‘One Receptor’ Rules in Sensory Neurons

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Introduction

A common phenomenon in sensory systems is the exclusive expression of a single receptor molecule in a given receptor cell. However, the molecular mechanisms involved in this exclusion process are largely unknown. We will discuss recent progress in our understanding of how the ‘single receptor molecule per receptor cell’ rule is established. The expression of only one receptor molecule per cell is necessary to prevent sensory overlap in the brain. Once a stimulus has been received, the animal must then respond appropriately. The presence of many receptors in one sensory cell projecting to the brain would complicate the process of determining the appropriate response. We will describe how this rule applies to the visual systems of Drosophila and of higher organisms, as well as other sensory systems, despite their differing anatomy and their varying degrees of complexity (more cells, more receptors and more regulations). We will focus on important findings recently described for the Drosophila visual system.

Through the Fly Eye

The fly compound eye is composed of approximately 800 individual eyes called ommatidia. Each ommatidium is composed of 20 cells, including 8 photoreceptor cells (PR) named R1–R8 (fig. 1a). Each PR contains a stack of apical microvilli, the rhabdomere, which is filled with a
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photosensitive receptor molecule rhodopsin (Rh) and forms the light-gathering structure of the PR. The rhabdomeres of the 6 outer PRs R1–R6 form an asymmetric trapezoid whose center is occupied by the rhabdomeres of the inner PRs R7 (distal cell) that sits on top of R8 (proximal cell) [1]. Five rhs are expressed in the fly eye, each with distinct spectral sensitivities to light (fig. 1). The most abundant Rh in the adult eyes is the broad spectrum Rh1 [2]. Four other Rhs are found in the eye: Rh3 and Rh4 have similar sensitivities in the UV range while Rh5 and Rh6 are maximally sensitive in the visible range; Rh5 is blue-sensitive and Rh6 has its peak of sensitivity in the green (fig. 1) [3–9]. Each of these rhs is specifically expressed in particular subsets of PRs: Rh1 in the outer PRs R1–R6, rh3 and rh4 in R7 cells and rh5 and rh6 in R8 cells (fig. 1b). A sixth rh, the short-wavelength-sensitive Rh2 [10, 11] is expressed in the ocelli, a photoreceptive organ on the top of the fly’s head involved in maintaining balance during flight.

Fig. 1. Organization of the Drosophila eye. a Schematic representation of one of the ~800 ommatidia that compose the fly eye. Six outer PRs surround R7 and R8 cells located in the center on top of each other, sharing the same light path. b The Drosophila eye is composed of three different types of ommatidia. In the dorsal most ommatidia, the DRA, both R7 and R8 cells express rh3 and are involved in measuring the angle of polarized light. The two other sub-types are randomly distributed in the eye. The pale subset comprises 30% of the retina. In this class, R7 cells express rh3 and R8 cells express rh5. In the remaining 70%, the yellow subset, R7 cells express rh4 while R8 cells express rh6. c Fluorescent confocal image of an antibody staining on a whole-mounted retina showing Rh5 in red, Rh6 in green and the outer PRs expressing Rh1 in blue. The outer PRs express always rh1, while the inner R8 cells express either rh5 or rh6, in a 30/70% ratio. The PRs always express only one Rh respecting the rule ’one receptor per cell’. d The Drosophila genome contains six identified Rhs with absorption maxima ranging from UV to green. Rh1 is the broad absorption Rh most likely involved in motion detection. rh2 is only expressed in the ocelli located on top of the head and not in the eyes. Rh involved in color vision have a more restricted absorption spectrum. Both R7 rhodopsins are UV sensitive, while the R8 opsins Rh5 and Rh6 are blue and green sensitive, respectively. The absorption maxima are 478 nm (Rh1), 420 nm (Rh2), 345 nm (Rh3), 375 nm (Rh4), 437 nm (Rh5) and 508 nm (Rh6) [9, 13].
The visual system is composed of a pair of organs, Bolwig’s organ (BO), present in the head of the larva. Each BO contains 12 PRs which express one of 2 rhodopsins, rh5 or rh6, again in a non-overlapping pattern [Pichaud et al. unpubl. observation]. In flies, BO does not get transformed into the adult compound eye, but it probably persists through metamorphosis and gives rise to extra-retinal PRs known as the ‘Hofbauer-Buchner’ eyelet [16]. One major function of the BO and eyelet is to entrain the circadian clock [17].

How General Is the Rule ‘One Receptor Molecule per Receptor Cell’?

There is no PR that expresses more than one particular Rh gene in the fly. For instance, when R7 expresses stochastically rh3, rh4 is always totally repressed. This is not unique to Drosophila, as most known visual systems respect the rule ‘one Rh per cell’. For example, the honeybee (Apis mellifera), like Drosophila, has compound eyes composed of ~6,000 ommatidia each containing 9 PRs [18]. Recordings from honeybee retinas suggest that there are three types of PRs with different maximal sensitivities [19]. When the bee opsins are expressed and analyzed in a heterologous system, they have absorption maxima that are in perfect agreement with the cellular recordings from the eye supporting the idea that only one opsin is expressed in each PR subtype [20].

The Japanese yellow butterfly P. xuthus is also a well-studied model system for color vision. The anatomical organization of the eye is similar to the honeybee eye, having 9 PRs per ommatidium. Intracellular recordings demonstrated the presence of five different receptor cells distributed in different subtypes of ommatidia [21]. Their distribution seems to be random as it is in Drosophila, but immunostainings and in situ experiments indicate that some PRs coexpress two opsins [22]. Furthermore, different non-opsin accessory pigments are also present along with Rh. Hence the spectral sensitivity of these PRs depends on both the particular opsin(s) they express, and the particular absorbance of the accessory pigment [23]. Thus, two PRs can express the same opsin, but paired with two different pigments, they will have different absorption maxima [22]. This strategy is not unique to butterflies or even to insects. For example, two cone populations in the chicken retina express the same Rh but contain differently colored oil droplets that change their spectral tuning [24]. Thus, the combination of a Rh and an accessory pigment will determine the spectral sensitivity of the PR. There is no clear biological explanation for

The outer and inner PRs represent two overlapping visual systems with different functions: the outer PRs, the equivalent of vertebrate rods, are involved in motion detection and project to the lamina part of the optic lobe; the inner PRs, the equivalent of the vertebrate cones, appear to be involved in color discrimination [1]. The organization of the inner PRs, with R7 in the distal half of the retina and R8 occupying the proximal half underneath, gives the fly the appropriate hardware to discriminate between colors (i.e. to compare the activity of two different pigments). Since the two rhabdomeres share the same visual axis, the different spectral sensitivities of R7 and R8 allow comparison of the wavelength of light [1]. The R7 and R8 axons bypass the lamina and project to two closely apposed layers of the medulla, with the R8 axons projecting just before the R7 terminations. Inputs from R7 and R8 cells are probably compared in this part of the optic lobe [12].

Although all the facets of the fly eye appear to be identical externally, there are clear physiological differences among ommatidia. The main part of the retina consists of a mosaic of two stochastically distributed types of ommatidia. Seventy percent, called the ‘yellow’ type (y), have an R7 cell with Rh4 and an R8 cell with Rh6 [3, 6]. The remaining 30% (the ‘pale’ type, p) have Rh3 in R7 and Rh5 in R8 (fig. 1). This distribution of Rhs presumably allows color discrimination over a broader range of wavelengths. Although the R7 Rhs (Rh3 and Rh4) are present in two non-overlapping subsets of R7, they have only slightly different absorption spectra in the UV range [13]. However, a blue filtering pigment in yR7 sharpens the absorption of Rh4 and filters the light that reaches the green-sensitive underlying R8 [1] (fig. 1d). Thus, it is likely that the p ommatidia discriminate better over short wavelengths, while the y ommatidia discriminate colors extending to the green.

A very specialized region of the eye of many insects, including Drosophila, is the dorsal rim area (DRA). The DRA is a row of ommatidia located in the dorsal-most part of the eye. These ommatidia face the sky and detect the vector of polarization of sunlight reflected by the sky, which the fly uses for navigation [14]. Specification of this subclass of ommatidia in a restricted domain of the eye is controlled differently from the rest of the eye. Only in the DRA, R7 as well as R8 cells express the same rhodopsin, rh3 [15] (fig. 1b).

The visual system of the Drosophila larva, although different in structure from the adult compound eye, utilizes some of the same Rh proteins. This simple larval visual system is composed of a pair of organs, Bolwig’s
this coexpression, but one can speculate that the expression of more than one pigment tunes the cells to colors that cannot be perceived using a single visual pigment.

The human retina contains rods and cones, which are dedicated to distinct visual tasks. Rods express rod opsin and are involved in image formation under dim light conditions, while the three types of cones express one of the three ‘color’ opsins, blue-, green-, or red-sensitive opsins and are involved in color discrimination [25]. As in Drosophila, a single cone PR expresses only one opsin. Besides the rod PRs, the nocturnal mouse has two types of cones, with the UV cones being more abundant ventrally and the green cones being more abundant dorsally. However, these cones also coexpress UV- and green-sensitive opsins at different levels along this regional dorso-ventral gradient [26]. In rats, coexpression of cone opsins is only observed in the ‘transition zone’, while dorsal green cones and ventral UV cones only express one opsin [27]. The loss of exclusive expression, which is incompatible with color vision, is presumably due to the degeneration of the color visual system in these nocturnal animals. In some cases, coexpression of two visual pigments is a mid-step during development. For example, in the human retina, there is a short period of time when cone cells coexpress blue and green or red opsins [28]. While the blue cones initially populate most of the fovea, later on, their number decreases as the green/red cone population increases [28]. This spatio-temporal pattern of expression suggests that cones are first born as blue cones and then switch to either a green or red cone fate. A similar phenomenon has recently been reported for the salmon, which appears to switch its retina from UV to blue sensitivity when its habitat changes from surface to deeper water [29]. Therefore, PRs are usually sensitive to only one peak of wavelength, most often due to the expression of one type of photo pigment per cell. Although there are some exceptions to the rule, these might represent particular adaptations to the environment or a transient stage in development.

**Receptor Exclusion in Other Sensory Systems**

Odorant receptors (ORs), like Rhs, are G-protein-coupled seven trans-membrane receptors [30, 31]. The ORs generally respect the same rules as the Rhs, in that only one receptor gene is expressed per cell, although the system is much more complex, comprising hundreds of ORs [32]. In the fly, some ORs are expressed in the antenna (the fly’s nose), some in the maxillary palp and some of them in both areas. No cells expressing more than one particular OR have been identified. However, most of the neurons express an additional receptor (OR83b) that has been postulated to function as a coreceptor [32, 33]. This might be a peculiarity of Drosophila. In mice, OR cells generally express only one given OR with a roughly random distribution within a particular zone of the olfactory epithelium [31]. This enables the olfactory system to respond to a vast amount of individual odors. As in the visual system, there are exceptions to the exclusion rule as some cells of the mouse olfactory epithelium express two different ORs [33]. This coexpression might broaden the sensitivity to different odorants, as might coexpression of opsins for colors in butterfly PRs. As it was shown for human opsin genes, recent findings indicate that during development, mice OR cells might coexpress more than one OR [34, 35].

The vomeronasal organ of mammals detects pheromone odorants important for social and sexual behavior. Two receptor families, V1Rs (30–100 receptors) and V2Rs (140 receptors), encode the vomeronasal receptors [36–38]. Although most of the time OR cells express only a single receptor, it has recently been reported that some vomeronasal sensory neurons express more than one of either V1R or V2R receptors. Using antibodies raised to several V2Rs, it was shown that V2Rs are broadly distributed and coexpressed in the same cells as other V2Rs [39]. However, these two subclasses of receptors are never coexpressed in the same cell, indicating that they presumably respond to different odorants.

An extreme exception to the one sensory receptor exclusion rule is the nematode C. elegans, where multiple olfactory and chemosensory receptors are expressed in the same receptor cell. Here, the identity of the sensory receptor neuron rather than the receptor repertoire of a single neuron determines the behavior of the animal, allowing it to be either attracted or repulsed by a particular odorant [40]. This was shown by elegant experiments where the diacetyl receptor (ODR-10) that is expressed in the cells that are responsible for attraction was misexpressed in a ‘repulsive’ neuron. The diacetyl receptor then mediated a repulsive response when exposed to diacetyl, indicating that the identity of the neuron is the determinant of the final response [40].

Recent studies have shown that taste receptor cells also detect a single taste modality (sweet, sour, salty, umami, bitter) [41–44]. Detailed expression studies of putative sweet, umami and bitter taste receptors have established that they are expressed in distinct, non-overlapping sets of taste receptor cells. Functional studies have shown that
sweet taste is mediated by a small family of 3 receptors, the T1Rs. The bitter receptors are encoded by the slightly larger family of T2Rs. As there is no coexpression of sweet, umami or bitter receptors in the same taste receptor cell, bitter receptor cells do not respond to sweet or umami stimuli and vice versa [41, 45].

Together, these data indicate that sensory systems share a common characteristic: each sensory cell is sensitive to a given stimulus by expressing only one functional sensory receptor molecule per cell. While in some sensory systems it is sufficient to express only one type of receptor molecule to respond to the environmental stimulus, others need to express a combination of receptors to be able to distinguish among the wide range of stimuli. The expression of a particular receptor or receptor ‘set’ requires a high degree of regulation. Therefore, efforts trying to understand the precise control of expression of a specific sensory receptor will have a profound impact toward better understanding sensory perception.

Repression of an rh Gene by Misexpression of Another

The visual system of Drosophila presents a number of advantages to study the mechanisms used by sensory systems to express one particular receptor per cell. In this genetic model system, only 5 Rhs are present in the eye and all have well-characterized promoters. The eye is also very accessible and its optics allows easy in vivo visualization of individual PRs [46]. Until recently, the molecular players responsible for the stochastic choice between y-(Rh4/Rh6) and p-type (Rh3/Rh5) of ommatidia, and for the exclusion and the coordination of rh expression between R7 and R8 were not known. However, it was clear that the stochastic choice to become one type of ommatidium was made in R7 cells: in a sevenless mutant (which lacks R7 cells), rh6 expands to nearly all R8 cells. In contrast, the distribution of R7 rhodopsins appears unaffected when R8 cells are missing [6, 47]. These results indicate, when an R7-p cell chooses to express rh3, a signal is sent to the underlying R8 to induce expression of rh5. R8 seems to play a passive role in this process and expresses rh6 by default. Therefore, it has been proposed that Rh4/Rh6 is the default state while Rh3/Rh5 is the acquired state [6, 47].

Recent findings in PR cell specification have shed some light on the process of rh expression. The inner R7 and R8 cells are distinguished from the outer PRs (and thus are prevented from expressing rh1) by the spalt (sal) genes [48]. After acquiring a generic inner default state, R7 and R8 further differentiate into functional R7 and R8 cells. This process appears to occur at least partly by preventing R8 characteristics from being acquired in R7 cells. This function is performed by the homeobox gene prospero (pros), pros is uniquely expressed in R7 cells, and directly represses the expression of R8 rhodopsins, rh5 and rh6 [49]. In loss-of-function pros eyes, the R8 rhodopsins are now expanded to subsets of R7 cells. In this genetic background, the immediate question that arises is whether the endogenous R7 rhs are coexpressed with R8 rhs, or whether the misexpression of R8 rhs corresponds to the exclusion of R7 rhs from these pros− R7 cells: in fact, only few pros− R7 cells maintain expression of R7 rhodopsins, and these cells never coexpress an R8 rhodopsin. R7 rhodopsin expression does not appear to be regulated by Pros directly, but instead, it seems that their expression is repressed by the presence of the R8 rhodopsin molecules themselves: removing pros together with the Rh6 protein (using a rh61 mutant) leads to a derepression of rh4 in pros− rh6− R7 and coexpression of Rh4 and an rh6 promoter-lacZ reporter (fig. 2). As coexpression of two functional rhs is never observed, this suggests that the Rh proteins themselves play a critical role by somehow repressing each other to achieve the expression of only one sensory receptor per cell. As described below, a similar mechanism has recently been proposed for ORs in mice and suggests an evolutionarily conserved feedback mechanism for ensuring mutual exclusion.

Lessons from Vertebrates

Interesting findings regarding receptor exclusion have emerged from analyzing the genomic structure of the human color visual pigment genes. Trichromacy (blue/green/red) in humans and old-world monkeys seems to have evolved from dichromacy (blue/green) by duplication of the gene encoding the green visual pigments located on the X chromosome. Therefore, the duplicated genes are now expanded to subsets of R7 cells. In this genetic background, the immediate question that arises is whether the endogenous R7 rhs are coexpressed with R8 rhs, or whether the misexpression of R8 rhs corresponds to the exclusion of R7 rhs from these pros− R7 cells: in fact, only few pros− R7 cells maintain expression of R7 rhodopsins, and these cells never coexpress an R8 rhodopsin. R7 rhodopsin expression does not appear to be regulated by Pros directly, but instead, it seems that their expression is repressed by the presence of the R8 rhodopsin molecules themselves: removing pros together with the Rh6 protein (using a rh61 mutant) leads to a derepression of rh4 in pros− rh6− R7 and coexpression of Rh4 and an rh6 promoter-lacZ reporter (fig. 2). As coexpression of two functional rhs is never observed, this suggests that the Rh proteins themselves play a critical role by somehow repressing each other to achieve the expression of only one sensory receptor per cell. As described below, a similar mechanism has recently been proposed for ORs in mice and suggests an evolutionarily conserved feedback mechanism for ensuring mutual exclusion.
Along with the aforementioned similarities between the visual and the olfactory systems, a similar LCR region has recently been described upstream of an array of four ORs in mice [52]. As described for the human cone opsins, this region might allow the activation of expression of only one of the genes in the array. Similar to the fly eye, there is also evidence that a functional odorant receptor is required to repress other OR in the array within a particular cell [49, 52, 53]. Together, these studies show that only genes coding for a functional OR are expressed with the 'single receptor per cell' rule: genes without a full-length open reading frame (pseudogenes or reporter-only constructs, which do not encode a receptor) are coexpressed with a second receptor, since, when a cell chooses to express this gene, the repression mechanism is not activated and an additional gene is chosen, driving expression of a second gene in these cells [52, 53].

These experiments have led to the model in which a positive, stochastic choice is made at the level of the promoter to express a specific receptor. Once this choice has been made, the receptor itself seems to negatively feedback to prevent another choice (fig. 3). This allows sensory information to be decoded at the level of the sensory epithelium. Thus, activation of a sensory cell through its particular receptor leads to an output with a discrete meaning. Understanding how the receptor accomplishes this task is a question that remains to be answered. Many research groups using different sensory systems and model organisms are working not only to understand how a particular sensory receptor is expressed in one cell, but also how its exclusive expression is maintained. As evidences from the visual and olfactory systems indicate,
this mechanism may be more complex than simple transcriptional activation and repression, and appears to involve negative feedback from the proteins themselves. This kind of regulation is used by the immune system when selecting a functional immunoglobulin. In this case, once a functional receptor has been made and expressed at the surface of the cell, signals are sent to the cell via a negative-feedback pathway involving downstream signaling molecules [54].

The signaling cascade initiated by sensory receptors to ensure that a single functional receptor is expressed is distinct from the phototransduction or olfactory transduction pathways. Mutations in the phototransduction pathway do not affect rh gene expression in the fly eye [Mazzoni and Desplan, unpubl.]. Similarly, with loss-of-function experiments of critical olfactory signal transduction, components like the G protein Golf do not seem to have an effect on OR gene expression [55]. Characterizing this pathway will answer one of the most fundamental questions in sensory neuron development. Ultimately, it will be important to learn more about how the information gathered at the periphery by the PR is processed and decoded by the brain. Therefore, the challenge for the future is not only to get some insights into the mechanism that coordinates sensory receptor expression and how this unique sensory receptor expression is maintained, but ultimately how these sensory receptor cells communicate the type of sensory input they detect to the brain.

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