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Research Report

Onset and dynamic expression of S100 proteins in the olfactory organ and the lateral line system in zebrafish development

Corina M. Sandulescu[a,1], Ru Yi Teow[b], Melina E. Hale[b], Chunbo Zhang[a,*]

[a]Department of Biological, Chemical and Physical Sciences, Illinois Institute of Technology, Chicago, IL 60616, USA
[b]Department of Organismal Biology and Anatomy, University of Chicago, Chicago, IL 60637, USA

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ABSTRACT

In the zebrafish olfactory epithelium, three morphologically distinct olfactory neurons express different marker proteins. We utilize this feature to access developmental dynamics of one of the neuron types, the crypt cells, to determine whether they are differentiated at a stage similar to other olfactory neurons. Immunohistochemical studies using an S100 antibody that specifically recognizes crypt cells showed that S100-positive cells appear in olfactory rosettes as early as at 2 day postfertilization (dpf). However, some of the rosettes did not have any S100-positive cells until 4 dpf. The number of S100-positive cells in individual rosettes increased steadily over the next 3 days before it decreased significantly. There were 7.8 S100-positive cells per rosettes on average in larvae at 7 dpf. The number reduced to 2.2 at 9 dpf. A recovery to a pre-reduction level was detected in 12 dpf larvae. We also observed S100-positive cells in neuromasts of the lateral line system in 2 dpf larvae, suggesting that the crypt cells and sensory cells in the neuromasts have similar onsets of differentiation. Our data have provided a time line of differentiation of crypt cells in development of the olfactory system and demonstrated that this type of cell is differentiated at a stage similar to ciliated and microvillous olfactory neurons. A nonlinear growth trajectory of the crypt cell population in the first nine days of zebrafish development implicates a possible functional significance of crypt cells in early life stages of zebrafish.

1. Introduction

Although gross anatomy of the olfactory organ varies considerably among teleost species, the cellular and biochemical characteristics of the olfactory epithelium are more or less similar: the pseudostratified olfactory epithelium consists of supporting cells, olfactory neurons and basal cells. Olfactory neurons may be categorized into ciliated, microvillous and crypt olfactory neurons according to their morphology (Hansen and Zielinski, 2005). In zebrafish, these three types of olfactory neurons are arranged in patterns. Somata of ciliated neurons are imbedded in a deep sublayer of the epithelium while those of microvillous neurons are normally found in the upper portion of the epithelial layer (Hansen et al., 2004; Hansen and Zielinski, 2005; Sato et al., 2005). Crypt cells reside at the apical surface of the epithelium (Hansen and Finger, 2000; Hansen and Zeiske, 1998; Sato et al., 2005).

* *Corresponding author at:* Department of Biological, Chemical and Physical Sciences, Illinois Institute of Technology, 3101 S. Dearborn Street, Rm 182 LS, Chicago, IL 60616, USA. Fax: +1 312 567 3494.
E-mail address: ZhangC@iit.edu (C. Zhang).
[1] Current address: Rosalind Franklin University, 3333 Green Bay Road, North Chicago, IL 60064, USA.

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Recent anatomical, cellular and genetic studies have shown that these three types of neurons express different signal transduction molecules and project to segregated regions in the olfactory bulb and telencephalon (Hamdani and Doving, 2007; Hansen et al., 2003; Sato et al., 2005). In zebrafish, ciliated olfactory neurons express olfactory marker protein (OMP), a protein uniquely expressed in mature olfactory neurons and vomeronasal neurons in rodents (Margolis, 1980; Mombaerts et al., 1996; Sato et al., 2005). These neurons express G-protein $G\alpha_{olf}$ and rhodopsin-like olfactory receptors (Hansen et al., 2004; Sato et al., 2005). On the other hand, microvillous neurons express transient receptor potential channel C2 (TRPC2), an ortholog of mammalian TRPC2 that is expressed in vomeronasal neurons and is important for pheromone signal transduction in mammals (Liman et al., 1999; Sato et al., 2005; Stowers et al., 2002). They express G-protein $G\alpha_o$ and possibly other types of G-protein subunits (Hansen et al., 2004). The V2R family of olfactory receptors is expressed in these neurons (Hansen et al., 2004; Sato et al., 2005). Oval shaped crypt cells exhibit strong S100 protein-like immunoreactivity and are sparsely scattered over the olfactory epithelium (Germana et al., 2004b; Sato et al., 2005). Although it is unknown which types of olfactory receptors are associated with crypt cells, electrophysiological studies in Pacific jack mackerel (*Trachurus symmetricus*) suggest that a majority of them are sensitive to amino acid stimuli and the activation is mediated by the cAMP pathway (Vielma et al., 2008). It was found that in adult crucian carp (*Carassius carassius*), the number of crypt cells in the apical surface of the olfactory epithelium increased during spawning season, suggesting a possible role for crypt cells in pheromone detection (Hamdani et al., 2008).

Olfactory neuron differentiation and function start early in development. In zebrafish, olfactory placodes first appear at 14–16 hour postfertilization (hpf) (6–10 somites) as bilateral thickenings of subepidermal ectoderm at the rostral–ventral region of the head, between the developing forebrain and the optic vesicles (Hansen and Zeiske, 1993). Placodal cells start to differentiate into olfactory neurons, as indicated by expression of neuronal markers and axonal projections, around 24–48 hpf (Barth et al., 1996; Yoshida et al., 2002). Expression of olfactory receptors is first detected in the placodes at 24–30 hpf (Argo et al., 2003; Barth et al., 1996). Interestingly, onsets of olfactory receptor expression occur at different time points for different sets of receptors. It was shown that a group of olfactory receptors, including ZOR1, ZOR2, ZOR9 and ZOR10, was expressed by 30 hpf while some of the receptors, such as ZOR6, were not expressed until 120 hpf (Barth et al., 1996). Similar results were reported by Argo et al. (2003). In this study, we investigate the onset of crypt cell differentiation in the zebrafish olfactory epithelium in early developmental stages to determine whether the timing for differentiation of crypt cells is similar to that for ciliated and microvillous olfactory neurons. We take the advantage of the fact that expression of S100-like protein is selective to crypt cells within the olfactory epithelium and immunolabeling of S100 is robust (Germana et al., 2004b; Sato et al., 2005). Using immunohistochemical methods, we report onset and dynamic growth of olfactory neurons expressing S100 in development. Our study also demonstrates that expression of S100-like proteins in crypt cells parallels their expression in the lateral line system.

2. Results

2.1. *Expression dynamics of the S100 proteins in the olfactory epithelium*

In adult zebrafish, the olfactory epithelium is arranged into olfactory lamellae that are extended from a midline raphe to become a rosette-like structure. In the olfactory epithelium a special group of olfactory neurons is immunoreactive to the polyclonal rabbit anti-S100 (Dako North America, Inc, Carpinteria CA). According to the manufacturer, this antibody was raised with bovine brain tissue as the immunogen and strongly reacts with human S100B but weakly with S100A1 and very weakly with S100A6. Several studies have demonstrated that this antibody is specific to crypt cells in the olfactory epithelium (Germana et al., 2004b; Sato et al., 2005). Using this antibody for immunoreactivity, we found that in adult zebrafish, crypt cells were sparsely and unevenly distributed in the apical sublayer of the olfactory epithelium (Fig. 1). They have oval shaped somata that are unlike other cells in the epithelium. In zebrafish, they are the only cells in the olfactory epithelium that are immunoreactive to the above described antibody. Their morphological structure, immunoreactivity to the S100 antibody, and positioning in the olfactory epithelium make it possible for them to be distinguished from ciliated or microvillous neurons.

During olfactory system development, onsets of olfactory receptor expression vary (Argo et al., 2003; Barth et al., 1996). This variation may be associated with ontogeny or the function of receptors. It is hypothesized that crypt cells might be specialized to sense pheromones because the number of crypt cells in the apical sublayer of the olfactory epithelium has been shown to increase during the spawning seasons (Hamdani et al., 2008). If crypt cells detect pheromones associated with reproductive behaviors, will these cells have a late onset of expression and differentiation in development? To answer this question, we

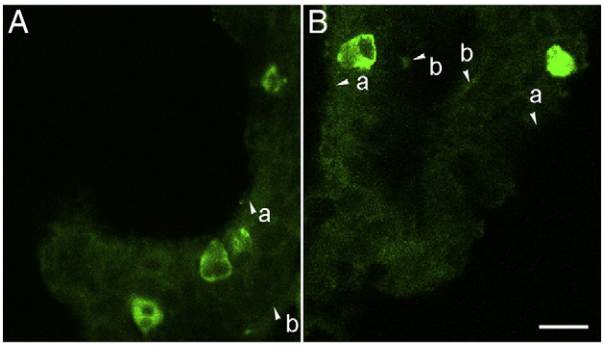

Fig. 1 – Sparsely scattered distribution of crypt cells in the olfactory epithelium of adult zebrafish. (A) and (B) are confocal images sampled from sections of the adult olfactory epithelium showing that randomly dispersed crypt cells at the apical surface are immunoreactive to the S100 antibody. Image (A) was acquired from the basal of a lamella adjacent to the midline raphe, center of the rosette. Image (B) shows the tip of a lamella. Locations of the apical surface (a) and the basal lamina (b) are pointed by arrowheads. The bar is 10 μm.