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Functional development of the olfactory system in zebrafish

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ABSTRACT

The olfactory system has become a popular model to study the function of neuronal circuits and the molecular and cellular mechanisms underlying the development of neurons and their connections. An excellent model to combine studies of function and development is the zebrafish because it not only permits sophisticated molecular and genetic analyses of development, but also functional measurements of neuronal activity patterns in the intact brain. This article reviews insights into the functional development of the olfactory system that have been obtained in zebrafish. The focus is on the specification of olfactory sensory neurons (OSNs), the mechanisms controlling odorant receptor expression and OSN identity, the pathfinding of OSN axons towards target glomeruli in the olfactory bulb (OB), the development of glomeruli and functional topographic maps in the OB, and the development of inhibitory interneurons in the OB.

1. Basic organization of the olfactory system in vertebrates

Odors are detected by olfactory sensory neurons (OSNs) in the nose, each of which expresses one odorant receptor (OR) from a repertoire of hundreds or, in some species, >1000 different genes (one neuron–one receptor rule). OSNs expressing the same OR converge their axons onto a specific target glomerulus in the first processing center in the brain, the olfactory bulb (OB), establishing a discrete and precise map of OR expression (one glomerulus–one receptor rule) (Axel, 1995; Buck, 2000). Because individual odorants activate multiple ORs with different efficacy, information about an odor is encoded in the input layer of the OB by a specific pattern of activation across the glomerular array (Friedrich and Korsching, 1997).

Odor-evoked activity patterns are processed by a network of neurons in the OB and conveyed by the principal neurons, the mitral cells, to multiple higher brain areas including the piriform cortex, anterior olfactory nucleus, olfactory tubercle, cortical amygdala and entorhinal cortex. In rodents, projections to some of these areas exhibit a coarse topographic organization but the projection to piriform cortex, the largest target of the OB, is widespread and diffuse (Ghosh et al., 2011; Miyamichi et al., 2011; Sosulski et al., 2011). The map of OR expression in the OB is thus not preserved by the secondary olfactory projection to piriform cortex. Rather, neurons in piriform cortex integrate inputs from topographically and

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functionally different mitral cells and respond to odors with widespread, yet stimulus-specific, activity patterns (Stettler and Axel, 2009; Yaksi et al., 2009).

The development of this system encompasses multiple steps including the specification of OSNs, the selection of an individual OR for expression, the pathfinding towards the target glomerulus, the assembly of neuronal circuits in the OB, and the establishment of higher-order projections. Remarkably, the olfactory system is functional already long before its development is complete, raising the question how the function of neuronal circuits is initially established and subsequently refined. A favorable animal model to study this complex of questions is the zebrafish because it allows for the combination of molecular, genetic, and developmental approaches with neurophysiological measurements in transparent larvae (Friedrich et al., 2010). This article reviews insights into the molecular, anatomical and functional development of the olfactory system in zebrafish.

2. Formation and development of the olfactory placode

2.1. Specification of the pre-placodal region

In all vertebrates, the cranial sensory organs including the olfactory epithelium develop from the placodes, transient structures of thickened ectoderm in the developing head (Baker and Bronner-Fraser, 2001). It has been suggested that all placodal precursors arise from a common territory surrounding the anterior neural plate, the pre-placodal region (Fig. 1A) (Schlosser, 2006; Streit, 2004). In the developing zebafish, several transcription factors such as *dlx3b* (formerly *dlx3*) (Akimenko et al., 1994), *dlx4b* (formerly *dlx7*) (Ellies et al., 1997), *eya1* (Sahly et al., 1999), *six1b* (formerly *six1*) (Bessarab et al., 2004), and *six4b* (formerly *six4.1*) (Kobayashi et al., 2000) are expressed in a horseshoe-like continuous stripe around the edge of anterior neural plate, which coincides with the proposed pre-placodal region. Signaling molecules that induce the pre-placodal region emanate from the underlying mesoderm and the adjacent neural plate. FGF signaling together with antagonism of both BMP and Wnt signaling are proposed to specify the pre-placodal region as a narrow band at the neural plate border (Schlosser, 2006; Streit, 2004).

The initially broad expression of these genes in the pre-placodal region becomes restricted to some, but not all, of the placodes. *dlx3b* and *dlx4b*, for example, gradually show restricted patterns of expression in the otic and olfactory placodes as they become morphologically evident (Ellies et al., 1997). Antisense morpholino oligonucleotide-mediated knockdown of both *dlx3b* and *dlx4b* functions causes severely reduced expression of early marker genes for the otic and olfactory placodes (Solomon and Fritz, 2002), suggesting that these two genes could concertedly induce and/or maintain the competence to differentiate into the otic and olfactory placodal cells. A recent study has reported that *dlx3b* and *dlx4b* assist in specifying the pre-placodal region through attenuating BMP activity (Esterberg and Fritz, 2009). Together these observations suggest that the transcription factors broadly expressed in the pre-placodal region could first play an important role in specifying the pre-placodal region itself and then could contribute to the induction of particular subsets of placodes from the common territory. To date it is still uncertain what combinations of transcription factors are required for inducing individual placodes including the olfactory placode and what downstream target genes they control.

2.2. Olfactory placode assembly

A refined fate-mapping study in zebrafish has revealed that the olfactory placode arises from a large cellular field

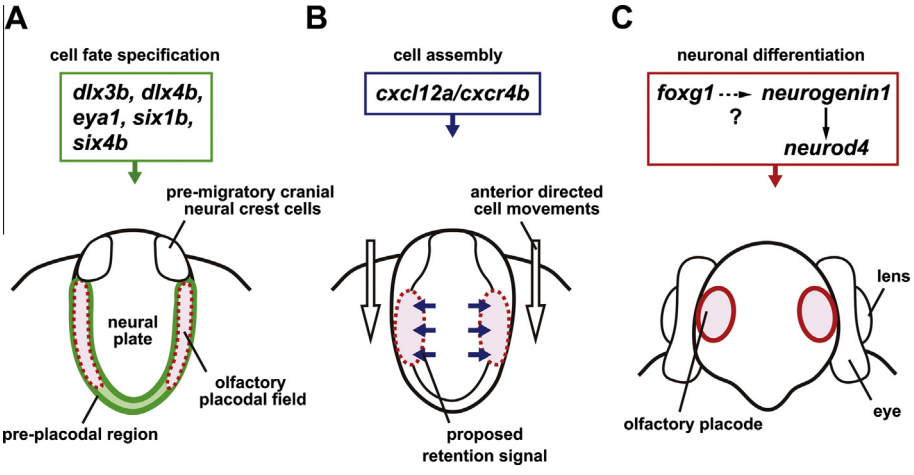

Fig. 1 – Schematic summary of olfactory placode development. (A) The cranial sensory organs develop from a common territory at the border of neural plate, the pre-placodal region (area with a green border), which is specified by the expression of several transcription factors (green box). The olfactory placode arises from a large cellular field (olfactory placodal field; area with a red dashed border) within the pre-placodal region. (B) The neural plate-derived Cxcl12a provides the Cxcr4b-expressing olfactory placodal precursors (area with a red dashed border) with a retention signal (blue arrows) to withstand the anterior-directed movement of neighboring cells such as cranial neural crest cells. (C) Differentiation of OSNs within the olfactory placode (area with a red border) is regulated by forkhead family and bHLH family transcription factors (red box).