross-regulatory interactions among the TFs disappear. This TF network is therefore substantially altered for maintenance. Thus, it is important to compare differentiation and maintenance networks in different cell types carefully. The resulting insights will guide future studies and will further our understanding of the network logic of SR specification and maintenance.

Acknowledgements

We thank J. Corbo, D. Didiano, S. Fuss, R. J. Johnston Jr, D. Jukam, A. Ray, S. Sprecher and M. Tsachaki for comments on an earlier version of the manuscript. We also thank S. Haverkamp, B. Mormann and A. Ray for providing images of the mouse retina, fly retina and olfactory epithelium.

Funding

This work was supported by a German Research Foundation (DFG) fellowship [to J.R.]; and National Institutes of Health grants [to C.D. and D.V.]. Deposited in PMC for release after 12 months.

Author contributions

The authors declare no competing financial interests.

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