Gene Expression Patterns 7 (2007) 274–281

www.elsevier.com/locate/modgep

# Localization of anosmin-1a and anosmin-1b in the inner ear and neuromasts of zebrafish

Sylvain Ernest [a,b], Stéphanie Guadagnini [c], Marie-Christine Prévost [c], Nadia Soussi-Yanicostas [d,e,*]

[a] *INSERM, U784, Paris, F-75005, France*
[b] *Ecole Normale Supérieure, Paris, F-75005, France*
[c] *Institut Pasteur, Microscopie électronique (Plate-forme), Paris, F-75015, France*
[d] *INSERM, U616, Equipe Avenir, Paris, F-75651, France*
[e] *Université Pierre et Marie Curie, Laboratoire du développement normal et pathologique du cerveau, Paris, F-75651, France*

Received 1 August 2006; received in revised form 5 September 2006; accepted 6 September 2006
Available online 10 September 2006

**Abstract**

Anosmin-1, encoded by the *KAL-1* gene, is the protein defective in the X-linked form of Kallmann syndrome. This human developmental disorder is characterized by defects in cell migration and axon target selection. Anosmin-1 is an extracellular matrix protein that plays a role, *in vitro*, in processes such as cell adhesion, neurite outgrowth, axon guidance, and axon branching. The zebrafish possesses two orthologues of the *KAL-1* gene: *kal1a* and *kal1b*, which encode anosmin-1a and anosmin-1b, respectively. Previous *in situ* hybridization studies have shown that *kal1a* and *kal1b* mRNAs are expressed in undetermined cells of the inner ear but not in neuromast cells. Using specific antibodies against anosmin-1a and anosmin-1b, we report here that both proteins are expressed in sensory hair cells of the inner ear cristae ampullaris and the lateral line neuromasts. Accumulation of these proteins was observed mainly at the level of the hair bundle and also at the cell membrane. In neuromast hair cells, immunogold scanning electronmicroscopy demonstrated that anosmin-1a and anosmin-1b were present at the surface of the stereociliary bundle. In addition, anosmin-1a, but not anosmin-1b, was detected on the track of the ampullary nerve. This is the first report of anosmin-1 expression in sensory hair cells of the inner ear and lateral line, and along the ampullary nerve track.


*Keywords:* Zebrafish; Kallmann syndrome; *Kal1a*; *Kal1b*; Anosmin-1a; Anosmin-1b; Neuromast; Inner ear; Hair cells; Kinocilia; Stereocilia; Lateral line; Crista; Anosmia; Hypogonadism; Anti-anosmin-1a; Anti-anosmin-1b; Scanning electron microscopy

## 1. Results and discussion

Kallmann syndrome is a human developmental disorder in which cell migration and axon target selection are affected (De Morsier, 1954; Kallmann et al., 1994; Maestre de San Juan, 1856; Naftolin et al., 1971; Quinton et al., 2001). In this disease, the olfactory neuron axons and the gonadotropin-releasing hormone (GnRH) synthesizing neurons do not reach their targets in the brain (Schwanzel-Fukuda et al., 1989), thus leading to anosmia and gonadotropic hypogonadism. *KAL-1* encodes anosmin-1, an extracellular matrix protein that displays cell adhesion, neurite outgrowth, axon guidance, and axon branch-promoting activities *in vitro* (Hardelin et al., 1999; Soussi-Yanicostas et al., 1996, 1998, 2002). In zebrafish (Danio *rerio*), two orthologues of the *KAL-1* gene have been identified: *kal1a* and *kal1b* (Ardouin et al., 2000), which encode anosmin-1a and anosmin-1b, respectively.

Expression patterns of *kal1a* and *kal1b*, as determined by *in situ* hybridization (Ardouin et al., 2000), are largely non-overlapping. *kal1a* and/or *kal1b* are transcribed in the olfactory system, the inner ear, the lateral line primordium, the retina, areas of the central nervous system, and

* Corresponding author. Tel.: +33 142162676; fax: +33 145709990.
*E-mail address:* soussi@chups.jussieu.fr (N. Soussi-Yanicostas).

1567-133X/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.modgep.2006.09.004

several non-neural structures (Ardouin et al., 2000). In the otic vesicle: *kal1a* was observed between 16.5 and 72 h post-fertilization (hpf) and *kal1b* was detected by 22 hpf until the end of the observation window (120 hpf). However, identity of the cells expressing *kal1a* and *kal1b* in the inner ear remained unknown. In the lateral line, *kal1a* and *kal1b* transcripts were seen in the posterior lateral line primordium (PLLP) throughout its migration towards the caudal region (i.e. between 20 and 42 hpf). However, neither *kal1a* nor *kal1b* expression was detected later in newly deposited or fully differentiated neuromasts.

In this work, using specific antibodies, we performed the first analysis of the cellular and sub-cellular distribution of anosmin-1a and anosmin-1b in adult inner ear and in differentiated neuromasts.

### *1.1. Expression of anosmin-1 in hair cells of the cristae ampullaris*

In order to further investigate the distribution of anosmin-1a and anosmin-1b in the inner ear of zebrafish, we followed the accumulation of each protein, by confocal microscopy, on whole-mount micro-dissected adult inner ear, using antibodies generated against peptides specific to each of the two proteins. Previous studies have shown that anosmin-1 was not expressed in inner ear sensory epithelia of human and zebrafish embryos (Ardouin et al., 2000; Hardelin et al., 1999), In contrast with these results, our present data demonstrated that anosmin-1a and anosmin-1b are present in sensory patches of the adult zebrafish inner ear (Figs. 2 and 3). The zebrafish adult inner ear contains seven sensory patches (Fig. 1A and B): the three main macula of the utricule (u), sacule (s) and lagena (l), the small macula neglecta (mn), and the three crista ampullaris (ac, anterior; lc, lateral; pc, posterior) located at the base of their semi-circular canal. In zebrafish, the different macula are involved in the detection of linear acceleration as well as in hearing (Nicolson, 2005; Popper and Fay, 1993; Popper and Platt, 1993; Whitfield et al., 2002). The crista ampullaris of the semi-circular canals are responsible for the control of posture and angular acceleration (Ernest et al., 2000; Nicolson, 2005; Popper and Fay, 1993; Popper and Platt, 1993; Whitfield et al., 2002).

No significant accumulation of anosmin-1a and anosmin-1b was detected in the macula of adult inner ear. Although we cannot rule out that anosmin-1a and anosmin-1b are present in macular sensory patches, the signal detected in these organs was not high enough, as compared to control samples, to be considered as specific (see Supplementary Figure 1).

In contrast, a strong staining was observed in the crista ampullaris of semi-circular canals. Both anosmin-1a and anosmin-1b were present at the two distal extremities of the cristae (Fig. 2A–D, Fig. 3A–D). However, the median region of the cristae, although composed of similar cells, does not express anosmin-1a or anosmin-1b. In the region of the cristae ampullaris where anosmin-1a and anosmin-1b

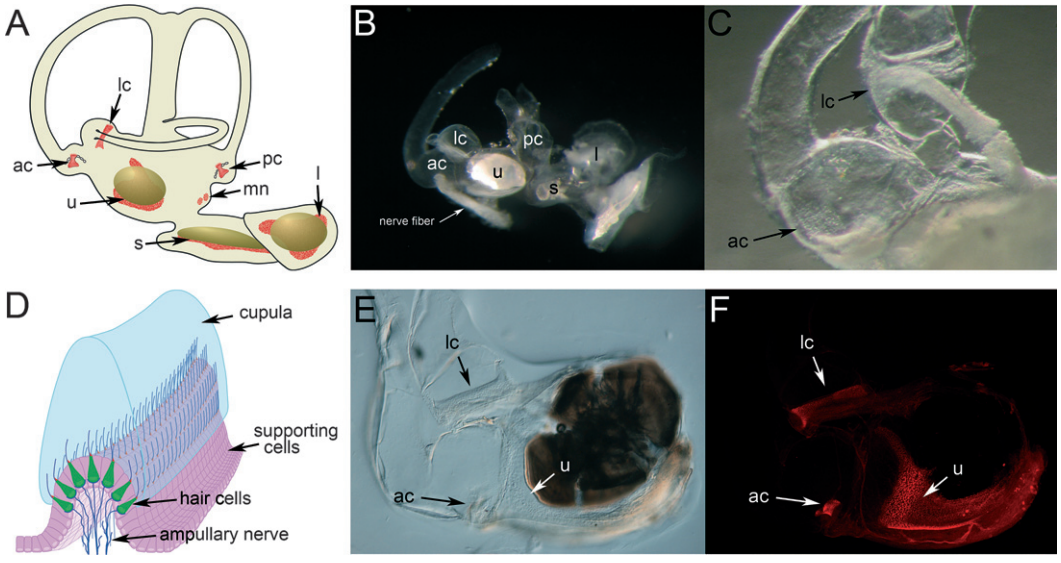


Fig. 1. Organization of the adult zebrafish inner ear. (A) Scheme of an adult zebrafish inner ear showing all sensory epithelia (in red). The three main macula are the utricule (u), the saccule (s) and the lagena (l), the fourth, smaller one, is the macula neglecta (mn); the three crista of the semi-circular canals are the anterior cristae (ac), the lateral cristae (lc), and the posterior cristae (pc). (B–C) Micro-dissected adult zebrafish inner ear. Whole adult inner ear showing the localization of the three main macula and of the three crista, as well as innervation of the anterior cristae (B) (note that the semi-circular canals were partially broken during hand dissection). Detail of the anterior portion of the inner ear, showing the anterior and the lateral crista together with the innervation of the lateral cristae (C). (D) Schematic representation of a cristae ampullaris with a cross section at one extremity of the cristae; the colors used in this scheme correspond to the colors shown in immunofluorescence experiments (hair cell body, kinocilium and stereocilia are depicted in green, blue, and red, respectively ; ampullary nerves are shown in blue). (E–F) Sensory patches of the anterior part of the inner ear. The anterior and lateral crista and the utricular maculae depicted here were stained with rhodamine–phalloidin and microphotographed using Nomarski optics (E) or a red fluorescence filter (F). The lateral cristae is shown longitudinally and the anterior cristae transversally.