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Expression of zebrafish *aldh1a3* (*raldh3*) and absence of *aldh1a1* in teleosts

Silke Pittlik, Susana Domingues, Axel Meyer, Gerrit Begemann *

Chair for Zoology & Evolutionary Biology, Department of Biology, University of Konstanz, Fach M617, 78457 Konstanz, Germany

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Abstract

The vitamin A-derived morphogen retinoic acid (RA) plays important roles during the development of chordate animals. The Aldh1a-family of RA-synthesizing enzymes consists of three members, Aldh1a1–3 (Raldh1–3), that are dynamically expressed throughout development. We have searched the known teleost genomes for the presence of Raldh family members and have found that teleost fish possess orthologs of Aldh1a2 and Aldh1a3 only. Here we describe the expression of *aldh1a3* in the zebrafish, *Danio rerio*. Whole mount in situ hybridization shows that *aldh1a3* is expressed during eye development in the retina flanking the optic stalks and later is expressed ventrally, opposite the expression domain of *aldh1a2*. During inner ear morphogenesis, *aldh1a3* is expressed in developing sensory epithelia of the cristae and utricular macula and is specifically up-regulated in epithelial projections throughout the formation of the walls of the semicircular canals and endolymphatic duct. In contrast to the mouse inner ear, which expresses all three Raldhs, we find that only *aldh1a3* is expressed in the zebrafish otocyst, while *aldh1a2* is present in the periotic mesenchyme. During larval stages, additional expression domains of *aldh1a3* appear in the anterior pituitary and the swim bladder. Our analyses provide a starting point for genetic studies to examine the role of RA in these organs and emphasize the suitability of the zebrafish inner ear in dissecting the contribution of RA signaling to the development of the vestibular system.

Keywords: Retinoic acid; RA synthesis; Raldh1; Raldh2; Raldh3; Zebrafish; Ear development; Otic vesicle; Semicircular canal; Crista; Utricular macula; Endolymphatic duct; Retina; Pituitary; Swim bladder; Adenohypophysis

1. Results and discussion

All-trans retinoic acid (RA), the major biologically active metabolite of vitamin A, acts as a signal to regulate gene expression by controlling the activity of members of the RA-regulated nuclear receptor family, the RA receptors (RARs) and the retinoid X receptors (RXRs) (reviewed in: Begemann and Meyer, 2001). RA biosynthesis involves a two-step process, in which the precursor vitamin A (retinol) is first oxidized by cytosolic alcohol dehydrogenases to retinaldehyde. In a second step, retinaldehyde is converted to RA by cytosolic retinal dehydrogenases, which are members of the aldehyde dehydrogenase (ALDH) family. In vertebrates, three enzymes have been described, Aldh1a1–3 (formerly called Raldh1–3) (Duester, 2000; Sophos and Vasiliou, 2003), that are highly specific for the synthesis of RA and are expressed in tissues with a high retinoid content (Niederreither et al., 1997; Berggren et al., 1999; Haselbeck et al., 1999; Li et al., 2000; Begemann et al., 2001; Chen et al., 2001). Of these, *Aldh1a3* has been identified and its developmental roles have been partially resolved in *Xenopus laevis*, the chick (Aldh6) and mouse (Raldh3) (Grün et al., 2000; Li et al., 2000; Mic et al., 2000; Suzuki et al., 2000; Lupo et al., 2005). A recent survey of the *Aldh1a*-gene family in deuterostomes demonstrated that zebrafish, in addition to the well-characterized *aldh1a2* gene, possess *aldh1a3*, but lack *aldh1a1* (Canestro et al., 2006). Here we show that the lack of *aldh1a1* is a general

* Corresponding author. Tel.: +49 7531 882881; fax: +49 7531 883018.
E-mail address: gerrit.begemann@uni-konstanz.de (G. Begemann).

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trait of teleosts and describe the embryonic and early larval expression patterns of zebrafish *aldh1a3*.

1.1. Phylogeny analysis of teleost retinaldehyde dehydrogenases

We have amplified a fragment spanning three exons of a novel retinaldehyde dehydrogenase (Raldh), present in 24 h post-fertilization (hpf) zebrafish embryos, that we identified by homology screening with sequences of known vertebrate Raldhs among genomic sequences published in Ensembl (Hubbard et al., 2007). During the course of this work, an expressed sequence tag covering the full open reading frame of this gene had become available in GenBank and was provisionally named *aldh1a3*. To determine the presence of Raldhs in teleosts other than the zebrafish, we identified all genes from the close to complete pufferfish (*Takifugu rubripes*, *Tetraodon nigrovirides*) and stickleback (*Gasterosteus aculeatus*) genomes that exhibit significant sequence similarities to Aldh1a1–3. A phylogeny analysis of the encoded proteins, including the known human, mouse, chicken and *Xenopus* sequences, assigned the identified sequences to three branches representing the Raldh-family members Aldh1a1 (Raldh1), Aldh1a2 (Raldh2) and Aldh1a3 (Raldh3), respectively (Sophos and Vasiliou, 2003), and identify the second zebrafish Raldh as Aldh1a3 (Fig. 1). We note that members of the Aldh1a1 gene family are neither present in the fish species sampled, nor did we succeed in identifying Aldh1a1 genes among any other publicly available fish sequences (Table 1), suggesting that teleosts in general only possess Aldh1a2 and Aldh1a3. Zebrafish *aldh1a3* is located on chromosome 7 (mapping data were produced at the Sanger Institute and were obtained from the World Wide Web at http://www.sanger.ac.uk), with a total of 13 exons extending over a length of 81,835 nucleotides (positions 8,113,850–8,195,685 in zebrafish assembly version 7; Ensembl release 46; August 2007). Zebrafish *aldh1a3* is therefore positioned 478 Mb away from *aldh1a2* on the same chromosome, which corresponds to the localization of the human orthologous genes *Aldh1a2* and *Aldh1a3* in Hsa15q22.1 and Hsa15q26.3, respectively

Table 1
Accession numbers of Aldh1a gene family members in teleosts

Species	Gene-accession
Zebrafish (*Danio rerio*)	aldh1a2: AF315691 aldh1a3: DQ300198
Stickleback (*Gasterosteus aculeatus*)	aldh1a2: ENSGACG00000015825[1)] aldh1a3: ENSGACG00000013986[1)]
Japanese Medaka (*Oryzias latipes*)	aldh1a2: DQ897366[2)]
Spotted green pufferfish (*Tetraodon nigroviridis*)	aldh1a2: CAAE01013867 aldh1a3: CAAE01014118
Torafugu (*Takifugu rubripes*)	aldh1a2: NM_001033639 aldh1a3: NEWSINFRUG00000146554[3)]

Accession numbers retrieved from GenBank and: 1) ENSEMBL, Assembly Broad S1 (Feb 2006); 2) ENSEMBL, Assembly HdrR (Oct 2005); 3) Assembly FUGU 4.0, Jun 2005.

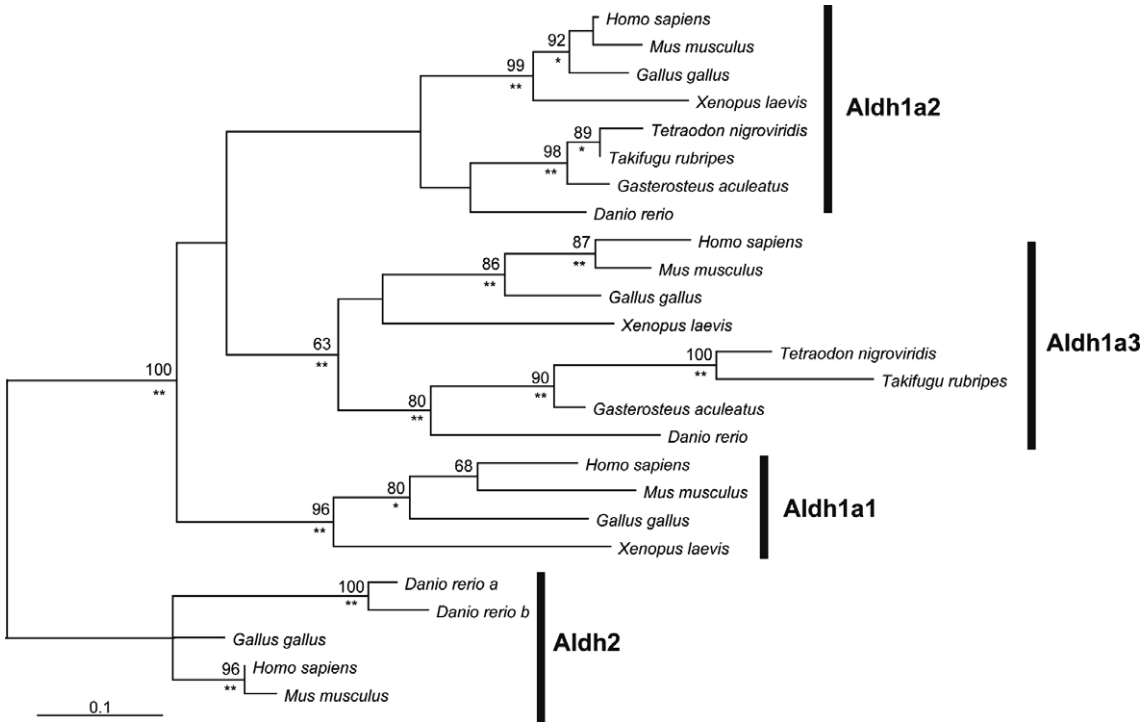

Fig. 1. Maximum likelihood tree of the Aldh1a gene family using PHYML. Numbers represent bootstrap values supporting each node, values lower than 60 are not shown. Posterior probabilities as obtained by MrBayes 3.1.1 are indicated by asterisks (**100%, *95–99%). The tree was rooted using closely related sequences of the Aldh2 gene family.