on the substrate. For instance, force-dependent activation of ion channels was initially considered a likely means of mechanotransduction at sites of adhesion. However, studies demonstrating that permeabilized cells retain the ability to recruit component of focal adhesions to the sites of adhesion suggested that it is unlikely that channels play a central role in the regulation of integrin-mediated adhesion (Tamada et al., 2004). Instead, these studies suggest that the submembrane components of focal adhesions contain all the basic elements of the mechanosensitive machinery. Other potential mechanisms include a "perturbation-reannealing process," in which mechanical force breaks protein-protein interactions, thereby stimulating their reassembly or the tension-dependent enhancement of formin-induced actin polymerization (Bershadsky et al., 2006).

It is also possible that applied force modulates the activity of potent kinases (e.g., Src-family kinases or FAK) or phosphatases by directly affecting their conformation, and consequently their enzymatic activity. Future work may establish whether other mechanosensory events occur at focal adhesions and could show how these events are coupled to the stretching of p130Cas.


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# Follow Your Nose: Axon Pathfinding in Olfactory Map Formation


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**Two new studies report how discrete identities of olfactory sensory neurons are converted into a spatial map of axonal connections (Imai et al., 2006; Serizawa et al., 2006). They find that levels of cAMP signals derived from olfactory receptors (ORs) can direct targeting of axons along an axis, and that ORs and neural activity regulate expression of adhesion/guidance molecules in mosaic patterns that can sort axons into discrete locations.**

Neural maps are a fundamental feature of brain architecture, forming the connections that transfer information from one area of the nervous system to another. These maps can be classified into two broad categories: continuous and discrete. In a continuous topographic map, such as the visual projection from the retina to the midbrain tectum, the spatial organization of the projecting neurons is maintained in the spatial order of their connections to the target, with nearest-neighbor relationships preserved. In contrast, in a discrete map, axons from spatially dispersed neurons with the same identity converge in one location in the target field, converting discrete information into a spatial representation. The best studied example of a discrete map is in the mammalian olfactory system.

Landmark work by Buck and Axel (Buck and Axel, 1991) identified the olfactory receptors (ORs), which transduce odorant information into electrical activity. In mice, there are approximately 1000 OR genes, which are thought to be expressed in a mutually exclusive manner in olfactory sensory neurons. Although neurons expressing the same receptor are dispersed in the olfactory epithelium, their axons converge into a pair of glomeruli at characteristic positions in the olfactory bulb. This results in a discrete chemotopic map that converts OR identity into a spa-

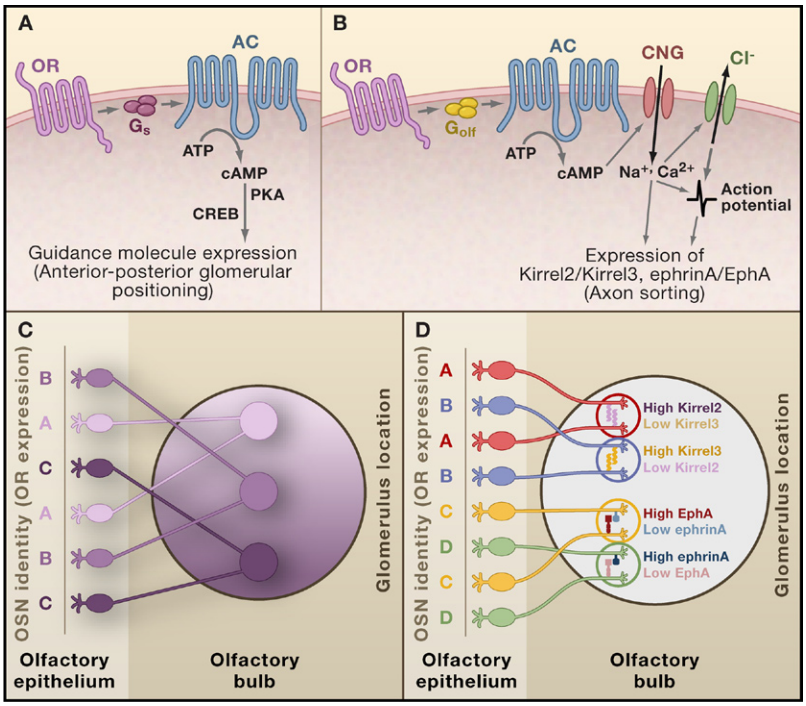


**Figure 1. Molecular Pathways and Models for Olfactory Map Formation**

(A) Proposed molecular pathway for coarse anterior-posterior mapping. Imai et al. (2006) find that $G_s$ couples olfactory receptors (ORs) with adenylyl cyclase (AC) for cAMP production and subsequent expression of guidance molecules, probably at an early stage of map development.

(B) Classical molecular pathway for OR signaling. Serizawa et al. (2006) provide evidence that components of the classical OR-signaling pathway are involved in the sorting of olfactory sensory neuron axons. $G_{olf}$ couples OR with AC for cAMP production, which opens a cyclic-nucleotide-gated (CNG) channel, allowing cation influx and membrane depolarization. Calcium-dependent chloride channel activation leads to further depolarization. CNG channel is required for OR-specific expression of adhesion/guidance molecules involved in axon sorting, suggesting activity dependence. This process probably occurs at a later stage of map development.

(C) Model of anterior-posterior glomerulus mapping by gradients. OR identity of each olfactory sensory neuron (OSN) is converted into a unique level of cAMP signals and guidance molecule expression, leading to the anterior-posterior positioning of glomeruli. An anterior low, posterior high gradient of guidance receptors such as neuropilin-1 is depicted in purple in the olfactory bulb (top is anterior, bottom is posterior).

(D) Model of axon sorting into glomeruli by discrete labels. OR-specific expression of adhesion/guidance molecules leads to the sorting of axons. Homophilic adhesive interaction of Kirrel2 and Kirrel3 can mediate fasciculation of axons expressing the same OR. Repulsive interaction between high EphA and high ephrinA may mediate segregation of axons with different ORs. Attractive interaction between high EphA and low ephrinA (or low EphA and high ephrinA) could also mediate fasciculation of axons with the same OR.

tial representation in the olfactory bulb (reviewed by Komiyama and Luo, 2006).

Whereas continuous maps can be generated by gradients of axon guidance cues (Flanagan, 2006), the specific mechanism for axon guidance in discrete maps, such as the olfactory system, has been unclear. How might this mapping be accomplished? A key observation is that when the coding region of one OR is substituted with another, axons expressing the inserted OR converge to a different glomerulus position, indicating that ORs play an instructive role in the targeting of axons (Mombaerts et al., 1996). Potentially, ORs could act as axon guidance receptors themselves, although there is no direct evidence for this yet. Alternatively, the expression of guidance receptors may be regulated by OR identity. Although classical guidance molecules have been implicated in olfactory mapping (Komiyama and Luo, 2006), it has not been clear how they could link OR identity to map formation. Now two new studies from the Sakano group reveal that OR-derived cAMP signals can direct glomerular positioning and that OR-regulated adhesion/guidance molecules can influence axon sorting (Imai et al., 2006; Serizawa et al., 2006). Together, they provide a molecular link between OR identity and axon guidance decisions,and offer an appealing model for the logic of discrete map formation.

In this issue, Serizawa et al. (2006) report identification of a set of OR-specific adhesion/guidance molecules that regulate the sorting of axons of olfactory sensory neurons. The authors sought to identify molecules involved in fasciculation (the bundling of axons) regulated by OR identity. By using transgenic mice in which the majority of olfactory sensory neurons express a particular OR, Serizawa et al. (2006) searched for genes whose expression correlates with this OR. Together with a candidate search for cell-recognition molecules, they identified the OR-specific adhesion molecules *Kirrel2*/*Kirrel3* and the guidance molecules *ephrinA5*/*EphA5*. These proteins were found to be expressed in a mosaic pattern, with different levels in different glomeruli. Importantly, these molecules showed complementary expression in olfactory sensory neurons: when Kirrel2 is high, Kirrel3 is low, and vice versa; and likewise when ephrinA is high, EphA is low, and vice versa (Figure 1D).

The authors then swapped the coding region of one OR with another and found a change in *Kirrel* expression levels, demonstrating that ORs are upstream of *Kirrel* expression. In addition, Kirrel2 and Kirrel3 were shown to mediate homophilic adhesion in cultured cells, suggesting that Kirrels could mediate axon sorting via homophilic adhesion between like axons. To test this idea directly, the authors conducted mosaic analysis by generating two sets of olfactory sensory neurons in the same transgenic animal: one with the endogenous level and the other with elevated Kirrel2. Strikingly, the two sets of neurons with the same OR now segregated into duplicated, adjacent glomeruli. This suggests that Kirrels mediate differential axon-

axon fasciculation, although a mechanism involving axon-target interaction cannot be ruled out. In either case, the authors provide convincing evidence that Kirrels function in OR-specific axon sorting.

Previous results had demonstrated that neurons with different ORs express different levels of ephrinAs on their axons, and ephrinAs are required for normal mapping (Cutforth et al., 2003). Serizawa et al. (2006) now find that *EphA5* shows complementary expression to *ephrinA5* in both the neuron and glomerulus. The authors propose that they act as contact-mediated repulsion molecules in axon segregation. It seems possible that ephrins could also have an attractant/adhesion function in olfactory mapping, since they can act as either attractants or repellents in other systems (Flanagan, 2006). In either case, these findings raise the possibility that olfactory axon sorting may involve multiple guidance molecules in a combinatorial code.

To investigate the role of activity in this process, Serizawa et al. (2006) analyzed mice mutant for *CNGA2*, an ion channel downstream of OR activation (Figure 1B). They provide evidence that OR-specific expression level of Kirrels and ephrinA/EphA is regulated in an activity-dependent manner. The precise role of this activity remains unknown. Is it spontaneous or evoked by odorants or other molecules? Is it random, or does it need to be patterned? These will be fascinating questions for the future.

In a separate study from the Sakano lab, Imai et al. (2006) studied how ORs may influence the anterior-posterior (A-P) positions of glomeruli. ORs are G protein-coupled receptors that were thought to transmit signals by activating the olfactory-specific G protein ($G_{olf}$) (Figure 1B). Mice deficient for $G_{olf}$ are anosmic but form an apparently normal glomerular map, which led Imai et al. (2006) to search for another G protein involved in targeting of olfactory sensory neurons. They found that constitutively active $G_s$ could rescue the axon targeting defect in an OR mutant that fails to couple to G proteins, thus identifying an alternative G protein pathway (Figure 1A). Furthermore, using elegant genetic manipulations of ORs, $G_s$, and intracellular signaling components that change levels of the downstream second messenger cAMP, they provided evidence that levels of cAMP determine the A-P positioning of glomeruli. Reasoning that this effect may involve transcriptional control of downstream molecules, they indeed found that expression of molecules such as the guidance receptor *neuropilin-1* correlates with levels of cAMP and are expressed in an A-P gradient in the olfactory bulb. The authors therefore proposed a model in which OR-derived cAMP signals direct glomerular positioning by modifying expression levels of guidance molecules (Figure 1C). Although it remains to be tested whether each OR correlates with a unique level of cAMP, these data offer a specific model of how OR identity could be converted into a spatial map.

An attractive aspect of these new papers is that they would help explain several previously surprising results. For instance, it had been unclear why after replacement of OR-A with OR-B, axons expressing OR-B converge to a glomerulus distinct from either the normal A or B position (Mombaerts et al., 1996). Additionally, in the same study, introduction of a reporter construct into an OR gene resulted in altered glomerular targeting. Also, a reduction of OR expression level caused an anterior shift of glomerulus position (Feinstein et al., 2004). These observations suggested that not only OR identity but also OR expression level may be important (Mombaerts et al., 1996; Feinstein et al., 2004), and in light of the new studies this may be explained by changed levels of cAMP and downstream guidance molecules. The new findings also might help explain the axonal convergence into a glomerulus when the β2 adrenergic receptor (*β2AR*) is expressed from an OR locus (Feinstein et al., 2004). β2AR is coupled to $G_s$ and thus is able to activate the same signaling pathway that Imai et al. (2006) identified as playing a role in glomerular mapping.

It is particularly appealing that the two new studies can lead to an overall two-step model to explain discrete mapping. First, axons find an approximate target by following a gradient of guidance molecules (Figure 1C) (Imai et al., 2006). Second, axons are further sorted in an activity-dependent manner, according to OR-specific adhesion/guidance molecules that can display sharp differences between neighboring glomeruli (Figure 1D; Serizawa et al., 2006). Why use gradients in a discrete map? First, gradients can be more economical, removing the need for a large family of ligand-receptor pairs of guidance molecules, because an individual graded molecule can specify multiple positional values. And second, molecular gradients can provide directional information during guidance, leading axons to converge efficiently toward the correct position without having to sample the entire target field (Gierer, 1998). But molecular gradients have the disadvantage that they may not provide the robustness and precision required for the diverse, discrete OR-specific axon targeting during olfactory map formation. How can this problem be overcome? Axon sorting by discrete cues (Figure 1D) may serve to ensure that like axons converge into a single glomerulus. Robustness and specificity could be further enhanced by the use of a combinatorial code of different guidance molecules, as suggested from the OR-specific expression of both Kirrels and ephrin/Eph proteins.

Interestingly, this two-step model for discrete mapping is analogous to the strategies used to specify continuous neural maps, where initial mapping by graded labels is followed by Hebbian activity-dependent refinement. Thus, nature may employ similar strategies to generate very different maps. Although much remains to be learned, the new work can provide an emerging logic for axon guidance in the formation of discrete neural maps. It smells right.

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