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The odor coding system of *Drosophila*

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Our understanding of the molecular and cellular organization of the *Drosophila melanogaster* olfactory system has increased dramatically in recent years. A large family of ~60 odorant receptors has been identified, and many of these receptors have been functionally characterized. The odor responses of olfactory receptor neurons have been characterized, and much has been learned about how odors are represented in olfactory centers in the brain. The circuitry of the olfactory system has been studied in detail, and the developmental mechanisms that specify the wiring and functional diversity of olfactory neurons are becoming increasingly well understood. Thus, functional, anatomical and developmental studies are rapidly being integrated to form a unified picture of odor coding in this model olfactory system.

Olfaction is a crucial sensory modality for many animals, mediating behavioral responses to food, mates and predators. Insects in particular possess highly sensitive and discriminating olfactory systems. Disease-carrying insects such as the malaria vector mosquito *Anopheles gambiae* rely primarily on olfactory cues for the localization of their human hosts [1], whereas in insects such as the sphinx moth *Manduca sexta*, males use pheromonal cues to navigate towards females [2]. The honeybee *Apis mellifera* exhibits robust olfactory learning [3], and odor-evoked navigational memories contribute to foraging behavior [4].

To support this diversity of olfactory-driven behaviors, the olfactory system must not only detect the presence of odors, in many cases with extraordinary sensitivity, but also distinguish among different odors. It must encode odor quality and quantity while contending with a formidable signal-to-noise problem: low concentrations of behaviorally relevant odorants must be perceived and interpreted against a background of other odorants. Ultimately, this information must be integrated to support a behavioral response.

New insight into the molecular mechanisms underlying these processes has come from recent work on the fruit fly *Drosophila melanogaster*, which provides a powerful model system for the study of olfaction. Its olfactory system is simple relative to vertebrate olfactory systems, the fly is amenable to facile genetic manipulation, the genome of the fly is small and is already sequenced, and its olfactory responses can be measured conveniently either physiologically or behaviorally [5].

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Anatomy of the *Drosophila* olfactory system

The fly has two pairs of olfactory organs, the antennae and the maxillary palps (Figure 1a). Each antenna contains ~1200 olfactory receptor neurons (ORNs), whereas each maxillary palp contains ~120 ORNs [6–8]. ORNs are compartmentalized into sensory hairs called sensilla, which can be subdivided into three major morphological types: basiconic, coeloconic and trichoid (Figure 1b). Each sensillum contains the dendrites of up to four ORNs. The antenna contains all three types of olfactory sensilla, whereas the maxillary palp contains only basiconic sensilla. The respective contributions of the antenna and maxillary palp to chemosensory-mediated behaviors are not yet clear.

ORNs send axons to the antennal lobe (AL), whose functional organization is remarkably similar to that of the olfactory bulb in vertebrates [9]. In the AL, ORNs synapse onto second order neurons called projection neurons (PNs) [6] (Box 1). The AL can be subdivided into ~43 spherical units called glomeruli [10]. Individual ORNs send axons to only one or a few glomeruli [6], and individual PNs typically innervate only a single glomerulus [11–13]. The glomeruli also contain the processes of local interneurons that branch in multiple glomeruli [6,14], providing a means for information transfer between glomeruli. The axons of PNs project to the mushroom body (MB) and lateral horn of the brain (Box 1).

Larvae of *Drosophila* also exhibit a robust olfactory response [15–17], which is mediated through the dorsal organ [18,19]. Each of the paired dorsal organs contains 21 neurons that project to the antennal lobe of the larval brain [20].

Olfactory receptor neurons

The ORNs of the antenna and maxillary palp generate action potentials in response to odor stimulation. The odor responses of many of these ORNs have been characterized through extracellular single-unit recordings from individual olfactory sensilla [21–24]. These recordings have revealed that different odorants elicit responses from different subsets of ORNs, and also that ORNs exhibit a remarkable diversity of response properties: responses can be either excitatory or inhibitory and can vary in both intensity and temporal dynamics, depending on the odorant and the ORN [22,23]. Similar ORN response properties have been described in other insects [25–28].

Extensive recordings from the antenna and maxillary palp have revealed that ORNs can be categorized into a limited number of functional classes based on their responses to a defined set of chemical odorants. The maxillary palp contains six functional classes of ORNs,

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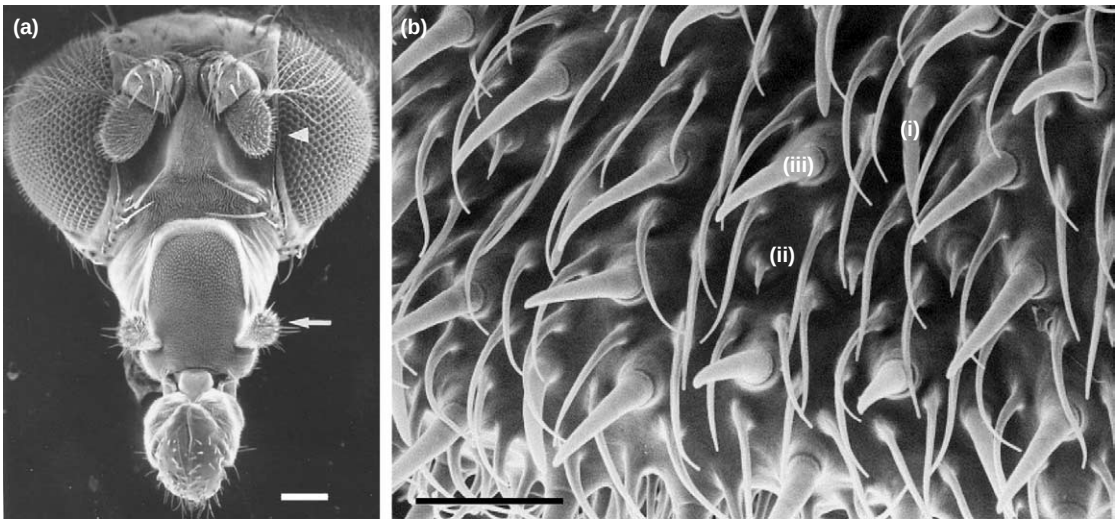

Figure 1. The *Drosophila* olfactory organs. **(a)** The adult head with antennae (arrow head) and maxillary palps (arrow). Scale bar = 100 μm. Figure 1a is reproduced with permission from Ref. [5]. **(b)** Three morphological types of olfactory sensilla on the antennal surface: (i) basiconic, (ii) coeloconic and (iii) trichoid. Scale bar = 10 μm. Figure 1b is reproduced with permission from Ref. [7].

which are found in stereotyped pairs within three classes of sensilla [22]. The antennal basiconic ORNs fall into 18 functional classes that are also found in stereotyped combinations within eight classes of sensilla [23,29]; the coeloconic and trichoid sensilla on the antenna also contain multiple kinds of ORNs [21] but a thorough characterization is not yet available.

Odorant receptor genes

Odorant receptors had been sought in insects for many years with a wide variety of genetic, biochemical and molecular approaches. A large family of candidate odorant receptor genes, the *Or* genes, was finally discovered in *Drosophila* in 1999 [30–32]. One successful approach to their isolation began with the assumption that odorant receptors in flies, like those in mammals [33] and *Caenorhabditis elegans* [34], were G protein-coupled-receptors (GPCRs), a superfamily of proteins whose members have extremely divergent sequences but a common structure composed of seven transmembrane domains. A computer algorithm was then devised that recognized proteins on the basis of structure and was trained to examine DNA databases for proteins with structures like those of GPCRs [30,35]. This algorithm identified members of the *Or* gene family from the genome of *Drosophila*.

Box 1. Organization of the *Drosophila* olfactory system

The adult fly has two pairs of olfactory organs, the antennae and the maxillary palps. The surfaces of these organs are covered with sensory hairs called sensilla. Each sensillum is innervated by the dendrites of up to four olfactory receptor neurons (ORNs). Odorant receptors are located in the plasma membranes of ORN dendrites [51,83]. The binding of odorants to odorant receptors results in the generation of action potentials in ORNs. Different subsets of ORNs project axons to different functional processing units called glomeruli in the antennal lobe (AL), where they synapse onto second order neurons called projection neurons (PNs). Local interneurons provide extensive lateral connections within the AL, typically branching in many, if not all, glomeruli (for simplicity, only a subset of these connections is depicted in Figure I) [6,14]. PN axons target the mushroom body (MB) and lateral horn in the brain. The MB has been shown to have a key role in olfactory learning and memory [63].

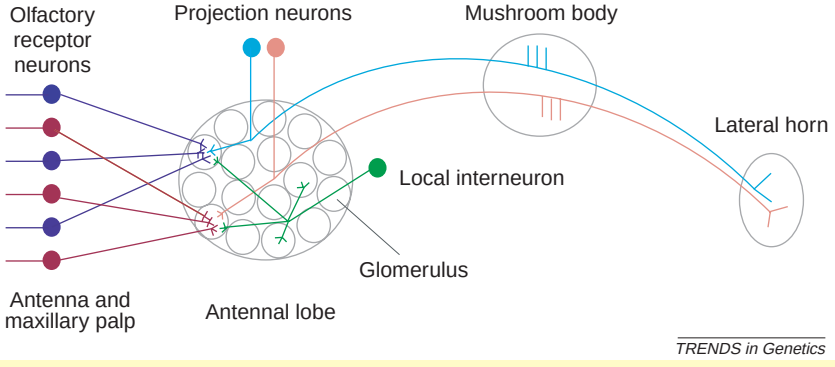

Figure I. The *Drosophila melanogaster* olfactory system. Adapted with permission from Ref. [80].

The *Or* gene family contains 60 members that are distributed throughout the genome, often in small clusters [30–32,36]. Two of these genes are alternatively spliced, resulting in a total of 62 odorant receptor proteins [36]. By comparison, humans are believed to have ~350 functional *OR* genes [37], mice have ~1000 *OR* genes [38,39] and mosquitoes have ~80 *Or* genes [40]. In general, *Drosophila* Or proteins are highly diverse, in many cases showing only ~20% identity to each other and no similarity to mammalian odorant receptors. This diversity among Or proteins is apparent throughout the length of the protein, although conserved residues shared by many of the genes have been identified [30,32,41]. However, closely linked genes often show a higher degree of similarity – the two most similar receptors, Or19a and Or19b, differ by only three amino acids – suggesting that *Or* gene clusters are likely to have arisen through recent genome duplication [36]. Each ORN expresses only one or a small number of *Or* genes, resulting in molecular diversity among ORNs [30,32,42].

Drosophila also contains a large family of gustatory receptor (*Gr*) genes that, like the *Or* genes, are predicted to encode seven-transmembrane domain GPCRs [43]. The *Gr* gene family consists of 60 genes that encode 68 proteins through alternative splicing [36]. The *Or* and *Gr* genes are believed to be evolutionarily related, together comprising a single chemoreceptor superfamily of which the *Or* genes represent a single highly expanded lineage within the more ancient *Gr* gene family [36,41,44]. Gr proteins are highly diverse, with many showing ~10% identity to each other. Many of these genes are expressed in gustatory organs [41,43,44] and some have been shown to function as taste receptors [45–47] or pheromone receptors [48]. However, at least three members of the *Gr* gene family are expressed in the antenna [41], raising the possibility that some *Gr* genes might encode odorant receptors.

Functional analysis of odorant receptors

Despite extensive data concerning *Or* gene expression, until recently little was known about *Or* gene function, in part because traditional genetic screens failed to identify odorant receptor mutants. The first *Drosophila* odorant receptor to be functionally characterized was the antennal receptor Or43a, which was initially characterized physiologically following antennal overexpression [49] and heterologous expression in *Xenopus* oocytes [50]. Both studies identified cyclohexanone, cyclohexanol, benzaldehyde and benzyl alcohol as ligands for this receptor.

Three additional odorant receptors – Or22a, Or22b and Or43b – were then characterized by genetic analysis. Deletion mutants were analyzed physiologically to identify ORNs lacking odor response. The ab3A neuron was found to lose odorant response in mutants lacking Or22a [51], whereas the ab8A neuron loses odorant response in mutants lacking Or43b [29]. (Or22b is coexpressed with Or22a in the ab3A neuron but does not appear to confer odor response upon the neuron and its function, if any, remains unclear [51].)

The deletion mutant lacking Or22a has been used as a 'decoder' in a large-scale analysis of odorant receptor function. Because the ab3A neuron of this mutant is still present on the antenna but lacks odorant response, it provides an *in vivo* expression system for odorant receptors: individual receptors are expressed in the mutant ab3A neuron, and the subsequent odor responsivity of the neuron is assayed by single-unit electrophysiology [51,52] (Figure 2a). This approach is simple: receptors can be easily expressed in the mutant ab3A neuron using the GAL4–UAS system [53], and large numbers of odorants can be rapidly screened for receptor activation. In addition, many different odorant receptors, including receptors from the distantly related mosquito *Anopheles gambiae*, have been shown to function in the mutant ab3A neuron [54]. Thus, this system is presumably useful for analyzing odorant receptors from other insects, including a variety of disease vectors and agricultural pests.

Nearly all of the *Drosophila* antennal odorant receptors have now been characterized using this approach, and by comparing the odor response spectra conferred by individual odorant receptors with the odor response spectra of wild-type ORNs (Figure 2b), many of these receptors have been mapped to the ORNs from which they are derived [52] (Figure 2c). This analysis has revealed that although many of the antennal receptors respond to common ligands, each has a unique odor response spectrum (Figure 2d). In addition, each receptor that was mapped to an ORN appeared to be sufficient to account for the full odor response spectrum of the ORN. Finally, the odor responses of mutant ab3A neurons that individually express different receptors were compared with those of the wild-type neurons from which the receptors are derived. The comparison revealed that the odorant receptor confers not only the odor response spectrum but also the spontaneous firing rate, response dynamics and signaling mode (excitation or inhibition) of the ORN [52]. Thus, much of the diversity among odor responses of ORNs is attributable to the odorant receptors they express.

The overlap in odor response profiles among receptors might explain why odorant receptor mutants were not isolated in genetic screens. Because many odor stimuli elicit responses from multiple receptors, mutations of single receptor genes might not have produced phenotypes strong enough to be detected in mutant screens, most of which were based on behavioral responses.

Odor representations in the antennal lobe

Odors are initially encoded in the diverse responses of the population of ORNs. How is odor information represented in the AL? The ~1320 ORNs of the antenna and maxillary palp converge onto ~43 glomeruli in the AL. Studies using *Or* promoters to drive expression of reporters have revealed that in *Drosophila*, as in mammals, axons of ORNs expressing the same odorant receptor converge onto only one or a few glomeruli [42,55,56]. The result is a highly precise spatial map of ORN projections, which exists despite the lack of a simple relationship between the location of an ORN on the surface of the olfactory organs and the location of its target glomerulus in the AL [42].

Different odorants activate distinct but overlapping subsets of glomeruli and the number of activated

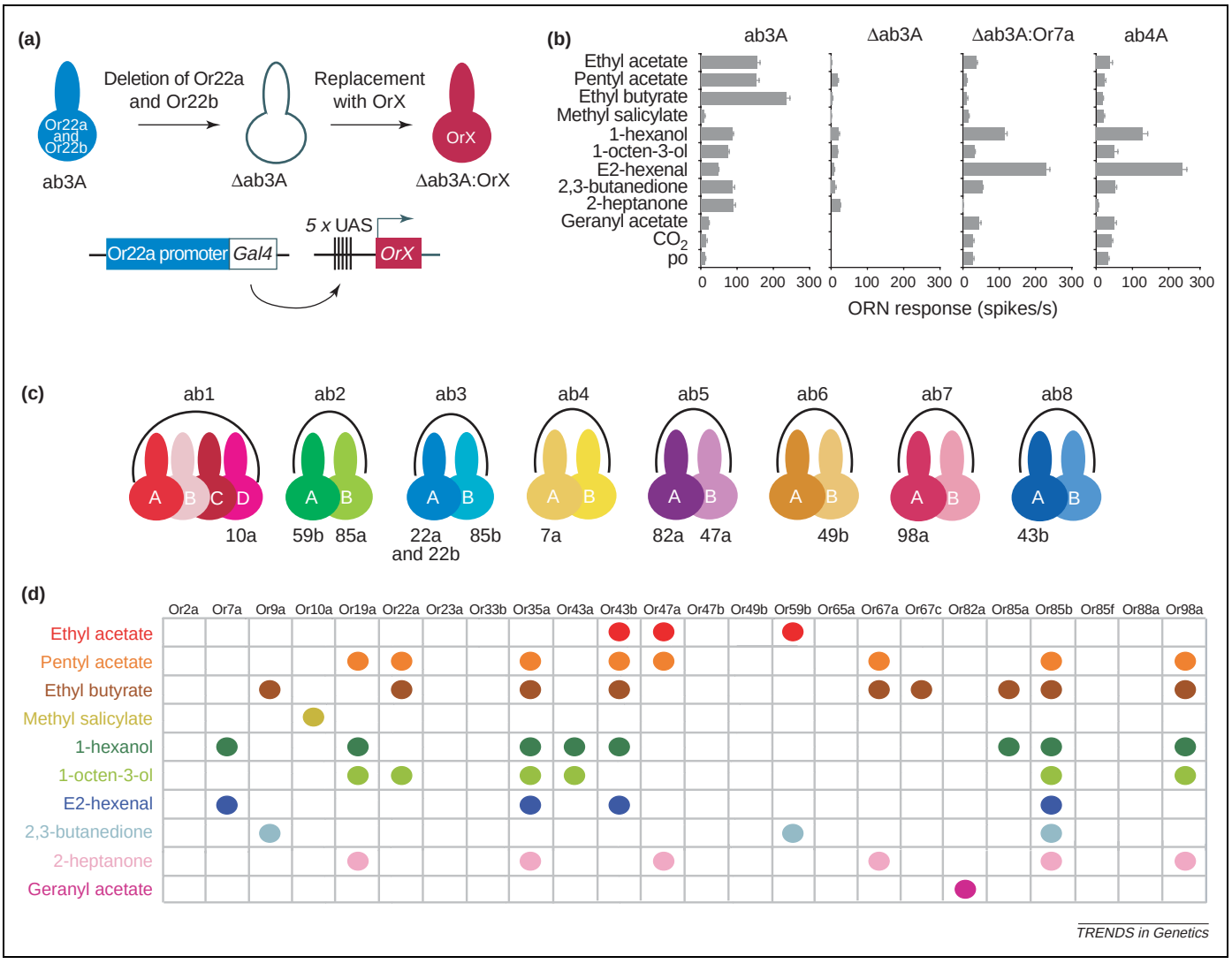

Figure 2. Analysis of odor response spectra of individual odorant receptors. **(a)** An *in vivo* expression system for odorant receptors. A mutant ab3A antennal neuron (Δab3A) lacks odor response due to the deletion of its endogenous receptor genes, *Or22a* and *Or22b*. Odorant receptors are introduced specifically into Δab3A using the GAL4–UAS system [53]; an Or22a–*GAL4* driver promotes transcription of the *Or* gene. The odorant response of the neuron (Δab3A:OrX) is then assayed electrophysiologically. **(b)** Mapping odorant receptors to olfactory neurons. The normal odor response of the ab3A neuron (first panel) is absent from the Δab3A neuron (second panel). Expression of Or7a in the Δab3A neuron (Δab3A:Or7a; third panel) results in an odor response spectrum resembling that of the wild-type ab4A neuron (fourth panel), indicating that E2-hexenal is a ligand for Or7a, and that Or7a is the odorant receptor in ab4A. Graphs depict the response of the neuron in spikes per second (spikes/s) to the diagnostic panel of odorants at the left. **(c)** Odorant receptors that have been mapped to functional classes of neurons. The eight different functional types of basiconic sensilla are designated ab1–ab8, and the neurons are named according to the sensillum in which they are found (for instance, the ab2 sensillum contains ab2A and ab2B neurons). Odorant receptors that have been mapped to basiconic neurons are indicated below the corresponding neurons. **(d)** Odor response spectra of antennal odorant receptors. The colored dots depict strong responses. Reproduced with permission from Ref. [52].

glomeruli increases with increasing odorant concentration, as revealed by optical imaging [57–59] and metabolic labeling [60] studies. Thus, odor coding in the AL appears to involve a spatial map of odorant receptor activation. An electrophysiological analysis of PNs similarly revealed that different odorants activate different populations of PNs [61]. In addition, like ORN responses, PN responses were found to differ in breadth of tuning, signaling mode and response dynamics [61].

An emerging question from these studies is how specific odor responses are represented in the antenna compared with the antennal lobe. Is the activity of a PN determined entirely by the activity of its pre-synaptic ORNs or is it influenced by the activity of other AL neurons? Ng *et al.* and Wang *et al.* compared the pre- and post-synaptic odor-evoked glomerular activity by driving optical reporters in either ORNs or PNs [57,59]. They found that a given odor evokes essentially the same activation pattern regardless of whether the reporter is driven pre- or post-synaptically, suggesting that activation of a PN simply reflects activation of its pre-synaptic ORNs [57,59].

Different results were obtained by Wilson *et al.*, who instead compared ORN with PN responses electrophysiologically [61]. This analysis found evidence that PNs are more broadly tuned than ORNs. A comparison of PN with presynaptic ORN activity in the same glomerulus showed not only that the PN was more broadly tuned but also that the temporal dynamics of its response to certain odorants differed from those of the ORN. In contrast to the results of Ng *et al.* and Wang *et al.*, these results suggest that PN output is shaped not only by ORN input but also by lateral connections within the AL [61].

Odor representations in higher brain centers

Spatial patterning of odor-evoked activity has also been reported in the MB. Calcium imaging of MB neurons revealed that different odorants evoke different patterns of spatial activity [58,62]. Higher odorant concentrations also evoke different patterns of spatial activity [62]. Interestingly, the spatial patterning in the MB appears to be highly variable between individual flies [62]. Consistent with this result, a lack of stereotypy among individual flies was observed in the branching patterns of individual PN axons within the MB [12,13]. Although the functional significance of this variability is unclear, the key role of the MB in olfactory learning and memory [63] raises the possibility that it might reflect experience-dependent plasticity.

A spatial map of odor representation is also likely to exist in the lateral horn, although a functional analysis of these neurons has not yet been reported. Genetic labeling of individual PN axons using either the mosaic analysis with a repressible cell marker (MARCM) [64] or FLP-out [65] techniques has revealed that PNs that connect to different glomeruli show stereotyped axon branching patterns within the lateral horn that are distinct but overlapping, thus, allowing for the integration of olfactory information from multiple AL glomeruli [12,13].

Olfactory sensillum development and the problem of receptor gene choice

The antenna and maxillary palp are highly precise and stereotyped in their organization. For example, ORNs of certain odor specificities are consistently paired in canonical combinations within individual sensilla (Figure 2c). How is this degree of precision established during development?

Sensory organ precursor cells, termed 'founder cells' [66,67], are specified in the antennal imaginal disc during the early stages of pupal development. These founder cells then recruit additional cells to form the pre-sensillum cluster, which undergoes one round of cell division before terminally differentiating into a single olfactory sensillum [66,67]. Two proneural genes, the basic helix–loop–helix transcription factors *amos* and *atonal*, are required for founder cell specification. The genes act on different subsets of precursor cells: while *amos* is required for specification of the antennal basiconic and trichoid sensilla [68,69], *atonal* is required for specification of the antennal coeloconic sensilla and for specification of the basiconic sensilla of the maxillary palp [70,71]. The prepattern gene *lozenge*, a Runt-domain transcription factor, also has a role in this process by regulating *amos* expression [66,68,72].

Crucial to the development of the olfactory system is the problem of receptor gene choice. Ultimately, the coding of odors depends on the existence of multiple classes of ORNs, each with a particular odor response profile. How do these ORNs select which receptors to express, from among a repertoire of 60 *Or* genes, and how are the choices of different ORNs coordinated to produce the stereotyped organization of ORNs? Remarkably little is known about this problem in any organism; however, in the fly the POU-domain protein Abnormal chemosensory jump 6 (Acj6) has been implicated in this process [73]. POU-domain proteins are transcription factors that contain a DNA-binding motif consisting of a homeodomain and a POU-specific domain, and many of these proteins have diverse roles in nervous system development [74]. In *acj6* mutants, some ORNs are normal, some lack odor response and some undergo alterations in odor-specificity. These mutants also lack expression of a subset of *Or* genes, suggesting that these neuronal alterations are a direct result of abnormal receptor gene expression [73]. The fact that Acj6 is required for the development of some but not all ORN functional classes suggests that it does not act alone to specify ORN identity but rather as part of a combinatorial code of transcription factors.

Axon pathfinding in the olfactory system

Little is known in *Drosophila* about the molecular cues that guide ORN axons to their target glomeruli. In vertebrates, the odorant receptor itself has been implicated in this process [75,76]. However, this does not appear to be the case in *Drosophila*, where ORNs whose *Or* genes have been deleted, in addition to ORNs expressing different *Or* genes ectopically, still target their cognate glomeruli [51]. Two signaling components, the adaptor protein Dock and the serine-threonine kinase Pak, have been implicated in this process [77]. In *dock* and *Pak* mutants, ORN axons enter the AL but then often show misrouting within the AL. Another molecule involved in this process is the cell-surface protein Down syndrome cell adhesion molecule (Dscam) [78]. In *Dscam* mutants, the axons of maxillary palp ORNs often fail to reach the AL, whereas the axons of antennal ORNs reach the AL but often mistarget within the AL. Interestingly, the *Dscam* gene is alternatively spliced to potentially encode more than 38 000 isoforms [79], raising the possibility that different isoforms might act combinatorially in different subsets of ORNs.

The post-synaptic partners of ORNs, the PNs, extend dendrites into the glomeruli and axons into the MB and lateral horn. PN identity is prespecified by neuroblast lineage and birth order rather than by ORN connectivity [11]. Two POU-domain transcription factors have been shown to be involved in this process: Acj6 and Drifter [80]. These are expressed in distinct subsets of PNs, where they are required for dendritic targeting in the AL; Acj6 also regulates axonal arborization in the lateral horn [80]. Thus, Acj6 acts at multiple stages of the olfactory pathway to generate functional diversity and wiring specificity.

Finally, the cell adhesion molecule N-cadherin was also recently found to have a role in the wiring of the olfactory system. In *N-cadherin* mutants, ORN axons target the appropriate region of the AL but fail to intermingle with PN dendrites [81]. PNs lacking *N-cadherin* successfully innervate their cognate glomeruli, but branch inappropriately, often resulting in the ectopic innervation of neighboring glomeruli [82]. The axons of mutant PNs also branch abnormally within the MB and lateral horn [82]. Thus, like Acj6, N-cadherin is required for multiple aspects of olfactory system development.

Conclusions and future directions

Our understanding of odor coding has increased dramatically in recent years, yet many questions remain. The mechanism by which an ORN selects a particular odorant receptor gene is likely to involve both a combinatorial code of transcription factors and a combinatorial code of *cis*-acting regulatory sequences adjacent to the odorant receptor genes. A major challenge for the future is to identify these *trans*- and *cis*-acting factors and to understand how they operate together to define the functional organization of the olfactory organs. Similarly, little is known about the mechanisms by which ORN axons find their targets in the brain. The question of how the odor code is translated at each stage of the olfactory pathway so as to ultimately support behavioral responses also remains unanswered. These questions have not been resolved in any organism but are now being addressed in *Drosophila* and intriguing answers are likely to be available soon.

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