Genetic Control of Wiring Specificity in the Fly Olfactory System

Weizhe Hong*,[1] and Liqun Luo[†]

*Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California 91125, and [†]Department of Biology, Howard Hughes Medical Institute, Stanford University, Stanford, California 94305

ABSTRACT Precise connections established between pre- and postsynaptic partners during development are essential for the proper function of the nervous system. The olfactory system detects a wide variety of odorants and processes the information in a precisely connected neural circuit. A common feature of the olfactory systems from insects to mammals is that the olfactory receptor neurons (ORNs) expressing the same odorant receptor make one-to-one connections with a single class of second-order olfactory projection neurons (PNs). This represents one of the most striking examples of targeting specificity in developmental neurobiology. Recent studies have uncovered central roles of transmembrane and secreted proteins in organizing this one-to-one connection specificity in the olfactory system. Here, we review recent advances in the understanding of how this wiring specificity is genetically controlled and focus on the mechanisms by which transmembrane and secreted proteins regulate different stages of the *Drosophila* olfactory circuit assembly in a coordinated manner. We also discuss how combinatorial coding, redundancy, and error-correcting ability could contribute to constructing a complex neural circuit in general.

The Larry Sandler Memorial Lecture was established in recognition of Dr. Larry Sandler's many contributions to Drosophila genetics and his dedication to the training of Drosophila biologists. The recipient of the Larry Sandler Award for the most outstanding Ph.D. dissertation submitted presents the Larry Sandler Memorial Lecture at the Annual Genetics Society of America Drosophila Research Conference. As the 2013 Larry Sandler Award winner, Weizhe Hong was invited to write the following Review article on the subject of his dissertation.

THE precise assembly of neural circuits during development is required for the proper function of the nervous system. One of the key steps in establishing a functional circuit is to ensure that neurons make precise connections with appropriate synaptic partners. Deciphering the molecular mechanisms of how such connections are established and how neural circuits are assembled will contribute to our general understanding of the organization, development, and function of the nervous system. The olfactory system offers an intriguing model to study the mechanisms underlying wiring specificity. In this review, we focus our discussion on recent advances in the understanding of the *Drosophila* olfactory system and highlight the role of transmembrane and secreted molecules in providing fine control over the target selection process. For comprehensive reviews of olfactory system development and function in other organisms, see Hildebrand and Shepherd 1997; Wilson and Mainen 2006; Vosshall and Stocker 2007; Su *et al.* 2009; and Sakano 2010.

doi: 10.1534/genetics.113.154336
Manuscript received September 20, 2013; accepted for publication November 7, 2013
[1]Corresponding author: Division of Biology and Biological Engineering, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125. E-mail: whong@caltech.edu

Organization of the *Drosophila* Olfactory System

From insects to mammals, the olfactory system displays remarkable similarities with respect to circuit organization. In *Drosophila*, each of the ~1300 adult olfactory receptor neurons (ORNs) expresses only 1 or 2 of 60 olfactory receptors (ORs) or ionotropic receptors (IRs) (Clyne *et al.* 1999; Gao and Chess 1999; Vosshall *et al.* 1999; Goldman *et al.* 2005; Benton *et al.* 2009; Silbering *et al.* 2011). The axons of ORNs expressing a common OR or IR converge onto one specific glomerulus in the antennal lobe, although their cell bodies are dispersed in the olfactory epithelia (Gao *et al.* 2000; Vosshall *et al.* 2000; Couto *et al.* 2005; Fishilevich and Vosshall 2005; Silbering *et al.* 2011). The antennal lobe consists of

50 glomeruli, which can be uniquely identified by their stereotypical size, shape, and relative position (Laissue *et al.* 1999).

Olfactory information is relayed to higher brain centers by the second-order olfactory projection neurons (PNs), which send their dendrites to the antennal lobe and make synaptic connections with ORN axons. The majority of PNs send their dendrites to individual glomeruli in a stereotyped manner within the antennal lobe and project their axons to higher brain centers, including the mushroom body calyx and the lateral horn (Laissue *et al.* 1999; Jefferis *et al.* 2001; Marin *et al.* 2002; Wong *et al.* 2002). Thus, a given PN makes synaptic connections with axons of only one ORN class (Figure 1). This one-to-one organizational principle is shared from insects to mammals.

In most other sensory systems, neurons are interconnected in a spatially continuous manner along particular axes in space. For example, the visual centers in the brain are spatially organized by a continuous two-dimensional representation of photoreceptors. In contrast, the peripheral sensory organs in the olfactory system show much less spatial order, and the connections between olfactory neurons in the central nervous system are organized in a structurally discrete manner (reviewed in Luo and Flanagan 2007). The class-specific convergence of ORN axons and PN dendrites onto a single glomerulus and their precise one-to-one matching are among the most striking examples of targeting specificity in developmental neurobiology and provide a unique opportunity to study how these connections are established in discrete structural units.

Development of PN–ORN Connections

The organization of specific connections between ORNs and PNs emerges from sequential developmental events, which can be roughly divided into three phases (Figure 2) (Jefferis *et al.* 2004). In the first phase, PNs send dendritic processes to the antennal lobe at the beginning of puparium formation and elaborate diffuse dendritic processes at stereotypical positions in the proto-antennal lobe (Figure 2A). This phase occurs before the ORN axons arrive at the antennal lobe. In the second phase, the ORNs send axons from the antenna and maxillary palps to the antennal lobe, where the incoming axons defasciculate and primarily form axon bundles along two main trajectories surrounding the antennal lobe. ORN axons further converge onto subregions near the final target area. In the third phase, ORN axons recognize the earlier-arriving PN dendrites in the local vicinity and establish specific synaptic connections with their cognate PN partners. Meanwhile, both dendrites and axons are refined to form discrete glomeruli so that the processes of neighboring classes of PNs and ORNs do not overlap.

When ~50 classes of PN dendrites and ORN axons reach the proto-antennal lobe, they are faced with a complex environment. How each class of neurons uniquely responds to this complex environment and establishes class-specific wiring

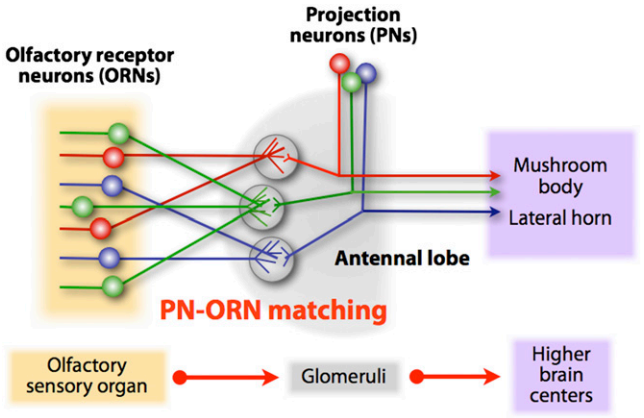

Figure 1 Organization of the olfactory neural circuit. The olfactory systems from insects to mammals display remarkable similarities with respect to their circuit organization. Individual classes of ORN axons make one-to-one connections with individual classes of second-order PN dendrites within one of ~50 discrete glomeruli in the antennal lobe. This specific one-to-one connection is referred to as PN–ORN synaptic partner matching. This illustration is modified from Jefferis and Hummel (2006).

patterns relies on differentially expressed transmembrane and secreted proteins, which serve as receptors and ligands to mediate interactions between different PNs, different ORNs, and extracellular cues from the antennal lobe. In the following sections, we collectively term transmembrane and secreted proteins as "cell-surface molecules" and review recent advances in the understanding of these molecules in regulating different wiring stages in a coordinated manner.

PN Dendrite Targeting

Mosaic analysis with a repressible cell marker (MARCM) studies demonstrated that PN dendrite target choice is specified by cell lineage and birth timing within the lineages (Jefferis *et al.* 2001). A number of transcription factors have been identified to link lineage and birth timing with dendrite targeting specificity of the 50 PN classes (Komiyama *et al.* 2003; Zhu *et al.* 2006b; Komiyama and Luo 2007; Spletter *et al.* 2007). Furthermore, genetic screens for genes regulating PN dendrite targeting isolated mutants involved in multiple biological regulatory processes, including chromatin remodeling (Tea *et al.* 2010; Tea and Luo 2011), microRNA processing (Berdnik *et al.* 2008), protein translation (Chihara *et al.* 2007), glycosylation (Sekine *et al.* 2013), and sumoylation (Berdnik *et al.* 2012). Thus, it is clear that a variety of processes contribute to the specification of PN target choice, presumably by regulating the expression of cell-surface molecules, the key effectors for cell–cell interactions.

Repulsive and attractive forces establish dendritic fields

As PNs elaborate dendrites in the proto-antennal lobe, dendrites of the same class are restricted to a subregion within the antennal lobe (Jefferis *et al.* 2004). Meanwhile, dendrites of the same class expand and fully cover this subregion. Expanding dendritic fields to cover a specific region

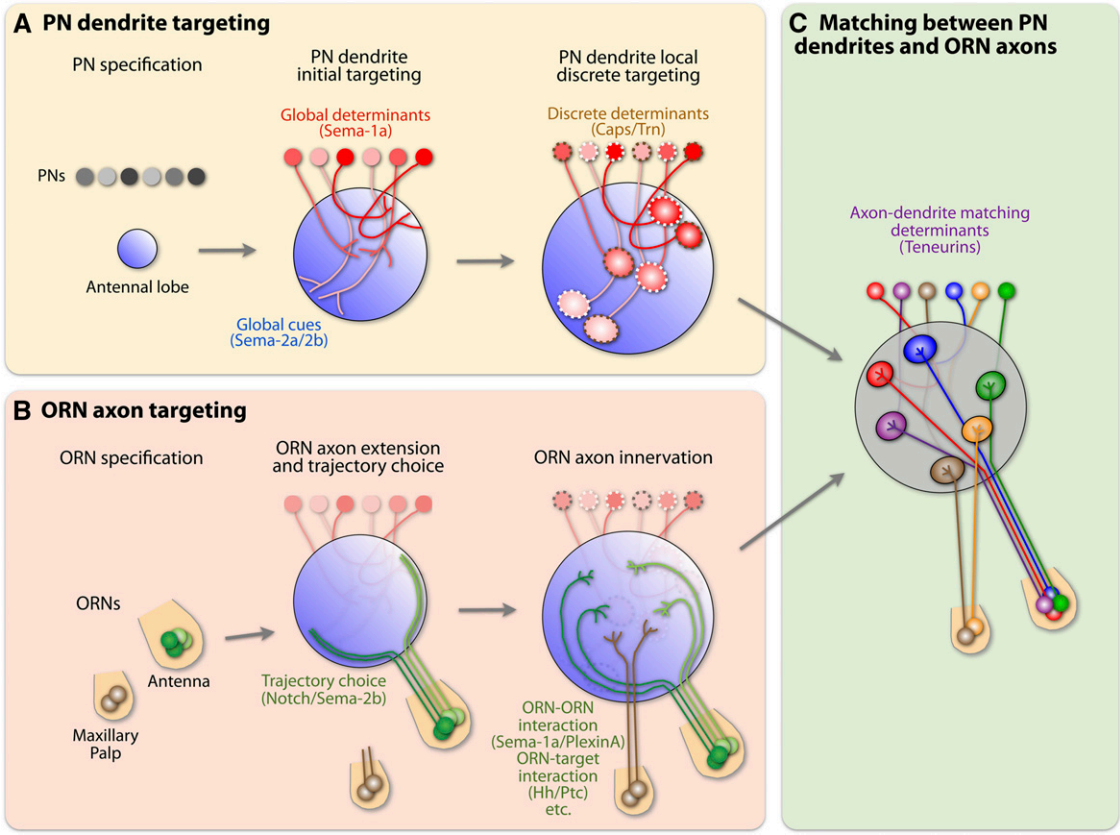

Figure 2 Assembly of the *Drosophila* olfactory circuit. The adult olfactory circuit starts to be assembled at the beginning of the pupal stage. (A) PN dendrite targeting. PNs are born in the embryonic and larval stages and specified by factors involving chromatin remodeling, transcription, microRNA processing, protein translation, glycosylation, and sumoylation. They start to extend their dendrites at the larval–pupal transition, which creates the proto-antennal lobe. Sema-1a cell-autonomously regulates PN dendrite targeting along the dorsolateral–ventromedial axis. Sema-2a/-2b proteins form countergradients to the Sema-1a gradient along the same axis and serve as the extracellular cues that direct this targeting. Subsequently, the differential Caps expression instructs the segregation of PN dendrites into discrete glomeruli. (B) ORN axon targeting. ORNs are born in the early pupa. Pioneering ORN axons arrive at the antennal lobe at 18 hr after puparium formation and choose different trajectories surrounding the antennal lobe. ORN axons require PN-independent mechanisms to converge to appropriate target regions, including ORN–ORN interactions mediated by Sema-1a and ORN target interactions mediated by Hh. By that time, PN dendrites already coarsely pattern the antennal lobe. (C) The independent PN and ORN targeting is coordinated by the one-to-one class-specific matching between ORN axons and PN dendrites. Ten-m and Ten-a, which are highly expressed in select PN and ORN matching pairs, instruct synaptic matching specificity between PNs and ORNs through homophilic attraction.

of a certain size is likely important for the further development of the antennal lobe, providing enough space and flexibility to interact with ORN axons and eventually establishing the morphology of individual glomeruli.

The cell adhesion molecule N-cadherin (Ncad) has been shown to restrict dendrites to the appropriate glomerular space. Loss of Ncad causes dendrites to spread beyond the developing antennal lobe or invade neighboring glomeruli (Zhu and Luo 2004). This phenotype was observed at an early developmental stage, suggesting that Ncad functions in initial confinement of dendrites to the appropriate glomerular space. Ncad likely mediates adhesion between PN dendrites (possibly between dendrites from the same classes) and in this capacity dendrite–dendrite adhesion would restrict dendrites of the same classes to a single glomerulus.

Dscam, a member of the Ig-domain superfamily of transmembrane proteins, was found to promote the elaboration of PN dendrites to occupy individual glomerular space (Zhu *et al.* 2006a). Loss of Dscam in PNs leads to a drastic reduction of their dendritic field size, suggesting that Dscam plays a repulsive role between sister dendrites of the same PN class. This suggests that Dscam functions in PN dendrites to expand and occupy the local space, while also preventing them from collapsing onto each other. Both Dscam and Ncad do not appear to play instructive roles for class-specific targeting, as they equally affect all PN classes examined (Zhu and Luo 2004; Zhu *et al.* 2006a).

Molecular gradients determine coarse positions

How is glomerulus-specific targeting of PNs achieved? It is likely that a set of cell-surface receptors, differentially expressed in the various PN classes, enables them to uniquely respond to extracellular cues. A transmembrane semaphorin, Sema-1a, was found to directly regulate PN dendrite targeting specificity (Komiyama *et al.* 2007). Sema-1a protein forms a concentration gradient along the dorsolateral–ventromedial axis of the

developing antennal lobe, and the removal of Sema-1a in dorsolateral PNs cause their dendrites to mistarget to ventromedial glomeruli (Figure 2A). This suggests that the levels of Sema-1a in PN dendrites instruct their coarse target positions along this Sema-1a-specific axis. Interestingly, the function of Sema-1a in regulating dendrite targeting is cell autonomous and requires its cytoplasmic domain, suggesting that it functions as a receptor. Its putative ligand is likely also distributed in a gradient along the same dorsolateral–ventromedial axis.

Two secreted semaphorins, Sema-2a and Sema-2b, direct PN dendrite targeting along the dorsolateral–ventromedial axis and are candidate extracellular ligands for Sema-1a (Sweeney *et al.* 2011). Sema-2a/-2b proteins form countergradients to the Sema-1a gradient along the same axis, and loss of Sema-2a/-2b also causes ventromedial mistargeting of PN dendrites that normally target to dorsolateral glomeruli, similar to what was observed in Sema-1a loss-of-function mutants. Thus, the novel interaction between secreted and membrane-bound semaphorins generates a coarse map of PN dendrites along one axis (Figure 2A). Interestingly, Sema-2a is predominantly expressed by ORN axons of the larval antennal lobe, which undergoes degeneration during metamorphosis (Sweeney *et al.* 2011). The degenerating larval ORNs occupy a ventromedial position with respect to the developing adult antennal lobe, suggesting that secretion of Sema-2a by the larval axons could participate in patterning a developing adult neural circuit.

Discrete determinants constrain glomerular targeting

After the initial coarse targeting of PN dendrites along one axis, neighboring PN dendrites need to be segregated and confined to class-specific glomeruli. The leucine-rich repeat (LRR)-containing transmembrane protein Capricious (Caps) plays an essential role in this class-specific targeting process (Hong *et al.* 2009). Unlike semaphorins, which are distributed in continuous gradients, Caps is differentially expressed in a subset of PN classes that innervate intercalated glomeruli (Figure 2A and Figure 3A). The differential Caps expression instructs the segregation of Caps$^+$ and Caps$^-$ dendrites into class-specific, discrete glomeruli. Loss of Caps in Caps$^+$ PNs causes their dendrites to invade glomeruli occupied by Caps$^-$ PNs (Figure 3B), whereas misexpressing Caps in Caps$^-$ PNs causes their dendrites to make ectopic innervation of glomeruli occupied by Caps$^+$ PNs (Figure 3C). The function of Caps in PN dendrite targeting is likely mediated by PN–PN interactions and is independent of presynaptic ORNs. Furthermore, Caps does not mediate PN dendrite targeting through homophilic interactions. Identification of the putative heterophilic ligand(s) will provide new insight into this discrete targeting process.

Caps provides only a single discrete cell-surface identity code for the various PN classes. Since the antennal lobe contains ~50 different classes of PNs, other cell-surface molecules functioning together with Caps are likely required to further distinguish within the groups of Caps$^+$ or Caps$^-$

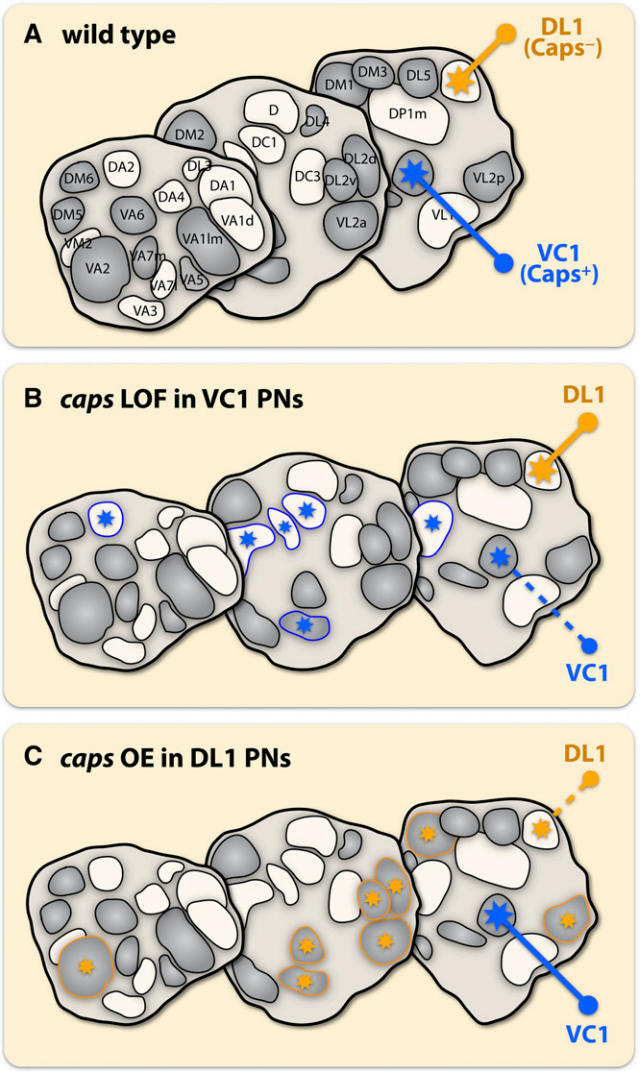

Figure 3 Discrete expression of Caps instructs PN dendrite targeting into class-specific glomeruli. (A) Schematic showing differential expression of Caps in a subset of PNs that innervate intercalated glomeruli (in gray) in the antennal lobe (*e.g.*, VC1 is Caps$^+$ and DL1 is Caps$^-$). (B) Loss of Caps in VC1 PNs (Caps$^+$) causes their dendrites to invade glomeruli innervated by Caps$^-$ PNs. (C) Misexpression of Caps in DL1 PNs (Caps$^-$) causes their dendrites to invade glomeruli innervated by Caps$^+$ PNs. Asterisks indicate the normal and ectopic targets of dendrites. This schematic is from Hong *et al.* 2009 and de Wit *et al.* 2011.

PNs. Indeed, a closely related LRR transmembrane protein Tartan is expressed in a distinct subset of PNs that partially overlap with Caps-expressing PNs (Hong *et al.* 2009). Tartan and Caps play partially redundant roles in regulating PN dendrite targeting. Additional cell-surface molecules are likely involved in determining the unique identity of each of the ~50 PN classes.

ORN Axon Targeting

ORNs are born in the olfactory sensilla that derive from an undifferentiated epithelium, the antennal disc. The sensillar types are initially specified by two transcription factors, Atonal

and Amos (Gupta and Rodrigues 1997; Goulding *et al.* 2000). In each sensillum, a common sensory organ precursor gives rise to ORNs and nonneuronal supporting cells (Endo *et al.* 2007). In contrast to the mouse olfactory system, *Drosophila* ORNs target their axons to the antennal lobe before the onset of their OR gene expression, and these two processes do not depend on each other. Both processes, however, are regulated by the common upstream Notch signaling pathway (Endo *et al.* 2007). ORNs with high Notch activity (Notch ON) and low Notch activity (Notch OFF) differentiate into distinct classes within each sensillum and target axons to distinct glomeruli.

Initial trajectory choice

When ORN axons reach the brain, they defasciculate and form two main trajectories to circumnavigate the developing antennal lobe: one goes ventromedially and the other dorsolaterally. Selecting the proper trajectory is essential for targeting specificity of certain ORN classes (Joo *et al.* 2013). A key determinant of this trajectory choice is the secreted semaphorin Sema-2b. Notch signaling restricts Sema-2b expression to ORN axons that take the ventromedial but not dorsolateral trajectory. Sema-2b functions cell autonomously in ORNs for trajectory choice in response to antennal lobe-produced Sema-2a and Sema-2b, which also regulate PN dendrite targeting along the ventromedial–dorsolateral axis just before ORNs arrive (see above). Sema-2b further mediates axon–axon interactions that consolidate trajectory choice and promote the formation of the ventromedial axon bundle (Figure 2B). Semaphorins thus demonstrate how the same molecules can coordinate multiple stages of neural circuit assembly along a common developmental axis.

Axon ingrowth and coarse targeting

In addition to Sema-2a/-2b, Hedgehog (Hh) is another central brain-derived cue that regulates axon targeting of ORNs (Chou *et al.* 2010b). Peripheral Hh signaling in the antennal disc divides ORNs into two distinct subsets: one subset expresses low levels of the Patched (Ptc) receptor, and the other expresses high levels of Ptc. Different Ptc levels in ORNs determine the responsiveness of ORN axons to brain-derived Hh. Only low-Ptc ORNs respond to brain-derived Hh for target selection (Figure 2B). Thus, the peripheral and central Hh signaling serves as a two-step mechanism to coordinate ORN cell body positions in the periphery and their axonal targets in the brain (Chou *et al.* 2010b).

The infiltration of ORN axons into the antennal lobe requires Ncad. When Ncad is removed from ORNs, their axons reach the vicinity of the antennal lobe, but the initial axonal convergence into the protoglomeruli is disrupted (Hummel and Zipursky 2004). This in turn affects subsequent steps of axon targeting and results in severe disorganization of the adult antennal lobe. Several additional molecules have been shown to be required for the initial coarse targeting of ORN axons to specific glomeruli. For example, Dscam and two downstream effectors of Dscam, the SH2/SH3 adaptor Dock and the serine/threonine kinase Pak, are broadly expressed in the developing antennal lobe, and their loss-of-function mutants display ectopic targeting of ORN axons (Ang *et al.* 2003; Hummel *et al.* 2003). The Robo receptors are also involved in the coarse targeting of ORN axons, as removal of Robo receptors results in widespread mistargeting phenotypes (Jhaveri *et al.* 2004). The mechanisms by which Dscam and Robo receptors regulate ORN axon targeting remain to be further investigated.

Axon–axon repulsion for sequential targeting

ORN axon–axon interactions not only regulate trajectory choice but also play a direct role in their proper targeting of specific glomeruli. For example, maxillary palp ORN axons enter the antennal lobe later than antennal ORN axons, and their target choice is constrained by early-arriving antennal axons. Sema-1a was found to mediate this interaction (Sweeney *et al.* 2007). Here, as opposed to its function as a receptor in PNs, Sema-1a functions as a repulsive ligand on antennal ORN axons to prevent late-arriving maxillary palp axons from invading regions already occupied by the antennal axons (Figure 2B) (Sweeney *et al.* 2007). PlexinA likely serves as the receptor for Sema-1a in ORN axon targeting.

Involvement of glial cells

Evidence suggests that glial cells are also involved in patterning the antennal lobe. The transient interhemispheric fibrous ring (TIFR) is a glial structure located between the antennal lobes, and its glial processes are closely associated with ORN axons (Simon *et al.* 1998). The Derailed (Drl) receptor tyrosine kinase is expressed in these glial cell processes near the antennal lobe, and its ligand, Wnt5, is expressed in ORN axons (Yao *et al.* 2007; Sakurai *et al.* 2009). Drl acts in glial cells to modulate the Wnt5 signaling, and this ORN–glia interaction contributes to the precise targeting of ORNs to specific glomeruli.

PN–ORN Matching

Evidence for axon–dendrite recognition

How do ORN axons make final connections with early-arriving PN dendrites within a particular glomerulus? Three scenarios can be envisioned: (1) ORN axons may initially form connections with many classes of PNs in the vicinity, after which the incorrect connections are pruned by sensory activity; (2) ORN axons and PN dendrites may independently target to precise locations in the antennal lobe where the final connections are made; and (3) ORNs and PNs may target to a rough target area and the mutual interaction between the two determines connection specificity.

The first hypothesis is unlikely because the olfactory receptors that produce sensory activity are not expressed until specific PN–ORN connections are formed (Jefferis *et al.* 2004). The second and the third hypotheses could not be distinguished until the following observation. When a *Dscam* transgene is

overexpressed in a subset of PNs, it causes PN dendrites to shift to a neighboring location (Zhu *et al.* 2006a). This dendritic position shift occurs early in development, before the PN dendrites contact the ORN axons. Interestingly, the cognate partner ORN axons subsequently arrive and follow the shifted PN dendrites to the new position, suggesting that the PN–ORN connection specificity is maintained despite the shift of the relative position of PNs. Therefore, late-arriving ORN axons could recognize cues on correct partner PN dendrites to finalize PN–ORN connections. This provides the first evidence supporting an essential role of axon–dendrite recognition in determining their final connectivity.

Matching determinants

Based on the above observations, two unbiased genetic screens were performed to search for molecules controlling PN–ORN matching (Hong *et al.* 2012). These screens identified two evolutionarily conserved EGF repeat-containing transmembrane Teneurins, Ten-m and Ten-a, as synaptic partner matching molecules (Hong *et al.* 2012). Ten-m and Ten-a are highly expressed in select PN and ORN matching pairs (Figure 4A). Loss- and gain-of-function experiments involving Teneurins cause class-specific ectopic connections between ORNs and PNs (Figure 4B). Specifically, increasing Teneurin levels in Teneurin-low PNs causes their dendrites to lose endogenous connections with Teneurin-low ORNs and mismatch with Teneurin-high ORNs, whereas overexpressing Teneurins in PNs that already express high levels of Teneurins does not disrupt the proper PN–ORN connections. Moreover, Teneurins mediate homophilic interactions *in vitro* and promote *trans*-cellular PN–ORN attraction via homophilic interactions *in vivo*. These findings suggest that Teneurins instruct synaptic matching specificity between PNs and ORNs through homophilic attraction, by matching Ten-m and Ten-a levels in PN and ORN partners (Figure 2C).

Teneurins are unlikely to be the only molecules regulating PN–ORN matching. Since the antennal lobe contains ~50 pairs of PNs and ORNs, additional cell-surface molecules most likely work together with Teneurins to further distinguish matching specificity among different Teneurin-high classes or among different Teneurin-low classes, so that each of the ~50 PN–ORN connections can be precisely established.

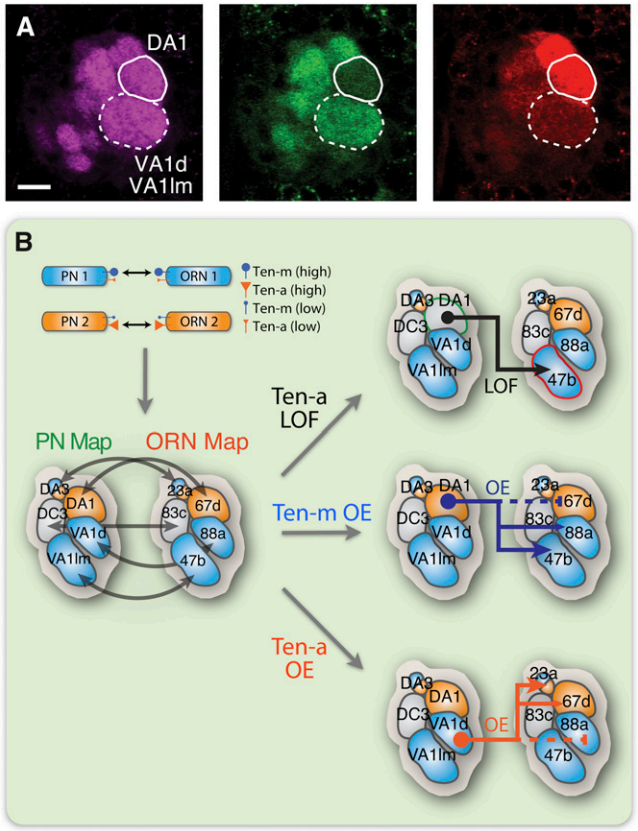

Figure 4 Teneurins instruct class-specific matching between PN dendrites and ORN axons. (A) Ten-m and Ten-a are highly expressed in select matching pairs of ORN and PN classes. Shown is a developing antennal lobe at 48 hr after puparium formation stained by antibodies against Ten-m, Ten-a, and a neuropil marker, N-cadherin. Solid lines encircle the DA1 glomerulus (Ten-m low, Ten-a high); dashed lines encircle the VA1d/VA1lm glomeruli (Ten-m high, Ten-a low). (B) High-level expression of Ten-m or Ten-a promotes homophilic attraction between specific pairs of PN dendrites and ORN axons to form proper and stable connections (blue, Ten-m high; orange, Ten-a high). The differential expressions of Ten-m and Ten-a in select PN–ORN pairs instruct the one-to-one class-specific matching. Loss of ten-a in DA1 PNs (Ten-a high) causes dendrites to mismatch with Or47b ORNs (Ten-a low). Misexpression of Ten-m in DA1 PNs (Ten-m low) causes dendrites to mismatch with Or88a and Or47b ORNs (Ten-m high). Misexpression of Ten-a in VA1d PNs (Ten-a low) causes dendrites to mismatch with Or23a and Or67d ORNs (Ten-a high). This schematic is from Hong *et al.* (2012).

Refinement, Maintenance, and Synaptogenesis

After converging onto individual glomeruli and making the proper connections, PN dendrites and ORN axons are restricted into single glomeruli with no overlap between neighboring classes. It is likely that in addition to restricting dendritic fields in an early developmental stage, N-cadherin continues to contribute to the refinement of dendrites to single glomeruli through interactions between dendrites from the same classes.

Once the adult antennal lobes are formed and proper axon–dendrite connections are established, the organization of the PN dendrites and ORN axons appears stable and insensitive to perturbations. Selective cell ablation or different olfactory experience leads to minimal changes in glomerular organization (Devaud *et al.* 2003; Berdnik *et al.* 2006).

Synaptogenesis occurs at a late stage of antennal lobe development. Ultrastructural analysis using electron microscopy suggests that the formation of presynaptic specialization occurs ~48–72 hr after puparium formation, although synaptobrevin-GFP, a synaptic marker, is already accumulated in the developing axons before this period (Devaud *et al.* 2003). This suggests that synapse formation follows after the establishment of connections between PN dendrites and ORN axons. Teneurins, the instructive molecules involved in matching proper PN and ORN partners, were found to be required for synaptogenesis in the *Drosophila* neuromuscular junction (Mosca *et al.* 2012); it will be

interesting to test whether they also contribute to the formation of synapses in the olfactory system.

Local Interneurons

In addition to the processes of ~1300 ORNs and ~200 PNs, the antennal lobe also contains neurites from ~200 local interneurons (LNs) (Das *et al.* 2008; Lai *et al.* 2008; Okada *et al.* 2009; Chou *et al.* 2010a). LNs make connections with both ORN axons and PN dendrites and play important roles in ORN-to-PN information transformation (Wilson 2013). In contrast to the one-to-one PN–ORN connectivity, LNs show a remarkable diversity and variability in their glomerular innervation patterns (Chou *et al.* 2010a). Some LN classes extend neurites throughout the antennal lobe, whereas other LN classes restrict dendrites only to a subset of glomeruli. Among LN classes that show restricted patterns, some innervate continuous glomerular regions and others innervate the antennal lobe in a patchy configuration. Surprisingly, certain LN classes display a striking variability in their glomerular innervation pattern across different animals.

Arborization of LN dendrites requires Dscam. Similar to its function in PN dendrites, Dscam plays a repulsive role between sister dendrites of the same LNs, causing the dendrites to expand and preventing them from collapsing onto each other (Zhu *et al.* 2006a). In addition, the establishment of LN arborization also requires interactions with ORN axons during development (Chou *et al.* 2010a). The molecular mechanism underlying the variability of the LN dendritic arborization, however, is still largely unknown. It would be interesting to further characterize the developmental process of LNs and to examine whether PN dendrites contribute to the glomeruli-specific arborization of LNs, and whether LNs contribute to the establishment of PN–ORN connectivity.

Interestingly, LN dendrites and ORN axons appear to occupy distinct subcompartments within a glomerulus (Hummel and Zipursky 2004). Dendrites of at least some LNs predominantly occupy only a small region in the central part of each glomerulus, and these dendrites are surrounded by synapses formed by ORN axons in the peripheral areas of a glomerulus. In contrast to the lack of overlap between LN dendrites and ORN synapses, PN dendrites extend throughout an entire glomerulus and overlap extensively with ORN axons (Hummel and Zipursky 2004). This subcompartmental specialization within a glomerulus would be an intriguing topic for further investigation.

Principles and Open Questions

A working model for olfactory circuit assembly

The advances summarized above suggest that a series of coordinated mechanisms regulate three major stages in the assembly of the fly olfactory system (Figure 2):

1. PN dendrite patterning precedes ORN axon targeting and relies on adhesive and repulsive molecules to establish their dendritic field. Their glomerulus-specific targeting is controlled by global gradients, including Sema-1a and Sema-2a/-2b, as well as local discrete determinants such as Capricious and Tartan that are distributed in a "salt-and-pepper" fashion on dendrites projecting to different glomeruli (Figure 2A).
2. Prior to arriving at their coarse target in the antennal lobe, ORN axons first make critical developmental trajectory choices through both axon-derived and central-brain-derived cues such as Sema-2a and Sema-2b. The initial targeting of axons into appropriate antennal lobe regions involves several cellular and molecular mechanisms that include axon–axon interactions mediated by Sema-1a and PlexinA as well as axon–target interactions mediated by Hh and its receptor Ptc (Figure 2B).
3. The final one-to-one matching between ORN axons and PN dendrites is coordinated by Teneurin-mediated homophilic attractive interactions between axons and dendrites (Figure 2C). These mechanisms act in a sequential and coordinated manner, which eventually leads to the precise one-to-one connectivity between 50 PN and ORN classes in 50 glomeruli.

Given the number of ORN and PN classes that need to be precisely wired in the olfactory circuit, the molecules identified so far are most likely incomplete. Future identification and mechanistic studies of additional players might reveal new principles that have not been uncovered thus far (discussed below).

Continuous vs. discrete neural maps

Spatial representation of the external world in neural maps is important for the nervous system to effectively process and integrate sensory information (McLaughlin and O'Leary 2005; Luo and Flanagan 2007). In many sensory systems, such as the visual and auditory systems, neurons connect nearby spatial/tonic inputs to nearby target regions in the brain and form a spatially continuous neural map. In the olfactory system, however, synaptic connections are organized into spatially separated structural units, thereby forming a discrete neural map.

The formation of continuous neural maps is typically mediated by graded expression of cell-surface molecules, which segregate axons or dendrites along the concentration gradient at the target. By contrast, studies of olfactory circuit development suggest that the formation of a discrete neural map usually arises from a sequential strategy. The first step involves an initial, coarse projection of neurites to broad target zones through the action of molecular gradients, a mechanism similar to the formation of continuous neural maps. This initial coarse targeting is followed by a local, precise targeting to distinct structural units via local discrete determinants. For example, an initial coarse map of PN dendrites is established by global gradients of Sema-1a and Sema-2a/-2b, followed by local binary choices specified by class-specific expression of Capricious. This sequential strategy

for neural map formation is also seen in the targeting process of the mammalian ORN axons to the olfactory bulb (Imai *et al.* 2010; Sakano 2010) and thus may represent a general solution to the problem of constructing a discrete map.

Multiple types of cellular interactions

Recent advances have highlighted several types of interactions between neurons in the developing antennal lobe, including interactions between axons from different ORN classes (Sema-1a/PlexinA and Sema-1b/PlexinB), repulsive interactions between dendrites from the same PN classes (Dscam), attractive interactions between dendrites from the same PN classes (Ncad), interactions between dendrites from different PN classes (Caps/Trn), and attractive interactions between axons and dendrites that target the same glomeruli (Teneurins), as well as the interactions between ORNs and glia (Wnt5/Drl). It should be noted that these previous works largely focused on part of the antennal lobe where reagents are available to genetically label and manipulate specific classes of neurons. It remains to be tested whether these previously identified mechanisms are generalizable to other classes of PNs and ORNs or whether new mechanisms are involved.

Other conceptually different types of interactions may also contribute to wiring specificity of the olfactory circuit. For example, axon–dendrite repulsions between nonpartner PNs and ORNs that innervate neighboring glomeruli could serve as a mechanism to avoid inappropriate PN–ORN connections and ensure the proper matching. In addition, dendrite–dendrite repulsions between neighboring PN classes may help confine dendrites of a single class of PNs into a single glomerulus and prevent them from entering neighboring glomeruli. Finally, LNs may interact with PNs to contribute to patterning of the antennal lobe or may directly regulate wiring specificity. These hypotheses remain to be tested.

Combinatorial Code in Constructing a Complex Circuit

Identifying individual genes and examining how each of them functions in select neurons is a common experimental approach to reveal the mechanisms underlying wiring specificity. In a complex system involving interactions among thousands of neurons, this bottom-up approach sometimes has limitations in understanding system-level features that could be critical in gaining a complete picture of the target selection process. In the following section, we make an attempt to consider this problem in a top–down approach and speculate how combinatorial coding, redundancy, and error-correcting ability could contribute to establishing identities and wiring patterns of different classes of neurons.

One neuron, many molecules

To specify targeting of different neuronal classes, the nervous system could utilize two alternative strategies: each neuron could be encoded (1) by expressing one unique molecule or (2) by expressing a unique combination of molecules. Several studies suggest that the olfactory system adopts the latter strategy; namely, a single neuron expresses a combination of molecules, and each molecule is expressed in multiple neurons. For example, both Caps and Tartan are expressed in multiple classes of PNs; some PNs express both whereas others express either one of them (Hong *et al.* 2009). This distinct and partially overlapping pattern of expression forms a combinatorial code to regulate PN dendrite targeting. Similarly, both Ten-m and Ten-a are expressed in distinct and partially overlapping subsets of PNs and ORNs; some PN–ORN pairs express both of them whereas others express only either one of them (Hong *et al.* 2012). In this capacity, Teneurins form part of a combinatorial code for PN–ORN matching. Both cases exemplify how combinatorial action of multiple molecules assigns identities to individual PN and ORN classes. The use of a combinatorial code could greatly reduce the number of molecules required to uniquely identify each neuronal class (Figure 5, A and B, Figure 6A, and Table 1, comparing models I and II).

Different ways of using a combinatorial code

We next discuss how combinatorial actions of multiple molecules determine the identities and wiring patterns of different neuronal classes. To simplify our discussion, we take only the presence or the absence of a molecule into account and propose three possible models (models II–IV, Figure 5 and Table 1). In all three models, each class of neurons expresses a combination of molecules. But these models have different minimum Hamming distance (Hamming 1950). Here, Hamming distance is defined as the molecular difference between any two classes of neurons. In model II, at least one molecule is different between any two classes; in model III, at least two molecules are different; and in model IV, at least three molecules are different. Although these models are simplified from the real target selection process, this simplification allows us to focus on a few important system-level properties, discuss how these properties may contribute to wiring specificity, and evaluate which models are more consistent with experimental observations.

Coding capacity and redundancy

The three models have different levels of coding capacity, redundancy, and robustness (Figure 6). Here, coding capacity is defined as the maximum number of identities a given number of molecules could theoretically encode, redundancy is defined as the minimum number of molecules a neuronal class needs to change to become identical to another class (*i.e.*, minimum Hamming distance), and robustness is defined as the ability of the entire system to resist molecular changes through redundancy (Table 1). If we assume when one class becomes identical to another class following perturbation they mistarget to the places of each other, we can measure coding robustness in three properties: percentage of classes unaffected by perturbation, average ectopic targets

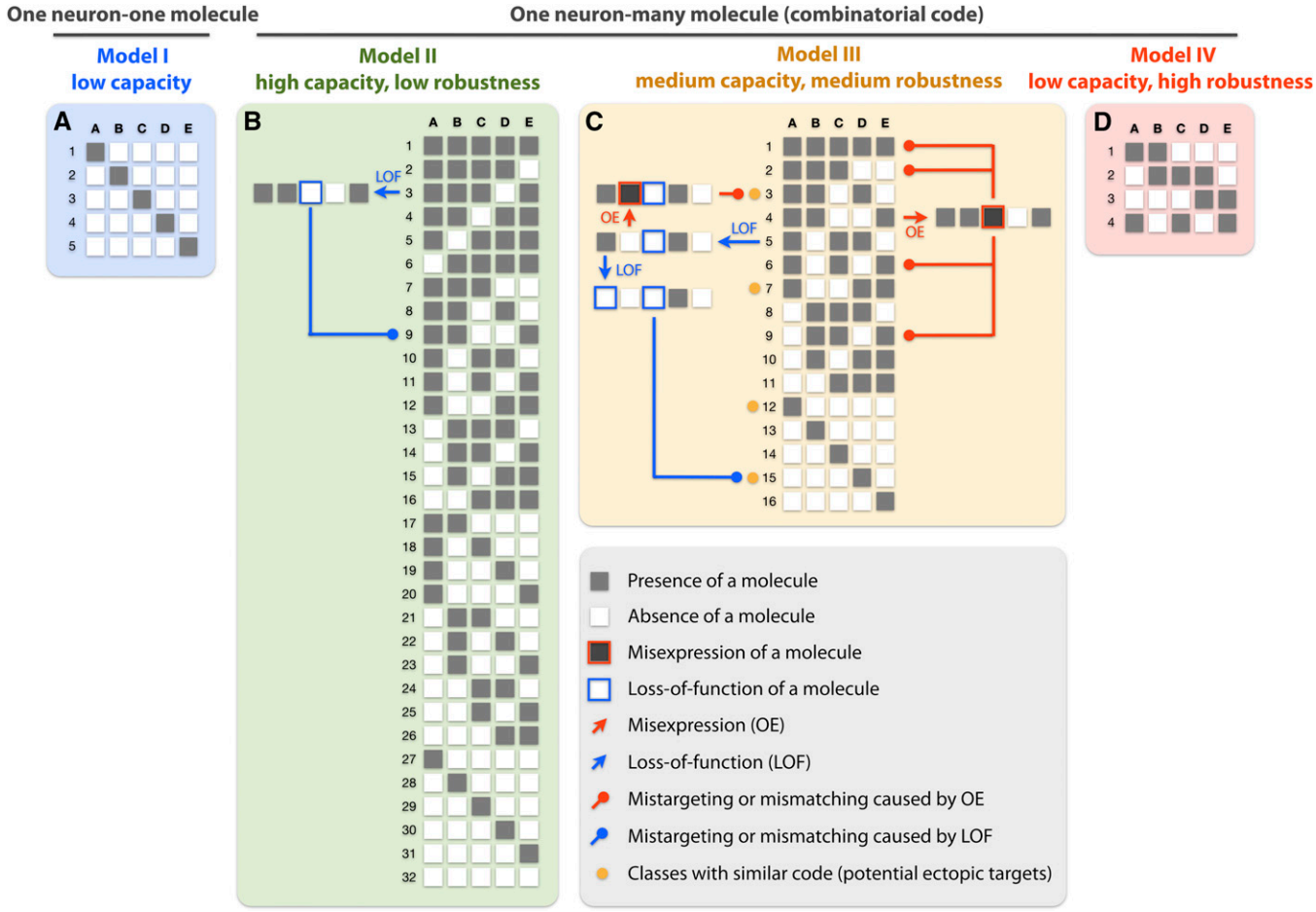

Figure 5 Four hypothetical models of encoding neuronal identities. (A–D) Illustrated are examples of four hypothetical models (illustrated for specific cases in Table 1) by which one molecule or a unique combination of multiple molecules determines the identity of different neuronal classes. (A) Model I. Each class of neurons expresses only one of the molecules. (B–D) Models II–IV. Each class of neurons expresses a unique combination of molecules. (B) Model II. At least one molecule is different between any two classes of neurons. This model has a low robustness. For example, removing molecule C from neuron 3 makes this neuron identical to neuron 9. (C) Model III. At least two molecules are different between any two classes of neurons. Model III has a medium redundancy that increases the robustness of the wiring specificity. For example, removing molecule C from neuron 5 does not make this neuron identical to any other neuronal classes. Removing molecule C from a subset of class 5 neurons may change this subset such that they have the same Hamming distance from the remaining unaltered class 5 neurons as to the classes of neurons that possess the closest identity (*i.e.*, classes with one molecule difference, marked by yellow dots), which could lead to a partial mistargeting. Simultaneous manipulation of two or more molecules (*e.g.*, removing both molecules A and C from neuron 5 or removing C and misexpressing B in neuron 5) may produce stronger phenotypes. If misexpression of a molecule overrides the action of one other molecule, misexpressing molecule C in neuron 4 may cause it to mistarget to places where neurons 1, 2, 6, and 9 are located. (D) Model IV. At least three molecules are different between any two classes of neurons. This model has high redundancy but low coding capacity.

a class may mistarget following perturbation, and total mistargeting events following perturbation (Table 2).

Among these three models, model II has the highest coding capacity, but is also the least robust, because altering one molecule may affect a large number of neurons expressing this molecule and render them indistinguishable from other classes that do not express this molecule (Figure 5B and Figure 6, B and C). This does not seem to be what was observed *in vivo*. All single-gene loss-of-function mutants of the cell-surface molecules studied thus far tend to cause weak phenotypes; dendrites or axons tend to still occupy their original glomeruli and only a small fraction of neurites innervate ectopic targets (Komiyama *et al.* 2007; Hong *et al.* 2009; Chou *et al.* 2010b; Sweeney *et al.* 2011). Loss of cell-surface proteins tends to produce phenotypes that are weaker than those produced by mutating nuclear factors (Tea and Luo 2011) or protein modification enzymes (Sekine *et al.* 2013). One possibility is that cell-surface molecules are partially redundant with each other, and mutating transcription factors or protein modification enzymes causes misregulation of multiple cell-surface molecules and leads to stronger defects.

Indeed, several cell-surface molecules previously identified play partially redundant roles in regulating targeting of axons and dendrites (Hong *et al.* 2009; Sweeney *et al.* 2011). Thus, a redundant coding system illustrated in model III, in which two or more molecules are different between any two classes of neurons, is more consistent with what was observed *in vivo* (Figure 5C). Compared to model II, model

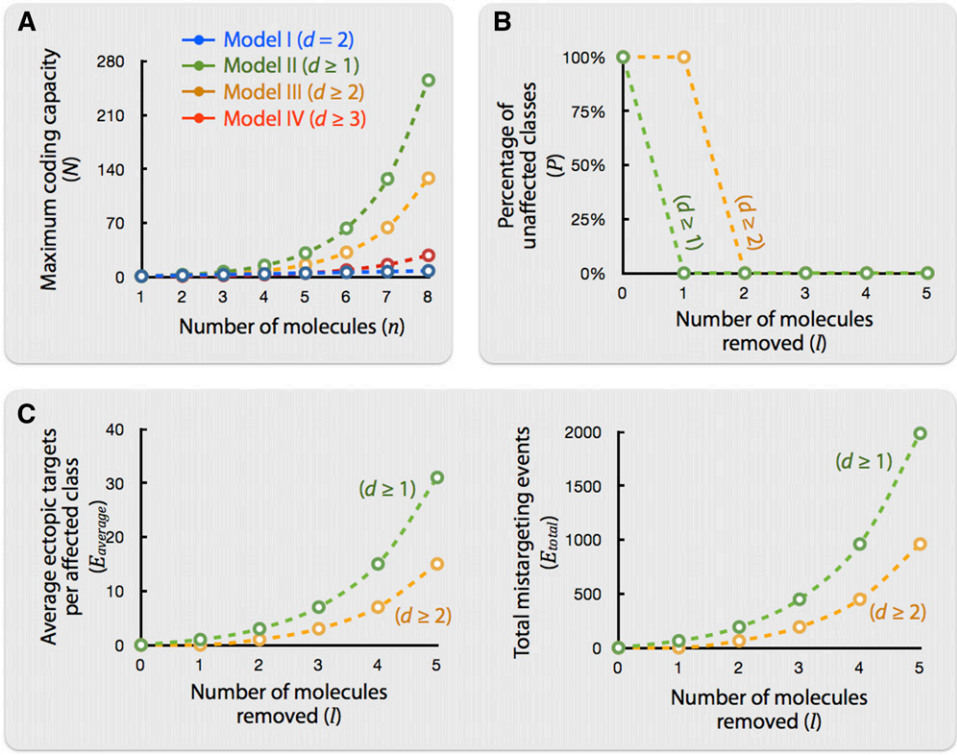

Figure 6 Coding capacity and robustness. (A) Relationship between maximum coding capacity and number of molecules in different models, calculated based on Table 1. The curve of model IV shows the Hamming bound, an upper bound of maximum coding capacity (Table 1). (B and C) Robustness of models II and III, measured by three properties calculated based on Table 2. (B) Percentage of classes unaffected after removal of a given number of molecules. (C) Left, average ectopic targets a class may mistarget following removal of a given number of molecules; right, total mistargeting events following removal of a given number of molecules. The properties in B and C were calculated using representative cases of models II and III in which six and seven molecules are used to encode 64 classes of neurons, respectively. In these two cases, the total numbers of encoded classes are the same, allowing a direct comparison of ectopic targets and mistargeting events between models II and III.

III keeps the entire system intact when removing one molecule and produces fewer average ectopic targets and fewer total mistargeting events when removing more molecules (Figure 6, B and C). This is reminiscent of what was observed in the loss of function of Tartan, Sema-2a, or Sema-2b alone (Hong *et al.* 2009; Sweeney *et al.* 2011). Thus, model III would not only achieve a marked reduction of molecules required but also increase the redundancy that makes wiring specificity more robust (Figure 6, A–C).

Note that during evolution there is less selection pressure on any gene that serves completely redundant functions, unless the gene is required elsewhere for the fitness of the organism. This may limit the degree of redundancy even though redundancy increases robustness.

Error-correcting ability

Wiring specificity could be impaired by two types of errors that may occur spontaneously or artificially. Adding/removing a molecule in all the neurons within a class or in all classes is considered type A. In this case, all neurons from a single class still possess a uniform combination of molecules. This occurs in a whole-animal mutant of a gene or in complete removal of a gene from a neuronal class. By contrast, adding/removing a molecule in a subset of neurons within a class is considered type B. In this situation, a single class of neurons is divided into two subpopulations of neurons that possess different combinations of molecules. This could occur when genes are mutated in a mosaic manner.

Model III ($d \geq 2$) offers redundancy for type A errors; a perturbed class still possesses a unique identity that can be distinguished from that of all other classes. But model III may not tolerate the type B errors, which may cause the perturbed subpopulation to have the same Hamming distance to the unchanged subpopulation of the same class as to other classes with the closest identity (yellow dots in Figure 5C), so that the system is unable to determine which class the perturbed neurons belong to and correct the error.

Since PNs and ORNs are separately specified, PN–ORN matching seems more susceptible to type B errors than PN dendrite targeting. However, misexpression screens of the same set of cell-surface molecules identified many fewer molecules affecting PN–ORN matching than those affecting PN dendrite targeting (Hong *et al.* 2009, 2012; W. Hong and L. Luo, unpublished results), suggesting the PN–ORN matching might possess a higher robustness, especially with respect to type B errors.

Model IV ($d \geq 3$) has the highest redundancy among all three models and offers correcting ability for type B errors based on relative Hamming distance. A neuron with one molecule removed has a shorter Hamming distance to its original class than to other classes with the closest identity. This error-correcting ability could contribute to the specificity of PN–ORN matching. Although the higher redundancy greatly limits the number of identities certain molecules are able to encode, as indicated by the Hamming bound (Hamming 1950) (Table 1, Figure 6A), this reduced capacity may not pose a problem for PN–ORN matching as matching occurs at the last stage of the wiring process after both PNs and ORNs arrive at the local target area where they only encounter a limited number of possible partners.

Table 1 Comparison of different models encoding neuronal identities

Model	Case	Coding redundancy[a] (d)	Total no. molecules (n)	No. molecules in a neuron class (m)	Maximum coding capacity (N)	Robustness
I. Each neuron class expresses a unique molecule	Specific	2	5	1	5	High
	General	2	n	1	n	High
II. Each neuron class expresses a unique combination of molecules; at least one is different between two classes	Specific	≥ 1	5	[0..5]	32	Low
	General	≥ 1	n	[0..n]	2^n	Low
III. Each neuron class expresses a unique combination of molecules; at least two are different between two classes	Specific	≥ 2	5	[0..5]	16	Medium
	General	≥ 2	n	[0..n]	$\sum_{i=0}^{[n/2]} \binom{n}{n-2i} = 2^{n-1}$	Medium
IV. Each neuron class expresses a unique combination of molecules; at least three or more are different between two classes	Specific	≥ 3	5	[0..5]	4	High
	General	$\geq d$	n	[0..n]	Hamming bound[b] $\left[\frac{2^n}{\sum_{i=0}^{t}\binom{n}{i}}\right]$ where $t = \left[\frac{d-1}{2}\right]$	High

[a] Defined as the Hamming distance between two classes.
[b] Upper bound for the maximum coding capacity of model IV. It is not reachable in some cases (Hamming 1950).

One hypothesis, which is the most consistent with our observations so far, is that model III ($d \geq 2$) is more broadly used in targeting of the same type of neurons in an initial stage (*e.g.*, PN dendrite targeting) and model IV ($d \geq 3$) is used locally to match multiple types of neurons in a final stage (*e.g.*, PN–ORN matching). To what extent and how broadly the error-correcting ability is involved in controlling wiring specificity of neural circuits is an intriguing avenue to explore in the future.

How to reverse-engineer a redundant system

Although redundancy enhances wiring robustness, it presents a greater challenge for geneticists to identify molecules and examine their loss-of-function phenotypes, as it is more difficult to perturb a redundant system using genetic experiments. How can we reverse-engineer a redundant system? First, it appears to be easier to cause a noticeable phenotype with misexpression compared with loss of function, partly because misexpression of a molecule may increase its expression to a level much higher than its endogenous level and this may create a stronger force that partially overrides other molecules (Hong *et al.* 2009, 2012). Second, simultaneous manipulation of two molecules could also potentially create a larger change of identity code and cause a stronger phenotype. Indeed, in PN dendrite targeting, loss of Caps or Trn alone causes only partial or no mistargeting of a single PN VC1, whereas removing both Caps and Trn causes VC1 dendrites to completely mistarget to an ectopic glomerulus (Hong *et al.* 2009). Third, when a class contains multiple neurons, removing or misexpressing a molecule from a single neuron or a subset of neurons using MARCM (type B errors) may produce a partial but noticeable mistargeting phenotype (Komiyama *et al.* 2007; Hong *et al.* 2009).

Although several molecules have been identified as part of the combinatorial code to determine targeting and connection specificity, they are clearly not enough for all ~50 pairs of PNs and ORNs. To overcome redundancy in future identification of additional wiring specificity molecules, enhancer screens in a sensitized background in which one molecule is removed and/or misexpression screens (Figure 5C and Figure 6, B

Table 2 Definition of properties related to robustness

Property[a]	Definition[b]
No. maximum identities encoded	$N(n)$
No. molecules removed	l
No. neuronal classes affected following the removal of l molecules	$A(n,m,d,l)$
Percentage of unaffected classes following the removal of l molecules	$P = \frac{N(n) - A(n,m,d,l)}{N(n)}$
Ectopic targets of a particular class following the removal of l molecules	$E_i(n,m,d,l)$
Average ectopic targets per affected class following the removal of l molecules	$E_{\text{average}} = \frac{\sum_{i=1}^{A} E_i(n,m,d,l)}{A}$
Total mistargeting events following the removal of l molecules	$E_{\text{total}} = \sum_{i=1}^{A} E_i(n,m,d,l)$

[a] Only type A errors are considered.
[b] n, m, and d are parameters defined by different models in Table 1.

and C) should be considered in addition to classic loss-of-function screens. Identification of combinatorial code in each of the ~50 PN and ORN classes remains a future challenge. It would also be important to discover how multiple molecules work together in the same neuron to respond to external cues and whether they regulate independent or common downstream signaling pathways.

It should be emphasized that all four models are drastically simplified in the sense that only the presence or the absence of a molecule is considered. In reality, molecules could be expressed at different levels, and identities could be specified in a spatial and/or temporal manner; all these contribute to the specificity. Moreover, the real biological system is unlikely to be designed via a top–down mechanism such that all classes use the same strategy. During evolution, different molecules and mechanisms were recruited gradually in an *ad hoc* basis. Nevertheless, the discussion of these simplified hypothetical models could guide our thinking about how the system could work, how we should design genetic experiments that allow us to perturb the specificity, and how we should interpret results and ultimately uncover the code.

Concluding Remarks

The convergence of ORN axons and PN dendrites onto single glomeruli and the precise one-to-one matching between ORNs and PNs are remarkable examples of targeting specificity in neural development. Recent studies have uncovered several key mechanisms that control different aspects of this target specificity. These mechanisms not only enhance the understanding of olfactory system assembly but also provide general insights into the principles by which complex neural circuits are assembled.

As discussed above, many questions still remain to be addressed. Combining sophisticated genetic manipulations, we could identify additional cell-surface molecules that work together with the ones that have been identified and uncover the combinatorial code for each of the ~50 pairs of PNs and ORNs. All of these joint endeavors will help create a more comprehensive picture of the olfactory circuit wiring process and help us better understand the principles governing wiring specificity in general.

Acknowledgments

The authors thank A. Ward, W. Joo, X. Gao, J. Charalel, F. Ding, B. Wu, E. Wu, and Y. Hong for commenting on the manuscript. W. Hong is a Helen Hay Whitney Fellow. L. Luo is an investigator of the Howard Hughes Medical Institute. Research on the olfactory circuit wiring in the Luo laboratory has been supported by National Institutes of Health grant R01 DC-005982.

Literature Cited

Ang, L.-H., J. Kim, V. Stepensky, and H. Hing, 2003 Dock and Pak regulate olfactory axon pathfinding in Drosophila. Development 130: 1307–1316.

Benton, R., K. S. Vannice, C. Gomez-Diaz, and L. B. Vosshall, 2009 Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell 136: 149–162.

Berdnik, D., T. Chihara, A. Couto, and L. Luo, 2006 Wiring stability of the adult Drosophila olfactory circuit after lesion. J. Neurosci. 26: 3367–3376.

Berdnik, D., A. P. Fan, C. J. Potter, and L. Luo, 2008 MicroRNA processing pathway regulates olfactory neuron morphogenesis. Curr. Biol. 18: 1754–1759.

Berdnik, D., V. Favaloro, and L. Luo, 2012 The SUMO protease Verloren regulates dendrite and axon targeting in olfactory projection neurons. J. Neurosci. 32: 8331–8340.

Chihara, T., D. Luginbuhl, and L. Luo, 2007 Cytoplasmic and mitochondrial protein translation in axonal and dendritic terminal arborization. Nat. Neurosci. 10: 828–837.

Chou, Y.-H., M. L. Spletter, E. Yaksi, J. C. S. Leong, R. I. Wilson *et al.*, 2010a Diversity and wiring variability of olfactory local interneurons in the Drosophila antennal lobe. Nat. Neurosci. 13: 439–449.

Chou, Y.-H., X. Zheng, P. A. Beachy, and L. Luo, 2010b Patterning axon targeting of olfactory receptor neurons by coupled hedgehog signaling at two distinct steps. Cell 142: 954–966.

Clyne, P. J., S. J. Certel, M. de Bruyne, L. Zaslavsky, W. A. Johnson *et al.*, 1999 The odor specificities of a subset of olfactory receptor neurons are governed by Acj6, a POU-domain transcription factor. Neuron 22: 339–347.

Couto, A., M. Alenius, and B. J. Dickson, 2005 Molecular, anatomical, and functional organization of the Drosophila olfactory system. Curr. Biol. 15: 1535–1547.

Das, A., S. Sen, R. Lichtneckert, R. Okada, K. Ito *et al.*, 2008 Drosophila olfactory local interneurons and projection neurons derive from a common neuroblast lineage specified by the empty spiracles gene. Neural Dev. 3: 33.

de Wit, J., W. Hong, L. Luo, and A. Ghosh, 2011 Role of leucine-rich repeat proteins in the development and function of neural circuits. Annu. Rev. Cell Dev. Biol. 27: 697–729.

Devaud, J.-M., A. Acebes, M. Ramaswami, and A. Ferrús, 2003 Structural and functional changes in the olfactory pathway of adult Drosophila take place at a critical age. J. Neurobiol. 56: 13–23.

Endo, K., T. Aoki, Y. Yoda, K.-I. Kimura, and C. Hama, 2007 Notch signal organizes the Drosophila olfactory circuitry by diversifying the sensory neuronal lineages. Nat. Neurosci. 10: 153–160.

Fishilevich, E., and L. B. Vosshall, 2005 Genetic and functional subdivision of the Drosophila antennal lobe. Curr. Biol. 15: 1548–1553.

Gao, Q., and A. Chess, 1999 Identification of candidate Drosophila olfactory receptors from genomic DNA sequence. Genomics 60: 31–39.

Gao, Q., B. Yuan, and A. Chess, 2000 Convergent projections of Drosophila olfactory neurons to specific glomeruli in the antennal lobe. Nat. Neurosci. 3: 780–785.

Goldman, A. L., W. van der Goes van Naters, D. Lessing, C. G. Warr, and J. R. Carlson, 2005 Coexpression of two functional odor receptors in one neuron. Neuron 45: 661–666.

Goulding, S. E., P. zur Lage, and A. P. Jarman, 2000 amos, a proneural gene for Drosophila olfactory sense organs that is regulated by lozenge. Neuron 25: 69–78.

Gupta, B. P., and V. Rodrigues, 1997 Atonal is a proneural gene for a subset of olfactory sense organs in Drosophila. Genes Cells 2: 225–233.

Hamming, R. W., 1950 Error detecting and error correcting codes. Bell Syst. Tech. J. 29: 147–160.

Hildebrand, J. G., and G. M. Shepherd, 1997 Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. Annu. Rev. Neurosci. 20: 595–631.

Hong, W., H. Zhu, C. J. Potter, G. Barsh, M. Kurusu *et al.*, 2009 Leucine-rich repeat transmembrane proteins instruct discrete dendrite targeting in an olfactory map. Nat. Neurosci. 12: 1542–1550.

Hong, W., T. J. Mosca, and L. Luo, 2012 Teneurins instruct synaptic partner matching in an olfactory map. Nature 484: 201–207.

Hummel, T., and S. L. Zipursky, 2004 Afferent induction of olfactory glomeruli requires N-cadherin. Neuron 42: 77–88.

Hummel, T., M. L. Vasconcelos, J. C. Clemens, Y. Fishilevich, L. B. Vosshall *et al.*, 2003 Axonal targeting of olfactory receptor neurons in Drosophila is controlled by Dscam. Neuron 37: 221–231.

Imai, T., H. Sakano, and L. B. Vosshall, 2010 Topographic mapping—the olfactory system. Cold Spring Harb. Perspect. Biol. 2: a001776.

Jefferis, G. S., and T. Hummel, 2006 Wiring specificity in the olfactory system. Semin. Cell Dev. Biol. 17: 50–65.

Jefferis, G. S., E. C. Marin, R. F. Stocker, and L. Luo, 2001 Target neuron prespecification in the olfactory map of Drosophila. Nature 414: 204–208.

Jefferis, G. S., R. M. Vyas, D. Berdnik, A. Ramaekers, R. F. Stocker *et al.*, 2004 Developmental origin of wiring specificity in the olfactory system of Drosophila. Development 131: 117–130.

Jhaveri, D., S. Saharan, A. Sen, and V. Rodrigues, 2004 Positioning sensory terminals in the olfactory lobe of Drosophila by Robo signaling. Development 131: 1903–1912.

Joo, W. J., L. B. Sweeney, L. Liang, and L. Luo, 2013 Linking cell fate, trajectory choice, and target selection: genetic analysis of Sema-2b in olfactory axon targeting. Neuron 78: 673–686.

Komiyama, T., and L. Luo, 2007 Intrinsic control of precise dendritic targeting by an ensemble of transcription factors. Curr. Biol. 17: 278–285.

Komiyama, T., W. A. Johnson, L. Luo, and G. S. Jefferis, 2003 From lineage to wiring specificity. POU domain transcription factors control precise connections of Drosophila olfactory projection neurons. Cell 112: 157–167.

Komiyama, T., L. B. Sweeney, O. Schuldiner, K. C. Garcia, and L. Luo, 2007 Graded expression of semaphorin-1a cell-autonomously directs dendritic targeting of olfactory projection neurons. Cell 128: 399–410.

Lai, S.-L., T. Awasaki, K. Ito, and T. Lee, 2008 Clonal analysis of Drosophila antennal lobe neurons: diverse neuronal architectures in the lateral neuroblast lineage. Development 135: 2883–2893.

Laissue, P. P., C. Reiter, P. R. Hiesinger, S. Halter, K. F. Fischbach *et al.*, 1999 Three-dimensional reconstruction of the antennal lobe in Drosophila melanogaster. J. Comp. Neurol. 405: 543–552.

Luo, L., and J. G. Flanagan, 2007 Development of continuous and discrete neural maps. Neuron 56: 284–300.

Marin, E. C., G. S. Jefferis, T. Komiyama, H. Zhu, and L. Luo, 2002 Representation of the glomerular olfactory map in the Drosophila brain. Cell 109: 243–255.

McLaughlin, T., and D. D. M. O'Leary, 2005 Molecular gradients and development of retinotopic maps. Annu. Rev. Neurosci. 28: 327–355.

Mosca, T. J., W. Hong, V. S. Dani, V. Favaloro, and L. Luo, 2012 Trans-synaptic Teneurin signalling in neuromuscular synapse organization and target choice. Nature 484: 237–241.

Okada, R., T. Awasaki, and K. Ito, 2009 Gamma-aminobutyric acid (GABA)-mediated neural connections in the Drosophila antennal lobe. J. Comp. Neurol. 514: 74–91.

Sakano, H., 2010 Neural map formation in the mouse olfactory system. Neuron 67: 530–542.

Sakurai, M., T. Aoki, S. Yoshikawa, L. A. Santschi, H. Saito *et al.*, 2009 Differentially expressed Drl and Drl-2 play opposing roles in Wnt5 signaling during Drosophila olfactory system development. J. Neurosci. 29: 4972–4980.

Sekine, S. U., S. Haraguchi, K. Chao, T. Kato, L. Luo *et al.*, 2013 Meigo governs dendrite targeting specificity by modulating ephrin level and N-glycosylation. Nat. Neurosci. 16: 683–691.

Silbering, A. F., R. Rytz, Y. Grosjean, L. Abuin, P. Ramdya *et al.*, 2011 Complementary function and integrated wiring of the evolutionarily distinct Drosophila olfactory subsystems. J. Neurosci. 31: 13357–13375.

Simon, A. F., I. Boquet, M. Synguélakis, and T. Préat, 1998 The Drosophila putative kinase linotte (derailed) prevents central brain axons from converging on a newly described interhemispheric ring. Mech. Dev. 76: 45–55.

Spletter, M. L., J. Liu, J. Liu, H. Su, E. Giniger *et al.*, 2007 Lola regulates Drosophila olfactory projection neuron identity and targeting specificity. Neural Dev. 2: 14.

Su, C.-Y., K. Menuz, and J. R. Carlson, 2009 Olfactory perception: receptors, cells, and circuits. Cell 139: 45–59.

Sweeney, L. B., A. Couto, Y.-H. Chou, D. Berdnik, B. J. Dickson *et al.*, 2007 Temporal target restriction of olfactory receptor neurons by Semaphorin-1a/PlexinA-mediated axon-axon interactions. Neuron 53: 185–200.

Sweeney, L. B., Y.-H. Chou, Z. Wu, W. Joo, T. Komiyama *et al.*, 2011 Secreted semaphorins from degenerating larval ORN axons direct adult projection neuron dendrite targeting. Neuron 72: 734–747.

Tea, J. S., and L. Luo, 2011 The chromatin remodeling factor Bap55 functions through the TIP60 complex to regulate olfactory projection neuron dendrite targeting. Neural Dev. 6: 5.

Tea, J. S., T. Chihara, and L. Luo, 2010 Histone deacetylase Rpd3 regulates olfactory projection neuron dendrite targeting via the transcription factor Prospero. J. Neurosci. 30: 9939–9946.

Vosshall, L. B., and R. F. Stocker, 2007 Molecular architecture of smell and taste in Drosophila. Annu. Rev. Neurosci. 30: 505–533.

Vosshall, L. B., H. Amrein, P. S. Morozov, A. Rzhetsky, and R. Axel, 1999 A spatial map of olfactory receptor expression in the Drosophila antenna. Cell 96: 725–736.

Vosshall, L. B., A. M. Wong, and R. Axel, 2000 An olfactory sensory map in the fly brain. Cell 102: 147–159.

Wilson, R. I., 2013 Early olfactory processing in Drosophila: mechanisms and principles. Annu. Rev. Neurosci. 36: 217–241.

Wilson, R. I., and Z. F. Mainen, 2006 Early events in olfactory processing. Annu. Rev. Neurosci. 29: 163–201.

Wong, A. M., J. W. Wang, and R. Axel, 2002 Spatial representation of the glomerular map in the Drosophila protocerebrum. Cell 109: 229–241.

Yao, Y., Y. Wu, C. Yin, R. Ozawa, T. Aigaki *et al.*, 2007 Antagonistic roles of Wnt5 and the Drl receptor in patterning the Drosophila antennal lobe. Nat. Neurosci. 10: 1423–1432.

Zhu, H., and L. Luo, 2004 Diverse functions of N-cadherin in dendritic and axonal terminal arborization of olfactory projection neurons. Neuron 42: 63–75.

Zhu, H., T. Hummel, J. C. Clemens, D. Berdnik, S. L. Zipursky *et al.*, 2006a Dendritic patterning by Dscam and synaptic partner matching in the Drosophila antennal lobe. Nat. Neurosci. 9: 349–355.

Zhu, S., S. Lin, C.-F. Kao, T. Awasaki, A.-S. Chiang *et al.*, 2006b Gradients of the Drosophila Chinmo BTB-zinc finger protein govern neuronal temporal identity. Cell 127: 409–422.

Communicating editor: J. Rine