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ONSET OF ODORANT RECEPTORS

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Abstract

Odorant receptors are thought to be critical determinants of olfactory sensory neuron axon targeting and organization. Nonetheless, a systematic characterization of the onset of odorant receptor expression has not yet been done in the main olfactory epithelium. Here, we briefly review our current understanding regarding the onset of odorant receptor expression in the main olfactory epithelium and identify some of those questions which we believe must be of high priority for future study.

Keywords

olfactory epithelium; development; axon guidance

The appearance of the placode as a thickening of the ectoderm on the rostral-lateral head around embryonic day (E) 9–9.5 is the first sign of the development of the olfactory epithelium (OE). It is followed by the formation of the pit around E10 and a cavity by E12 that develops into a more complex structure with multiple turbinates (1,2,3 for review). During this process, the growth of multiple cranial-facial structures, such as the cheeks and the maxillary palps pushes the nostrils from the original lateral position towards the midline. The first putative olfactory sensory neurons (OSNs) appear around E9 and the number continuously increases as the surface area and thickness of the OE continue to increase. The first axons extend from OSNs at E10 (2) and appear to be dependent on the expression of an intact odorant receptor (OR) (4). The adult-like organization of the nasal cavity, including the delineation of turbinates and recesses, is apparent by approximately E13. The OSN axons first reach the nascent olfactory bulb (OB) by approximately E11.5–12 but the first synapses are not apparent in appreciable numbers until E15 (5). Proteins G_{olf} and olfactory cyclic nucleotide-gated channel 1 (OCNC1), which are present in the odor transduction cascade of mature OSNs, do not appear robustly until around E15, but adenylyl cyclase III (ACIII), downstream from the G-protein coupled OR, is present at E13 (6). Prior to E15 immature OSNs, appear to express G_S in lieu of G_{olf} (7).

Each OSN expresses only 1 out of ~1100 different ORs. Axons from OSNs expressing the same OR converge into 2–3 glomeruli per OB (8–11); and all the axons terminating in a glomerulus express the same OR (12). Because substitution of ORs disrupts or redirects the convergence of axons in the OB, changing the molecular/glomerular map of ORs, they have been proposed to be critical determinants of OSN axon targeting and organization (4,13,14). More recently, several reports suggest that downstream signaling mediated via ACIII and

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the production of cAMP are also involved and can supersede the OR to promote the coalescence and convergence of axons, perhaps through the regulated translation of cell adhesion molecules (7,15–17).

Given the overwhelming evidence that ORs, and perhaps members of their transduction cascade, are involved in the coalescence and glomerular termination of axons that express the same OR, we have asked when are ORs first expressed in the main OE. It seems plausible that if ORs regulate both axon extension and coalescence/targeting, their onset of expression in OSNs must correlate with axogenesis and more generally, differentiation and maturation of the OSNs. While the onset of OR expression is an important question in the field, the available data is somewhat conflicting. There are a limited number of articles regarding the onset of OR expression in the main OE (18–23). These groups characterized a small number of ORs (usually between 1 and 4), they employed different ways of defining conception (or embryonic day 0 (E0)) and thus staging is not consistent amongst them making it difficult to compare the data between them. Moreover, the methods used to analyze the onset of OR expression differ in many cases (isotopic versus chromogenic *in situ* hybridization or transgenic animals) which makes comparison between them complicated.

A more comprehensive study was recently performed in Dr. Minghong Ma's laboratory (24,25). Nine different ORs were characterized in the septal organ, a repertoire which were expressed in over 90% of the olfactory marker protein (OMP)-expressing cells. They showed that the onset of all these ORs is asynchronous; it takes place over a 3 day window, and by birth all the ORs are expressed. One caveat of this study, however, is that while the septal organ appears to offer a smaller simplified model of the olfactory system to study OR onset, the presumptive septal organ first appears around E16 in the mouse, which is relatively late in the development of the olfactory pathway. ORs are thought to be necessary for axon targeting of the OB, and by E16 some axons have already established synapses in the OB (5). As was mentioned above, the olfactory placode develops around E9 in the mouse, and axons first reach the presumptive olfactory bulb around E11.5–12. Thus the question of when OR expression occurs in the main olfactory epithelium remains unanswered.

Several central issues have not yet been systematically addressed, and as a first step we have begun to ask if the onset of OR expression in the main olfactory epithelium is dependent on, or correlates with, family class. There are some intriguing hints. For example, MOR256-17 was reported to be expressed as early as E10, by isotopic *in situ* hybridization (22), and as early as E11 by chromogenic *in situ* (19). Nonisotopic *in situ* showed that MOR256-11 expression is turned on by E11.5 (18). Despite the fact that it belongs to the same family as MOR256-17 the ORs are turned on with half a day difference. On the other hand, both genes are located on different chromosomes which raises the question of the importance of chromosomal gene sequence position. Even though there is a paucity of evidence, two reports have suggested that the onset of expression of two different chromosome 7 ORs, MOR18-2 and MOR263-5 is E11 and E12.5, respectively. Consequently, two new questions arise. First, is cluster position on the chromosome important for the onset of expression? Second, is zonal expression in the OE a determinant for OR onset? Because little systematic information is available to answer these questions, our ongoing analyses of OR expression consist of early developmental studies that include the careful selection of OR genes that differ based on family, chromosome, cluster and OE zone. We hope that as our studies progress, further insights into the mechanisms regulating the onset of OR expression will emerge.

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