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Sensory systems must map accurate representations of the external world in the brain. Although the physical senses of touch and vision build topographic representations of the spatial coordinates of the body and the field of view, the chemical sense of olfaction maps discontinuous features of chemical space, comprising an extremely large number of possible odor stimuli. In both mammals and insects, olfactory circuits are wired according to the convergence of axons from sensory neurons expressing the same odorant receptor. Synapses are organized into distinctive spherical neuropils—the olfactory glomeruli—that connect sensory input with output neurons and local modulatory interneurons. Although there is a strong conservation of form in the olfactory maps of mammals and insects, they arise using divergent mechanisms. Olfactory glomeruli provide a unique solution to the problem of mapping discontinuous chemical space onto the brain.

The olfactory system detects airborne organic and inorganic chemicals that originate from plant and animal metabolites and environmental and industrial sources. Odors mediate both innate and learned behaviors such as attraction and aversion, governing decisions to eat, mate, attack or flee from aggressors and predators. A remarkable feature of the olfactory system is the extraordinary diversity of possible odor molecules that exist. Although accurate measurements can be made of the detection range of visual wavelengths or auditory frequencies in humans, there are no reliable estimates of the number of odorous molecules that exist on earth or those that can be detected by a given animal species (Gilbert 2008). Nevertheless, the number of possible odorants is thought to be high, in the range of many thousands. Beyond odor detection lies the problem of odor discrimination: how can chemicals with slightly different physical properties be discriminated and associated with distinct odor percepts?

Experiments investigating the molecular logic of olfaction over the last 20 years have led to major insights into how animals solve the problem of detecting and discriminating a vast number of different odor stimuli (Axel 1995). Four organizational principles have emerged from this work. First, large numbers of distinct odorant receptor (OR) genes are dedicated

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to olfaction: ~1000 in mammals and ~100 in insects. Second, each olfactory sensory neuron (OSN) typically expresses only a single receptor out of this large repertoire. Each OR has a distinct odor ligand profile, such that there are ~1000 types of OSNs in the mouse and ~100 types in the typical insect. Third, OSNs expressing the same receptor extend axons that converge on the same glomerulus in the brain, forming a map of ORs in the brain. Fourth, each odor is encoded in a combinatorial manner: One odor can activate multiple ORs, and each OR can respond to multiple odors (Malnic et al. 1999; Hallem and Carlson 2006). As a result, odor information is spatially encoded in the first relay station of the brain (reviewed in Mori et al. 2006). Strikingly, these four principles along with the anatomical organization of the olfactory system are quite similar in mammals and insects, suggesting a mechanism of convergent evolution (Hildebrand and Shepherd 1997).

Recent studies in two genetic model organisms, the mouse and the fly, *Drosophila melanogaster*, have elucidated mechanisms governing how the topographic yet discrete olfactory map is established in the brain (see Luo and Flanagan 2007 for an excellent recent review). Although mice and flies have multiple olfactory subsystems and additional classes of chemosensory receptors (reviewed in Brennan and Zufall 2006; Touhara and Vosshall 2009), this article will focus on comparative mechanisms used to map ORs and OSNs from the main olfactory epithelium in the mouse and adult chemosensory structures in the fly to glomerular targets in the brain.

PERIPHERAL OLFACTORY ORGANIZATION AND ODORANT RECEPTOR GENE CHOICE

Sensory Neurons and Odorant Receptor Proteins in Mammals and Insects

In the main olfactory system of mammals, inhaled odorants are dissolved in the olfactory mucosa in the main olfactory epithelium (OE) (Fig. 1A). OSNs in the OE extend a single dendrite into the lumen of the nasal cavity. The dendrite gives rise to 20–30 ciliary processes whose membranes contain OR proteins. Mammalian ORs are seven transmembrane G protein-coupled receptors (GPCRs) and were first discovered in rodents in 1991(Buck and Axel 1991). The number of functional ORs varies widely across mammalian species from ~1000 in mice (Zhang and Firestein 2007) to ~400 in humans (Glusman et al. 2001).

Insect OSNs are anatomically similar to mammalian OSNs but are housed in specialized sensory hairs called sensilla that decorate the surface of the major olfactory organ, the antenna (Stocker 1994)(Fig. 2A,B). Insect ORs are seven transmembrane proteins that are evolutionarily distinct from mammalian ORs (Benton et al. 2006; Wistrand et al. 2006). The total number of such ORs in recently sequenced insect genomes varies from ~60 in *Drosophila* (Robertson et al. 2003) to ~260 in the red flour beetle *Tribolium* (Engsontia et al. 2008). Insect ORs are rapidly evolving and show little sequence similarity even within a given insect species. A single member of the insect OR super family, in contrast, is strongly conserved in all sequenced insect genomes (Krieger et al. 2003; Pitts et al. 2004; Jones et al. 2005). This gene, called Or83b in *Drosophila*, does not interact with odors but functions as a chaperoning coreceptor that assembles into a heteromeric complex with the ligand-binding ORs (Larsson et al. 2004; Nakagawa et al. 2005; Neuhaus et al. 2005; Benton et al. 2006). Thus although mammalian and insect OSNs are anatomically similar, the OR repertoire is smaller in insects than mammals and insect ORs are evolutionarily unrelated to vertebrate ORs.

Divergent Olfactory Signaling Mechanisms in Mammals and Insects

The binding of odor ligands to ORs in both mammals and insects activates a signaling cascade that results in depolarization of the OSN, but very different mechanisms activate mammalian and insect ORs. Mammalian ORs use canonical GPCR signaling to convert odor binding to neuronal depolarization. (reviewed in Ma 2007). OR signal transduction begins

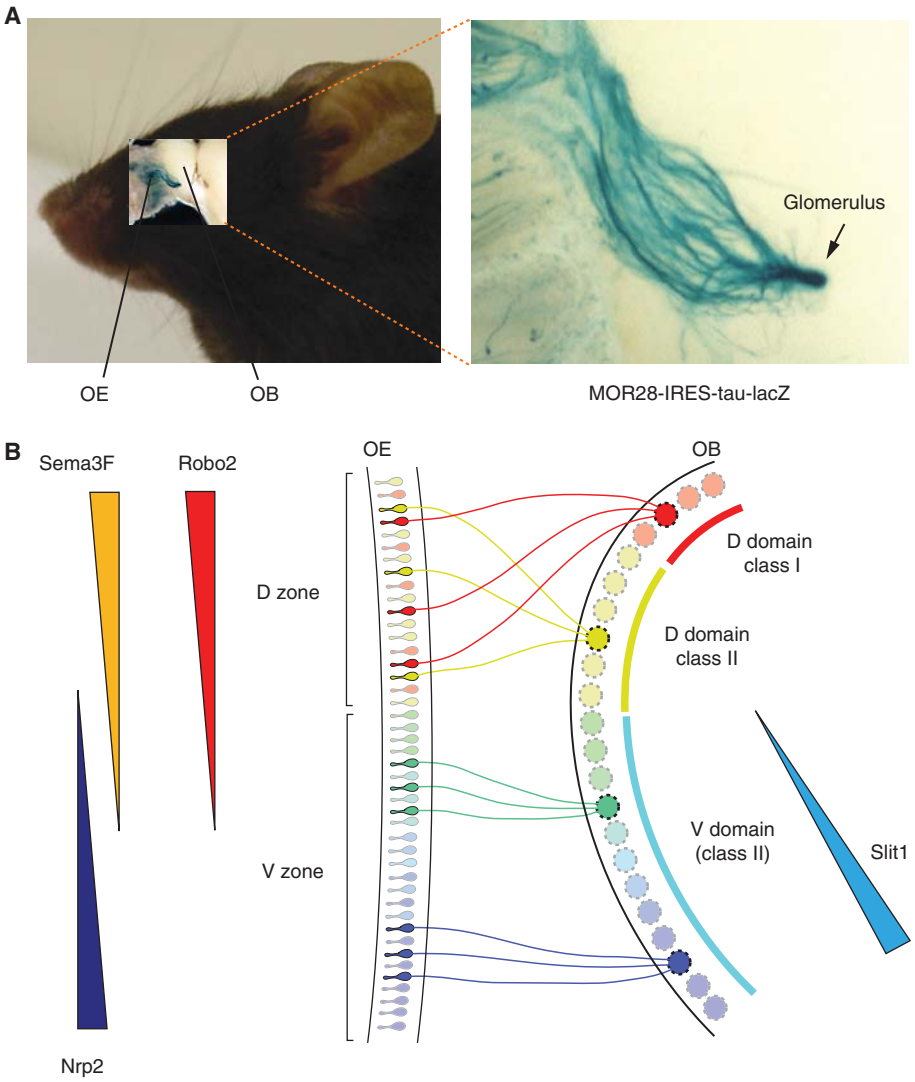

Figure 1. Olfactory sensory neuron projections along the dorsal–ventral axis in the mouse olfactory system. (*A*) Lateral view of a *MOR28-IRES-tau-lacZ* transgenic mouse (Serizawa et al. 2000) stained with X-gal to reveal OSNs in the OE and *tau-lacZ*-tagged *MOR28* axons projecting to a specific site forming a glomerulus in the OB. (*B*) OSNs in the dorsomedial zone (D zone) in the OE project their axons to the dorsal domain (D domain) of the OB. Class I ORs are mostly expressed by OSNs in the D zone in the OE, which target the anterodorsal cluster of the D domain in the OB (red). In the ventrolateral zone (V zone), each class II OR possesses its own unique expression area, which is distributed in a continuous and overlapping manner along the dorsomedial–ventrolateral axis in the OE. The dorsomedial–ventrolateral expression area in the OE corresponds to the glomerular positioning along the dorsal–ventral axis in the OB. Thus, the dorsal–ventral arrangement of glomeruli is roughly determined by the locations of OSNs in the OE. Axonal projection along the dorsal–ventral axis is determined by axon guidance molecules such as Nrp2, Sema3F, and Robo2 expressed in a graded manner in the OE. OSN, Olfactory sensory neuron; OE, olfactory epithelium; OB, olfactory bulb; OR, odorant receptor; D zone, dorsal zone; V zone, ventral zone.

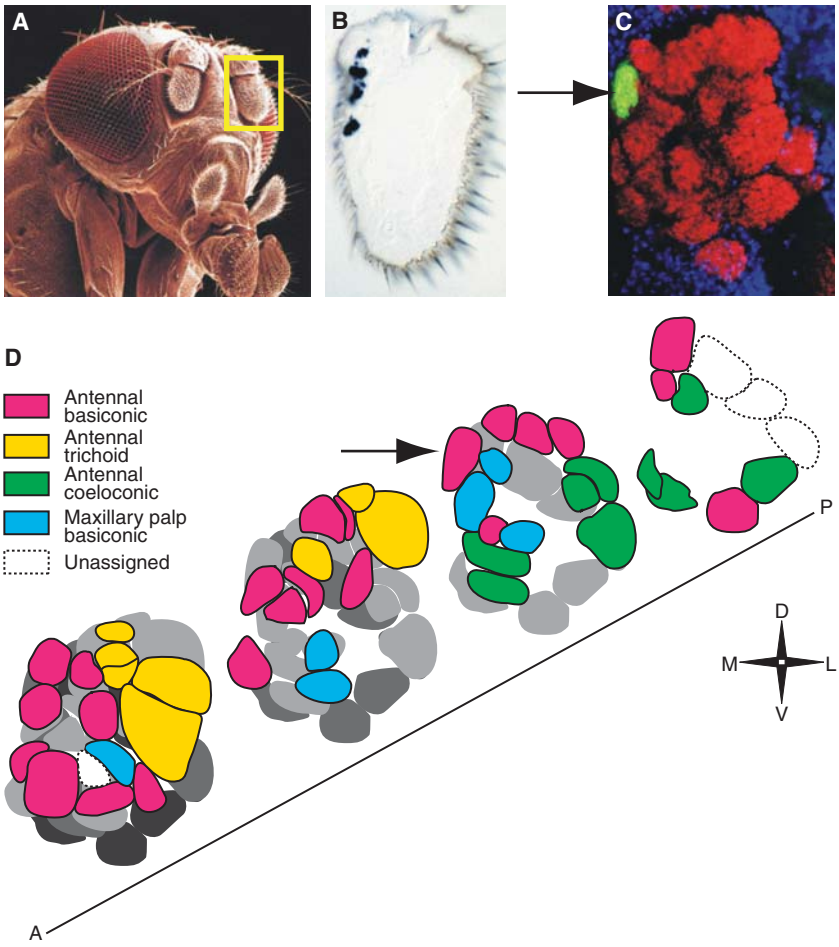

Figure 2. Functional connectivity in the *Drosophila* antennal lobe. (*A*) False-colored scanning electron micrograph image of a *Drosophila* head illustrating the major olfactory (antenna) and gustatory (proboscis) organs (photo: Jürgen Berger, Max Planck Institute for Developmental Biology, Tübingen, Germany). (*B*) Cross section of adult antenna hybridized with an in situ probe to reveal expression of *Or22a* mRNA. (C) Projection of Or22a-expressing axons to the DM2 glomerulus in the antennal lobe, as traced with n-synaptobrevin:GFP (green). Neuropil is counterstained with nc82 (red). (D) Connectivity map of the adult fly antennal lobe, with glomeruli color-coded according to OSN type at the periphery. The position of the *Or22a* glomerulus is marked by the arrow in panels (C,D). The antennal lobe is represented as four slices arrayed from anterior (A) to posterior (P), with glomerular position depth-coded such that white glomeruli are superficial, gray glomeruli intermediate, and black glomeruli deep.

with the heterotrimeric guanine nucleotide binding proteins G_{α}s and G_{α}olf, which stimulate adenylyl cyclase type III (ACIII), leading to cAMP production and the gating of a calcium permeable, tetrameric cyclic nucleotide gated (CNG) channel, which contains CNGA2 as an essential subunit. G_{α}olf, ACIII, and CNGA2 knockout mice are all anosmic (Brunet et al. 1996; Belluscio et al. 1998; Wong et al. 2000). Calcium influx through the CNG channel results in the opening of the chloride channel, ANO2 (Stephan et al. 2009), and the efflux of chloride, which depolarizes the OSN.

Insect ORs have seven transmembrane domains and although they lack sequence homology to mammalian ORs, they were generally

assumed to be GPCRs like mammalian ORs. Recent studies have questioned this assumption. Touhara and coworkers showed that expression of heteromeric insect olfactory receptors in heterologous cells was associated with an odor-gated cation current (Nakagawa et al. 2005). Further experiments that investigated the source of these currents led two groups to propose that insect ORs are odor-gated cation channels (Sato et al. 2008; Wicher et al. 2008), although the extent to which these channels are modulated by G-protein signaling remains controversial (Nakagawa and Vosshall 2009).

Odorant Receptor Gene Choice: Stochastic in Mice, Deterministic in Flies

In both mammals and flies, OR gene expression is tightly regulated to ensure that one (or in insects rarely more than one) ligand-binding OR is expressed per OSN. Such exclusion ensures that a given OSN has a limited range of chemical specificities. Interestingly, strict regulation to limit gene expression to one OR per OSN is achieved by entirely different mechanisms in mice and flies.

In the mammalian olfactory system, each OSN expresses only one OR from a large repertoire (Malnic et al. 1999) and expression is limited to either the maternal or paternal allele (Chess et al. 1994). This monogenic and monoallelic mode of receptor gene choice is known as the "one neuron—one receptor rule." Remarkably, this exclusion is observed even among OR-containing transgenes integrated in tandem on the same chromosome (Serizawa et al. 2000). How do rodent OSNs ensure that only a single allele of one OR is functionally expressed? Although vertebrate antigen receptor genes are stochastically chosen by irreversible DNA rearrangements, OR gene choice does not operate by such a mechanism (Eggan et al. 2004; Li et al. 2004). Monogenic OR expression appears to be established by the combination of positive and negative regulation (reviewed in Serizawa et al. 2004). Positive regulation is proposed to operate via a *cis*-acting locus control region (LCR) capable of activating only one OR gene promoter at a time by physical interaction (Serizawa et al. 2003; Lomvardas et al. 2006). Because there are more than 40 different OR gene clusters, each of which may contain its own LCR, the LCR model alone cannot account for monogenic OR expression (Fuss et al. 2007; Nishizumi et al. 2007).

Once a functional OR gene is expressed, the OSN must have a mechanism to prevent the expression of all other ORs, including the allelic OR expressed from the opposite chromosome. There is evidence that the functional OR protein itself contributes to the suppression OR expression from other loci. The deletion of, or a frame shift mutation within, the OR coding region permits the coexpression of a second OR gene (Serizawa et al. 2003; Feinstein et al. 2004; Lewcock and Reed 2004; Shykind et al. 2004). Forced expression of an OR using heterologous promoters can suppress the expression of endogenous ORs (Nguyen et al. 2007; Fleischmann et al. 2008). Because OR-mediated negative feedback does not require G protein signaling (Imai et al. 2006), the exact nature of the negative feedback signal is unclear. In young animals, a small number of OSNs misexpresses multiple ORs, which however are developmentally eliminated in an activity-dependent manner (Tian and Ma 2008).

The apparently stochastic mechanism of OR gene choice in the mouse contrasts with deterministic receptor gene choice in the fly. In *Drosophila*, there are approximately 1200 OSNs distributed across the two major sensory organs, the antenna and maxillary palp (Stocker 1994). These organs are covered with three different types of olfactory sensilla, the basiconic, trichoid, and coeloconic. ORs are expressed in all basiconic and trichoid sensilla and one class of coeloconic sensilla, each of which houses between 1 and 4 OSNs (Stocker 1994). The position of identified sensilla is stereotyped between individual flies (de Bruyne et al. 2001). Because the number of neurons is many orders of magnitude smaller than in the mouse, it has been possible to map in detail which OR is expressed in which OSN, to identify the corresponding sensilla type and position, and to determine which odors activates most fly ORs (de Bruyne et al. 2001; Dobritsa et al. 2003;

Hallem et al. 2004; Couto et al. 2005; Goldman et al. 2005; Hallem and Carlson 2006). From this comprehensive body of work, it is known that the identity of ORs in a given sensillum is tightly regulated (Dobritsa et al. 2003; Hallem et al. 2004; Couto et al. 2005) and depends on Notch signaling (Endo et al. 2007). Members of the POU homeodomain transcription factor family govern the selective expression of ORs in defined OSNs (Clyne et al. 1999a; Ray et al. 2007; Ray et al. 2008). Although *Drosophila* OSNs typically express a single OR together with the Or83b coreceptor, there are numerous instances of two or three ligand-binding ORs coexpressed in a single OSN (Goldman et al. 2005). Further, there is no evidence of either monoallelic expression or the exclusion mechanisms observed in rodents. OSNs in the fly can be transgenically altered to express ectopic ORs, which change the functional tuning of the reprogrammed OSN (Dobritsa et al. 2003; Goldman et al. 2005). Insect OR gene regulation therefore differs in being simpler and more deterministic than the poorly understood process occurring in mammals.

Spatial Organization of the Rodent Olfactory Epithelium and *Drosophila* Antenna

In both rodents and the fly, peripheral olfactory organs show functional organization. In rodents, OSNs expressing a given type of OR are scattered in a restricted area, or zone, in the OE (Ressler et al. 1993; Vassar et al. 1993). Using zone-specific marker genes, one can divide the OE into the dorsomedial (D) and ventrolateral (V) zones (Fig. 1B). The OR genes are divided phylogenetically into two different classes, I and II, based on homology of deduced amino acid sequences (Glusman et al. 2001; Zhang and Firestein 2002; Tsuboi et al. 2006). Although Class I ORs are mostly expressed within the D zone (Zhang et al., 2004; Tsuboi et al., 2006), class II OR genes are expressed in both D and V zones. Within the D zone, class I and class II expressing cells are intermingled and there is no restricted distribution for both classes (Tsuboi et al. 2006). In the V zone, however, each class II OR gene possesses its unique expression area, distributed in a continuous and overlapping manner along the dorsomedial–ventrolateral axis in the OE (Miyamichi et al. 2005).

In *Drosophila*, sensilla types are distributed across the antenna in a pattern reminiscent of zon**es** in the mouse OE. Trichoid sensilla are restricted to the lateral–distal portion of the antenna, whereas large basiconic sensilla are concentrated in the medial–proximal region of the antenna. Small basiconic and coeloconic sensilla are interspersed in other regions of the antenna. OR gene expression is dispersed among these sensilla types, with a given OR being expressed in ~10–50 OSNs (Clyne et al. 1999b; Vosshall et al. 1999). As in the mouse, OSNs expressing a given OR are interspersed among OSNs expressing different receptors.

OLFACTORY CIRCUITS IN THE BRAIN

Axonal Convergence and the Topographic Map in the Rodent Olfactory Bulb

How is odor information represented in the OB? Each OSN projects a single unbranched axon to the ipsilateral olfactory bulb (OB) of the brain, where it forms synapses with second-order neurons, the mitral and tufted cells. OSNs expressing the same OR converge their axons to two of ~2000 glomeruli located in stereotyped positions on the OB, one on the medial and the other on the lateral surface (Ressler et al. 1994; Vassar et al. 1994; Mombaerts et al. 1996). As a consequence of this topographic map formation, each OR—and the odors that activate it—is represented in the OB as the pattern of activated glomeruli.

In the OB, odorant signals originating in OSNs are relayed to two types of excitatory projection neurons, the mitral and tufted cells. Each glomerulus is innervated by 20–50 mitral/tufted cells, each of which innervates a single glomerulus with a single primary dendrite. Tufted cells project axon branches to the isotypic olfactory columns on the mirror-symmetrical positions, forming intrabulbar connections (Lodovichi et al. 2003). In addition

to the excitatory neurons, there are several types of inhibitory interneurons, the periglomerular cells and granule cells (reviewed in Wilson and Mainen 2006).

Topographic Mapping in the *Drosophila* Antennal Lobe

The antennal lobe in *Drosophila* is anatomically homologous to the rodent olfactory bulb, but significantly simpler because it houses only ~50 glomeruli (Laissue et al. 1999). OSNs in the antenna and maxillary palp each extend a single axon, which fasciculates into the antennal or maxillary nerve and initially targets the periphery of the antennal lobe before individual axons target stereotyped glomeruli (Fig. 2). Within glomeruli, OSNs synapse with projection neurons (PNs), the functional equivalent of mitral and tufted cells. Each PN extends a dendrite that branches within a single glomerulus and an axon that targets higher brain regions, the mushroom body and lateral horn of the protocerebrum. Local inhibitory neurons interact with both OSNs and PNs to modulate olfactory signals (reviewed in Wilson and Mainen 2006).

As in mice, all fly OSNs expressing a given OR target a single glomerulus (Gao et al. 2000; Vosshall et al. 2000; Couto et al. 2005; Fishilevich and Vosshall 2005) (Fig. 2B,C). Unlike in the mouse, there is no obvious relationship between the position of a group of OR-expressing OSNs in the periphery and its target in the antennal lobe (Fig. 2D). However, there is a rough organization of glomeruli according to sensilla type: trichoid OSNs tend to target the anterior–lateral antennal lobe, coeloconic sensilla mostly target the posterior antennal lobe, and basiconic sensilla the anterior medial-dorsal antennal lobe.

MECHANISMS OF OLFACTORY MAP FORMATION IN MAMMALS

Topographic Correlations between the Epithelium and Bulb

Wiring up several millions OSNs expressing up to 1000 ORs to 2000 OB glomeruli is a staggering organizational problem. One parameter that defines the axonal projection site is positional information in the OE. Antibody staining of zone-specific molecules showed a zone-to-zone correlation: OCAM-positive V zone OSNs project axons to the ventral (V) domain of the OB, whereas NQO-1-positive D zone OSNs project axons to the dorsal (D) domain of the OB (Alenius and Bohm 1997; Yoshihara et al. 1997; Yoshihara and Mori 1997; Gussing and Bohm 2004) (Fig. 1B). Systematic in situ hybridization and genetic labeling indicated that OSNs expressing class I ORs project their axons to the most anterodorsal areas (DI) within the D domain (Tsuboi et al. 2006; Kobayakawa et al. 2007; Bozza et al. 2009). The OR-specific expression area along the dorsomedial–ventrolateral axis of the V zone OE is well correlated with D–V positioning of glomeruli in the OB (Miyamichi et al. 2005). In contrast, no discernible correlation was found for the anterior-posterior (A-P) axis. These observations suggest that spatial information in the OE contributes to D-V glomerular positioning in the OB. This notion was also supported by transgenic experiments. When the expression areas of ORs were shifted or broadened, projection sites adjusted accordingly along the D-V axis in the OB (Vassalli et al. 2002; Miyamichi et al. 2005).

Neuropilin-2 (Nrp2) and Robo-2 are good candidate guidance molecules determining the D–V axis, because they show graded expression in the OE (Norlin et al. 2001; Cho et al. 2007). Nrp2 is expressed in a ventral-high and dorsal-low gradient, whereas Robo-2 expression shows a dorsal-high and ventral-low gradient in the OE and OSN axon termini (Fig. 1B). Mice deficient for Robo-2 have defective axonal projections along the D–V axis (Cho et al. 2007; Nguyen-Ba-Charvet et al. 2008).

Each OB has mirror-symmetric olfactory maps on the medial (M) and lateral (L) sides. OSNs in the anterior and lateral OE tend to project axons to the lateral OB, whereas OSNs in the medial and posterior OE tend to project axons to the medial OB (Levai et al. 2003). Disruption of IGF signaling affects OSN projections to the lateral OB, although neither

IGF nor IGF receptors show apparent M–L asymmetry (Scolnick et al. 2008). The molecules that distinguish the M–L OB are not known.

OR-Instructed Glomerular Positioning along the A–P Axis

An early and appealing proposal to solve the complex mammalian OSN wiring problem posited that the ORs themselves provided topographic information. The involvement of OR proteins in OSN projections was investigated using genetically engineered mutant mice (Mombaerts et al. 1996). When the coding region of a particular OR gene was replaced with that of another OR gene, axons expressing the swapped OR gene targeted a novel position in the OB (Wang et al. 1998; Feinstein and Mombaerts 2004). Thus, the OR protein appeared to play an instructive role in projecting OSN axons (Mombaerts 2006). Interestingly, OR proteins can be detected not only in olfactory cilia and soma, but also at axon termini (Barnea et al. 2004; Strotmann et al. 2004).

Later experiments complicated the simple view that OR proteins directly instructed guidance decisions. Instead, it appears that OR-instructed axonal projection is regulated by G protein-mediated cAMP signaling independent of the odor ligand specificity of a given OR (Imai et al. 2006; Chesler et al. 2007). Axons expressing a mutant OR-I7 that cannot activate G proteins remained in the anterior region of the OB and failed to converge to a specific glomerulus. However, coexpression of a constitutively active $G_{\alpha}s$ mutant restored axonal convergence and glomerular formation defects. Partial rescue was also observed with the constitutive active mutants of PKA and CREB. Thus, $G_{\alpha}s$ and PKA-mediated transcriptional regulation is required for OSN projections. This conclusion is consistent with earlier findings that $G_{\alpha}s$-coupled β2-adrenergic receptor can instruct OSN projection when swapped with OR-M71, whereas the $G_{\alpha}i2$-coupled vomeronasal receptor, V1rb2, cannot (Feinstein et al. 2004). Further, constitutively active $G_{\alpha}s$ or dominant-negative PKA, when coexpressed with wild-type OR-I7, causes a posterior or anterior shift of glomeruli, respectively (Imai et al. 2006). Topographic map formation in the OB is disrupted in ACIII mutant mice (Col et al. 2007; Zou et al. 2007). These findings suggest that the quantitative levels of OR-derived cAMP signals determine A–P positioning of OB glomeruli independent of the function of the OR as ligand-binding receptor (reviewed in Imai and Sakano 2007).

How do different levels of cAMP originating from G-protein signaling lead to differential A–P positioning of OSN axons in the OB? cAMP regulates the transcription of axon guidance molecules such as Neuropilin-1 (Nrp1) (Imai et al. 2006) (Fig. 3). When protein levels are measured in axon termini of OSNs, Nrp1 was found in an anterior-low and posterior-high gradient. Increases or decreases of Nrp1 caused posterior or anterior glomerular shifts, respectively (Imai et al. 2009). Consistent with the hypothesis that Nrp1 regulates A–P positioning, the A–P topography of the glomerular map is perturbed in mice deficient for Nrp1 or its repulsive ligand, Sema3A (Schwarting et al. 2000; Taniguchi et al. 2003; Imai et al. 2009).

Axon–Axon Interactions and Topography of the Olfactory Bulb Map

How do Nrp1 and Sema3A establish the A–P topography of the olfactory map? It is generally believed that the topography of the neural map is determined by positional cues present on the target. However, OSN axons projecting to distinct destinations are presorted in the axon bundle before arrival at the OB, suggesting that pretarget mechanisms also account for the topographic map formation. Indeed, A–P topography of OSN axons can be formed in *Gli3* mutant mice that completely lack the OB (St John et al. 2003; Imai et al. 2009). Gain-of-function and loss-of-function experiments revealed that Nrp1 levels determine the pattern of pretarget axon sorting in the bundles. Nrp1 and its repulsive ligand, Sema3A, are expressed in a complementary manner in OSNs (Fig. 3). OSN-specific knockout of Sema3A altered the

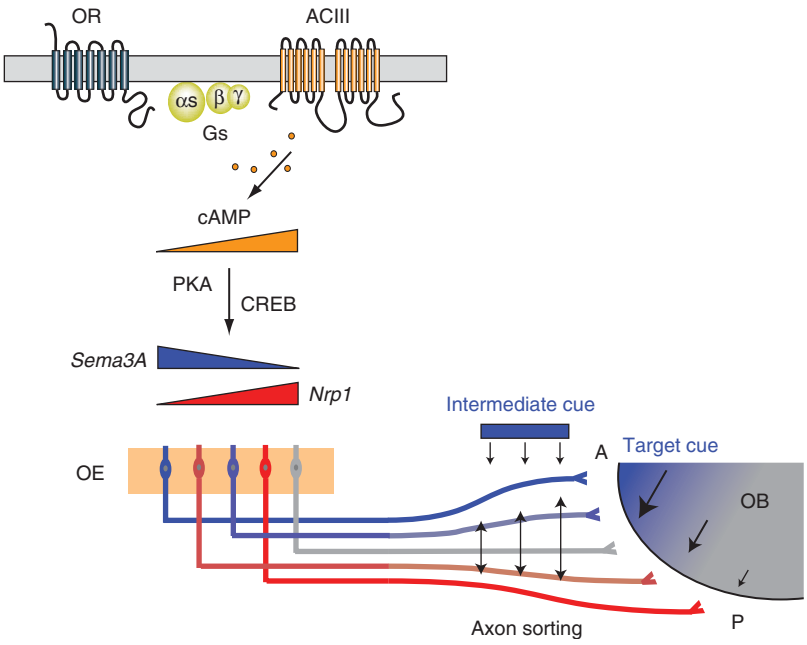

Figure 3. Olfactory sensory neuron projections along the anterior–posterior axis in the mouse olfactory system. Each OR is thought to generate a unique level of cAMP signaling, which is converted to relative expression levels of axon guidance molecules such as *Nrp1* and *Sema3A* via cAMP-dependent protein kinase A (PKA) and cAMP response element binding protein (CREB). An axon guidance receptor, Nrp1, and its repulsive ligand, Sema3A, are expressed in a complementary manner in OSNs and regulate pretarget sorting of axons. This axon sorting mechanism may be important to establish the topographic order in the OB based on the relative expression levels of guidance molecules expressed by axons. Once OSN axons are sorted, they need to be oriented along the correct axis before projecting onto the OB. This probably requires an intermediate cue, possibly encoded by Sema3A derived from the target or along the pathway between the OE and OB. A, anterior; P, posterior.

pretarget axon sorting as well as olfactory map topography (Imai et al. 2009). Thus, pretarget axon–axon interaction is important for topographic map formation (Imai et al. 2009). In the visual system, the "relative" expression levels of axon guidance receptors determine axonal projection sites (Brown et al. 2000). Axon–axon interactions may be a general strategy to pattern topographic maps based on relative expression level of axon guidance molecules expressed by axons.

Theoretically, however, axon–axon interactions alone cannot determine the axis of the map. Guidance cues on the target, as well as intermediary cues, may help orient the presorted axons to a correct orientation on the target. In the olfactory system, Sema3A expressed by ensheathing glia appears to function as an intermediate cue. Such intermediate guidance cues have been reported for the topographic projections of thalamo-cortical axons (Dufour et al. 2003; Seibt et al. 2003).

Axon–axon interactions may be important for the process of OSN regeneration as well (St. John and Key 2003). OSNs turn over continuously throughout life, and newly generated OSNs project axons to the preexisting map with high precision (Gogos et al. 2000). The ability of the olfactory map to rewire itself even in older animals provides a mechanism to maintain constancy in odor perception throughout life.

Neuronal Activity-dependent Local Axon Sorting to Form the Discrete Map

After OSN axons are sorted to approximate D–V and A–P positions in the OB, they are further sorted locally to establish the discrete

glomerular structures and such sorting is influenced by neuronal activity in OSNs. The CNGA2 gene is X-linked and, therefore, female heterozygous mutant of this gene are mosaic because of random X-inactivation (Zhao and Reed 2001). In these mice, glomeruli for some ORs are duplicated to produce one CNGA2-positive and one CNGA2-negative glomerulus (Zheng et al. 2000; Serizawa et al. 2006). Genetic silencing of neuronal activity in OSNs perturbs local fasciculation of OSN axons (Yu et al. 2004). These observations indicate that neuronal activity affects local sorting of OSN axons.

In OSNs, neuronal activity regulates various genes such as those coding for homophilic adhesive molecules Kirrel2/Kirrel3 and repulsive molecules ephrin-A5/EphA5, thereby regulating local axon sorting (Serizawa et al. 2006) (Fig. 4). In CNGA2 knockout mouse, *Kirrel2* and *EphA5* were down-regulated, whereas

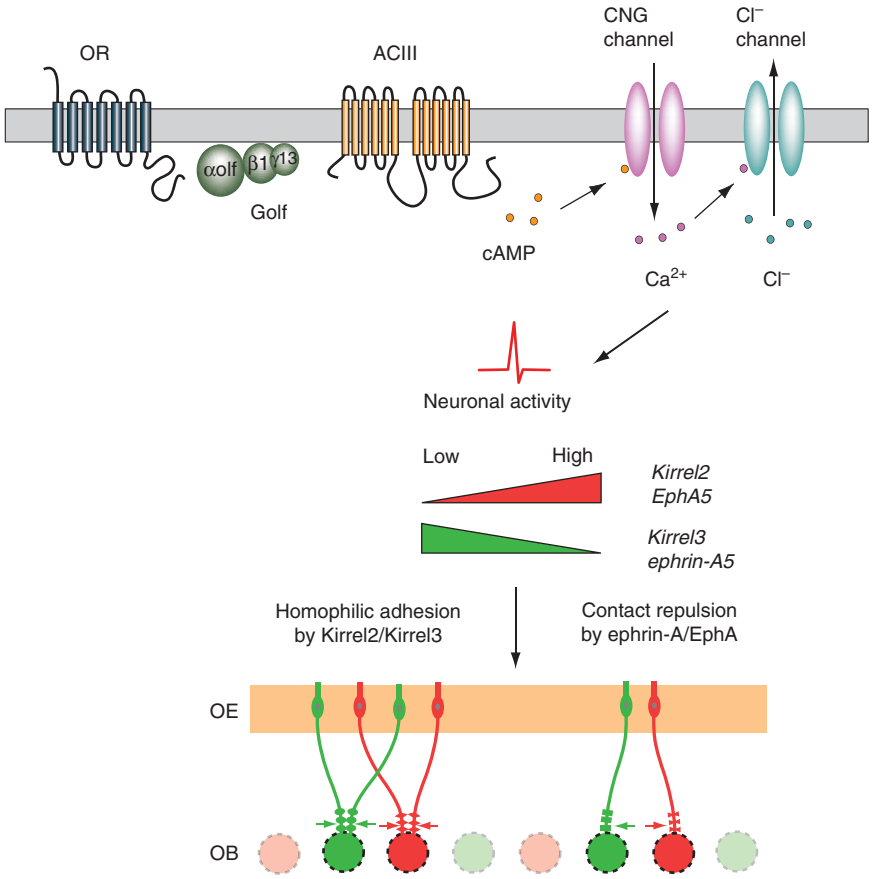

Figure 4. Activity-dependent local axon sorting to form a discrete map on the mouse olfactory bulb. Although OR/cAMP signals regulate coarse targeting of OSN axons along the A–P axis at an earlier stage of patterning, neuronal activity generated by OR/cAMP signals regulates local axon sorting at a later stage. This may indicate that cAMP signals are generated by different mechanisms in earlier and later stages (reviewed in Imai and Sakano 2008). Levels of neuronal activity determine different sets of axon guidance and adhesion molecules, including Kirrel2/Kirrel3, ephrin-A/EphA, and BIG2. Expression levels of these molecules are affected by nares occlusion, although those of Nrp1/Sema3A are not. Kirrel2/Kirrel3 mediates local fasciculation of like axons via homophilic adhesion activities, whereas ephrin-A/EphA is thought to segregate heterotypic axons via contact repulsion. BIG2 is involved in heterophilic adhesion. (CNG) cyclic nucleotide gated channel, (ACIII) adenylyl cyclase III.

Kirrel3 and *ephrin-A5* were up-regulated, indicating that these genes are transcribed in an activity-dependent manner. Gain of function of these genes with transgenic mosaic analysis causes duplication of glomeruli for some ORs (Cutforth et al. 2003; Serizawa et al. 2006). In the axon termini of OSNs, these molecules are expressed at differential levels in an OR-specific manner, showing a mosaic pattern of glomeruli with different expression levels. BIG2 is also expressed at axon termini of OSNs in an OR-specific manner, and facilitates local axon sorting with unknown heterophilic binding partners (Kaneko-Goto et al. 2008).

Activity-Dependent Refinement and Maintenance of the Olfactory Bulb Map

Activity-dependent refinement, which follows the initial targeting processes, plays an important role in many sensory systems during development. In the mouse olfactory system, satellite glomeruli are ectopically formed in young animals. Such glomeruli are gradually eliminated with age, refining the glomerular map. In mice deficient for *CNGA2* or whose nares are surgically occluded, these ectopic glomeruli persist longer or fail to be eliminated altogether (Zheng et al. 2000; Nakatani et al. 2003; Zou et al. 2004).

Neuronal activity is required for the maintenance of the glomerular map (Zhao and Reed 2001). In mosaic female mice, CNGA2-negative cells are eliminated when they face competition from neighboring functional OSNs, but survive under the noncompetitive conditions operating under nares occlusion. Thus refinement and maintenance appears to be regulated in part at the level of cell survival in the mouse.

MECHANISMS OF OLFACTORY MAP FORMATION IN *DROSOPHILA*

Same but Different: Divergent Mechanisms of Glomerular Targeting in Flies

Convergence of like axons to stereotyped glomeruli where synapses form with relay and local interneurons is a feature of both mammalian and insect olfactory systems. However, the underlying developmental mechanisms are remarkably different. First, there is a considerable difference in scale between mouse and fly olfactory systems, with the fly having ~4000-fold fewer OSNs, ~40-fold fewer glomeruli, and ~20-fold fewer ORs. Second, fly OSNs do not regenerate throughout the life of the animal. Antennal lobe connections formed during development are fixed throughout life. Third, ORs appear to play no functional role in wiring the glomerular map. Flies lacking the Or83b coreceptor, required for basal spontaneous and odor-evoked activity in OSNs, show normal map formation (Larsson et al. 2004). OSNs retaining Or83b but lacking the ligand-binding receptor Or22a target the appropriate DM2 glomerulus (Fig. 2B,C), and this is not altered by ectopic expression of another receptor, Or47a (Dobritsa et al. 2003). Further, there is also no evidence of OR protein localized to OSN axons (Elmore and Smith 2001; Dobritsa et al. 2003; Larsson et al. 2004). Fourth, there is no evidence for monogenic or monoallelic expression. All fly OSNs express at least two ORs, one or more ligand-binding ORs and the Or83b coreceptor, and there is no regulatory barrier preventing the transgenic expression of additional ORs in a given OSN. Fifth, there is no evidence of a requirement for neuronal activity for the establishment, refinement, or maintenance of the glomerular map. The antennal lobe map is remarkably stable even when all odor-evoked activity is eliminated (Larsson et al. 2004) or when populations of target PNs or afferent OSNs are ablated (Tanaka et al. 2004; Berdnik et al. 2006). Therefore the insect antennal lobe is patterned by hard-wired, OR- and activity-independent mechanisms distinct from those used by mammals.

Independent Developmental Specification of Input and Output Neurons

What are the rules that wire up the insect olfactory system, if the ORs themselves have no influence over map formation? The smaller scale of the *Drosophila* olfactory system, and the availability of excellent cell-type specific markers

such as the ORs, has made this a powerful system to study the rules of axon guidance. The ~50 *Drosophila* glomeruli have stereotyped shapes, sizes, and positions in the antennal lobe (Laissue et al. 1999) and forward genetics can be used to identify genes that disrupt any aspect of the wiring problem: The specification of OSN identity, the precise targeting of OSN axons, and the dendritic patterning of PNs.

An important early discovery in this field was that OSNs and PNs are independently specified to target a defined area in the antennal lobe. Asymmetric Notch signaling is important to specify the identity of OSNs in a given sensillum (Endo et al. 2007) (Fig. 5A). Notch signaling in OSN precursors initiates a series of lineage decisions that directs both the choice of an OR and glomerular target in the antennal lobe. Combinatorials of POU homeodomain BTB-zinc finger transcription factors govern both OR gene choice and wiring specificity of OSNs and PNs (Clyne et al. 1999a; Komiyama et al. 2003; Komiyama et al. 2004; Zhu et al. 2006b; Spletter et al. 2007). Cells that are physically adjacent in the antenna can target either adjacent glomeruli or widely separated glomeruli in the antennal lobe. Therefore, the spatial relationships that govern position in the rodent OE and targets in the OB are not evident in the fly.

For OSN axons to synapse appropriately with PN dendrites, two contrasting models can be envisioned: Either afferent or target neurons could set up a prepattern that then attracts the matching neuron or OSNs and PNs could be independently specified to target the appropriate region. To test these models, Luo and colleagues used the powerful MARCM (mosaic analysis with repressible cell marker) technique (Lee and Luo 1999) to label individual PN precursors during fly development. They discovered that PNs are prespecified by birth order and cell lineage to target a specific glomerulus and do so before the arrival of OSNs (Jefferis et al. 2001) (Fig. 5B). Careful analysis of the developmental timing of OSN axon extension further suggested that PNs establish a glomerular prepattern long before the arrival of incoming OSNs (Jefferis et al. 2004). This suggests that global patterning cues exist in the antennal lobe to permit a rough spatial patterning of PN dendrites that is later refined both by interactions between PNs and also the later influence of incoming OSN axons. One of these cues is Wnt5 signaling mediated by the derailed receptors, Drl and Drl-2. When these signaling pathways are disrupted, both OSN axons and PN dendrites are disorganized (Yao et al. 2007; Sakurai et al. 2009).

Mechanisms of Olfactory Axon Guidance and Dendritic Patterning in the *Drosophila* Antennal Lobe

Forward genetic screens in *Drosophila* have identified a number of cell adhesion and axon guidance molecules that instruct axon and dendrite patterning in the antennal lobe (Fig. 5B). Mutant animals in which all OSNs lack N-cadherin show anomalies in glomerular map formation. OSN axons arrest at the border of the antennal lobe and fail to invade their appropriate glomerular targets, and accordingly glomeruli fail to form in N-cadherin mutants (Hummel and Zipursky 2004). When subsets of N-cadherin-negative OSNs were examined, their axons were seen to target an area of the external surface of the bulb that corresponds roughly with the wild type glomerular position (Hummel and Zipursky 2004). Therefore, OSNs are likely to be specified to recognize global cues, independent of positional information encoded by PN dendrites. N-cadherin also plays an important role in patterning PN dendrites. PNs lacking N-cadherin target their target glomeruli appropriately, but show ectopic innervation of adjacent glomeruli (Zhu and Luo 2004). Therefore dendrite–dendrite interactions mediated by N-cadherin serve to restrict PN dendrites to a single glomerulus.

What are the mechanisms that restrict OSN and PN processes to a single glomerulus? Some clues come from analysis of flies mutant for the Ig-superfamily protein DSCAM, which uses alternative splicing to generate up to 38,000 distinct protein isoforms. The axonal targeting of a subset of OSNs requires DSCAM (Hummel et al. 2003; Hattori et al. 2007) and DSCAM

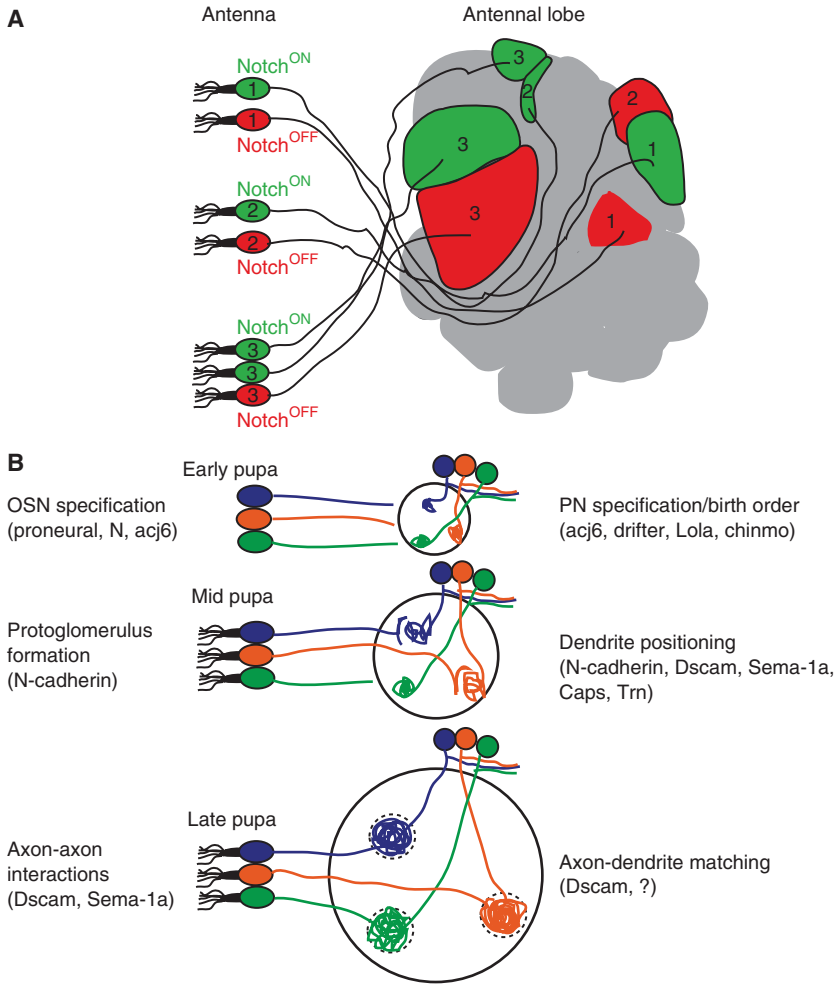

Figure 5. Molecular determinants of neuronal identity and glomerular targeting in the *Drosophila* antennal lobe. (*A*), Notch signaling specifies cell fate of OSNs housed in the same sensillum. Three arbitrary sensilla (1–3) housing either 2 or 3 OSNs are depicted with the corresponding axonal targets of the individual OSNs. Although Notch^{ON} and Notch^{OFF} cells are adjacent in a given antennal sensillum, their targets are broadly dispersed in the antennal lobe (Endo et al. 2007). (*B*), Schematic of developmental events and pathways patterning the fly olfactory system. In early pupal stages PNs have already targeted dendrites to appropriate positions where future glomeruli will form, whereas OSN axons have only reached the edge of the antennal lobe. In midpupation, some OSN axons begin to enter the antennal lobe and target the appropriate glomerular region. Late in pupal life, all OSNs have arrived and synaptic matching refines the connections of OSN axons and PN dendrites. The genes known to be involved in each of these steps are indicated and discussed in the text.

mutant PNs fail to elaborate fully branched dendrites (Zhu et al. 2006a). When DSCAM is overexpressed in a subset of PNs, their dendrites were shifted ventrally to a novel glomerulus and the OSNs that normally synapse with the PNs were correspondingly shifted to the new position (Zhu et al. 2006a). When the diversity of DSCAM isoforms was artificially reduced to a single protein, OSN axons were severely disordered and formed many ectopic connections (Hattori et al. 2007). This suggests that DSCAM organizes axonal projections of OSNs and coordinates matching between OSN axons and PN dendrites. It remains to be resolved how and

whether the diversity of DSCAM isoforms instructs glomerular specificity of either or both cell types, although an instructional role for DSCAM has been shown at other fly synapses (Hattori et al. 2009).

Gradients of signaling and axon guidance molecules are crucial for the correct patterning of the mammalian OB. Although Sema3A/Nrp1 controls A–P glomerular targeting of mouse OSNs, the *Drosophila* semaphorin Sema-1a plays a role in the patterning of both OSN axons and PN dendrites (Komiyama et al. 2007; Lattemann et al. 2007; Sweeney et al. 2007). In OSNs, Sema-1a/PlexinA-mediated axon–axon interaction is required to sort early-arriving and late-arriving axons and prevent mistargeting (Lattemann et al. 2007; Sweeney et al. 2007). In PN dendrites, Sema-1a is expressed in a D–L high to V–M low gradient that is important for proper dendritic targeting in a cell-autonomous fashion (Komiyama et al. 2007). Although Sema-1a shows continuous and graded expression in PN dendrites, recent work suggests a new mechanism for discrete patterning of PN dendrites. The transmembrane leucine-rich repeat proteins Capricious (Caps) and Tartan (Trn) were found to be expressed in subsets of PNs that target distinct glomeruli (Hong et al. 2009). When *caps* is deleted from $Caps^{+}$ PNs, their dendrites invade glomeruli occupied by $Caps^{-}$ PNs. Thus Caps provides a mechanism to assign a discrete cell–surface identity code to different PNs. Because Caps provides information to divide the antennal lobe into two populations, additional cell–surface codes still to be described would be required to specify a unique identity to each of the ~50 antennal lobe glomeruli.

CONCLUDING REMARKS

In both mammals and insects, olfactory information is organized according to the selective wiring of axons linked to OSNs expressing the same OR. This elegant organizational scheme arrays olfactory information in a receptor-topic fashion on the olfactory bulb and antennal lobe. In both experimental systems, axon–axon interactions play an important role as do gradients of axon guidance molecules that provide global positional information. Although the final pattern of the primary olfactory center is homologous in rodents and flies, the detailed molecular mechanisms that give rise to this pattern are largely distinct. This suggests mechanisms of convergent evolution acted to produce an optimal solution to wiring olfactory information into the brain. There has been great progress toward elucidating how large numbers of molecularly distinct OSN axons target distinct glomeruli in the brain. What remains to be solved is the exact molecular code that matches OSNs and postsynaptic neurons to unique glomeruli. Once solved, such a code would likely provide insights into synaptic patterning throughout the nervous system.

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