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Axon Guidance of Olfactory Sensory Neurons in Zebrafish

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The guidance of growing axons to their appropriate targets is a crucial process for the establishment of complex but highly ordered neural circuits and the proper functioning of the nervous system. The axonal convergence of olfactory sensory neurons (OSNs) expressing a given odorant receptor (OR) onto spatially invariant glomeruli (Ressler *et al.*, 1994; Vassar *et al.*, 1994; Mombaerts *et al.*, 1996) is one of the most exciting examples of precise axonal targeting. Genetic deletions or substitutions of specific OR genes in mice suggested that the ORs themselves play an instructive role in the guidance of OSN axons, but guidance molecules other than ORs are also required for precise glomerular targeting (Mombaerts *et al.*, 1996; Wang *et al.*, 1998). Indeed, a large number of axon guidance and cell recognition molecules are expressed in the developing olfactory system, with dynamic spatiotemporal patterns (Yoshihara *et al.*, 1997; Pasterkamp *et al.*, 1999; Astic *et al.*, 2002; St John *et al.*, 2002). However, functional evidence for their involvement in the precise glomerular targeting is limited. To learn about molecular basis of specific connectivity of the olfactory neural circuitry, we are studying molecular and cellular mechanisms underlying axon guidance of OSNs in zebrafish, an excellent vertebrate model system.

The zebrafish offers many advantages over other model organisms. It is well suited to forward genetics because of large clutch size and relatively short generation time. Externally fertilized eggs can be easily manipulated. Its nervous system has reduced complexity and high similarity to that of higher vertebrates: zebrafish olfactory bulb (OB) contains only ~80 glomeruli, as compared with ~18 00 in rodents (Baier and Korsching, 1994); optical imaging techniques revealed that individual odorants can elicit activity in specific subsets of glomeruli in the OB as in rodents (Friedrich and Korsching, 1997, 1998). A great advantage for studying axon guidance mechanisms is the optical transparency of zebrafish during early development. By combining transgenesis with the use of fluorescent proteins such as green fluorescent protein (GFP), it is possible to visualize dynamic axon behavior in living embryos (Dynes and Ngai, 1998; Higashijima *et al.*, 2000). Thus, we are taking a two-step strategy to identify genes that control precise axonal targeting of OSNs: (i) establishment of transgenic zebrafish lines in which GFP is expressed in specific subpopulations of OSNs and (ii) analysis of existing mutants by crossing with the GFP-expressing transgenic lines or application of reverse genetic approach using antisense morpholino oligonucleotides to the transgenic lines.

In many terrestrial vertebrates including rodents, there are two distinct olfactory systems: main and accessory. In the main olfactory system, ciliated sensory neurons lying in the olfactory epithelium (OE) sense volatile odorant molecules and transmit their information to the main OB. In the accessory olfactory system, on the other hand, microvillous sensory neurons populating the vomeronasal organ sense pheromone molecules and send their information to the accessory OB. In contrast, fish including zebrafish have a single OE and a single OB. Ciliated and microvillous OSNs occupy the same OE, and the somata of each type of OSNs are situated at different positions along the apical-basal axis (Yamamoto, 1982; Morita and Finger, 1998). We have established two transgenic zebrafish lines in which GFP is expressed in either ciliated or microvillous OSNs (unpublished data). Immunohistochemical analysis of the OB from each transgenic line revealed that ciliated and microvillous OSNs projected their axons to mutually exclusive sets of glomeruli in the adult OB. Furthermore, we were able to obtain high-resolution images of the axonal trajectories of each type of OSNs in living embryos throughout development. Our ongoing analysis of existing mutants under the transgenic background has identified an interesting one in which the navigation of OSN axons to the OB is perturbed. Furthermore, these GFP-expressing transgenic lines can be used as starting strains for mutagenesis to find new mutations that affect OSN axon pathfinding. We expect that these transgenic lines will allow us to discover many genes critical for the formation and maintenance of precise neural connectivity patterns in the zebrafish olfactory system.

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