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Expressions of Raldh3 and Raldh4 during zebrafish early development

Dong Liang [a], Mei Zhang [a,b], Jie Bao [a], Luqing Zhang [a], Xiaofeng Xu [b], Xiang Gao [a], Qingshun Zhao [a,*]

[a] *Model Animal Research Center, MOE Key Laboratory of Model Animal and Disease Studies, Nanjing University, Pukou High-tech Development Zone, 12 Xuefu Road, Nanjing, Jiangsu 210061, China*

[b] *School of Life Sciences, Nanjing Normal University, Nanjing 210097, China*

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Abstract

Retinoic acid (RA) plays crucial roles in vertebrate embryogenesis. Four retinal dehydrogenases (Raldhs) that are responsible for RA synthesis have been characterized in mammals. However, only Raldh2 ortholog is identified in zebrafish. Here, we report the identification of *raldh3* and *raldh4* genes in zebrafish. The predicted proteins encoded by zebrafish *raldh3* and *raldh4* exhibit 70.0% and 73.5% amino acid identities with mouse Raldh3 and Raldh4, respectively. RT-PCR analyses reveal that both *raldh3* and *raldh4* mRNAs are present in early development. However, whole mount *in situ* hybridization shows that *raldh3* mRNA is first expressed in the developing eye region of zebrafish embryos at 10-somite stage. At 24 hpf (hours post fertilization), *raldh3* mRNA is expressed in the ventral retina of eye. At 36 hpf, the mRNA is also expressed in otic vesicle in addition to ventral retina, and it maintains its expression pattern till 2 dpf (days post fertilization). At 3 dpf, *raldh3* mRNA becomes very weak in ventral retina but is present in otic vesicle at a high level. At 5 dpf and 7 dpf, *raldh3* is no longer expressed in eyes but is expressed in otic vesicles at a very low level. *raldh4* mRNA is initially detected in developing liver and intestine regions at 2 dpf embryos. Its expression level becomes very high in these two regions of embryos from 3 dpf to 5 dpf. Analysis on the sections of 5 dpf embryos reveals that *raldh4* is expressed in the epithelium of intestine. At 7 dpf, *raldh4* mRNA is only weakly expressed in the epithelium of intestinal bulb.

Keywords: Zebrafish; Retinal dehydrogenases; Raldh3; Raldh4; Retinoid signalling; Embryogenesis; Eye; Retina; Otic vesicle; Liver; Intestine

1. Results and discussion

Retinoic acid (RA) is a morphogen that is essential to vertebrate embryogenesis (Lander, 2007). *In vivo*, animals synthesize RA by first converting the precursor vitamin A into retinal using retinol dehydrogenase, then oxidizing retinal into RA irreversibly using retinal dehydrogenases (Raldh) (Ross et al., 2000). Four Raldhs including Raldh1, Raldh2, Raldh3 and Raldh4 have been characterized in mammals (Glover et al., 2006). Raldh2, a major retinoic acid generating enzyme in the early mouse embryo (Zhao et al., 1996), is mainly expressed in mesoderm during early gastrulation, and is later expressed in undifferentiated somites, the optic vesicles and differentiating limbs (Niederreither et al., 1997). Mice with *Raldh2* deletion die at midgestation with a shorter anterior–posterior axis, open neural tube, absence of limb buds (Niederreither et al., 1999), and asymmetric somitogenesis (Vermot et al., 2005; Vermot and Pourquie, 2005). Raldh3 is primarily localized in ventral retinas of the developing eyes, otic vesicles and olfactory placodes in early mouse embryos (Li et al., 2000; Mic et al., 2000). *Raldh3*-null mice die at birth from respiratory distress due to choanal atresia, displaying malformations in ocular and nasal regions (Dupé et al., 2003), and mild defects in retinas (Dupé et al., 2003; Molotkov et al., 2006). Raldh1 is expressed in dorsal retinas and mesencephalic flexures (McCaffery et al., 1991; Mic et al., 2000). Genetic ablation of *Raldh1* had no apparent defects in mouse embryo development (Fan et al., 2003;

* Corresponding author. Tel.: +86 25 58641527; fax: +86 25 58641500.
E-mail address: qingshun@nicemice.cn (Q. Zhao).

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Matt et al., 2005). However, double knock out of both *Raldh1* and *Raldh3* causes apoptosis and alters gene expressions in periocular mesenchymal cells, resulting in severe eye abnormalities in mice (Matt et al., 2005; Molotkov et al., 2006). Raldh4 is first expressed in liver at E14.5 stage during mouse embryogenesis and present in adult mouse liver and kidney (Lin et al., 2003). The role of Raldh4 in mice embryogenesis is unknown because targeted deletion of *Raldh4* in mouse has not been reported yet. Till now, only Raldh2 ortholog has been characterized in zebrafish. Zebrafish *raldh2*$^{-/-}$ mutants (*nls*, *nof*) display a truncation of the anterior–posterior axis anterior to the somites, absence of pectoral fins (Begemann et al., 2001; Grandel et al., 2002) and asymmetric somitogenesis (Kawakami et al., 2005), which are similar to defects of *Raldh2*$^{-/-}$ mouse. In this paper, we report the identification of *raldh3* and *raldh4* genes in zebrafish and describe their expression patterns during early development.

1.1. Zebrafish has raldh3 and raldh4 genes

BLASTing the zebrafish EST database with *Xenopus laevis* Raldh3 sequence (GenBank Accession No.: **AY692028**) using TBLASTN program, we found a candidate EST (**DR729671**) of zebrafish Raldh3. Employing RACE-PCR strategy, we cloned a cDNA of zebrafish *raldh3* with the length of 1590 base pairs (bp). The sequence was deposited in GenBank under Accession No. **EF375713**. The *raldh3* cDNA consists of a complete CDS (coding sequence) with 1542 bp long, predicted to encode a protein comprising 513 amino acids (Fig. 1). BLASTing GenBank protein database, we found that the predicted protein is identical to that encoded by **DQ300198** (Canestro et al., 2006) and the two cDNAs are mapped in the same contig of zebrafish chromosome 7, and it is encoded by 13 exons. However, the CDS of **EF375713** has nine nucleotides (9 of 1542) that are different from that of **DQ300198**. The difference indicates that single nucleotide polymorphisms (SNPs) are present in the gene. Protein alignment analysis reveals that zebrafish Raldh3 shares high identity with other members of Raldh family (Table 1). It exhibits 64.5% amino acid identity with zebrafish Raldh2, about 70% identity with mammalian Raldh3, 63% identity with mammalian Raldh1 and 34% identity with mammalian Raldh4. DNA sequence alignment shows that the CDS of zebrafish *raldh3* (**EF375713**) shares 65.5% nucleotide identity with that of zebrafish *raldh2*, 69% identity with that of mammalian *Raldh3*, about 65% identity with that of mammalian *Raldh1* and about 50% identity with that of mammalian *Raldh4*. Using Mega3.1, phylogenetic analysis on the vertebrate Raldh family shows that zebrafish Raldh3 is clustered into Raldh3 subfamily (Fig. 2). Additionally, synteny analysis reveals that zebrafish *raldh3* gene is surrounded by *asb7* gene in chromosome 7. Similarly, mouse and rat *Raldh3* genes are also linked with *Asb7* and are located in mouse chromosome 7 and rat chromosome 1, respectively (http://www.ncbi.

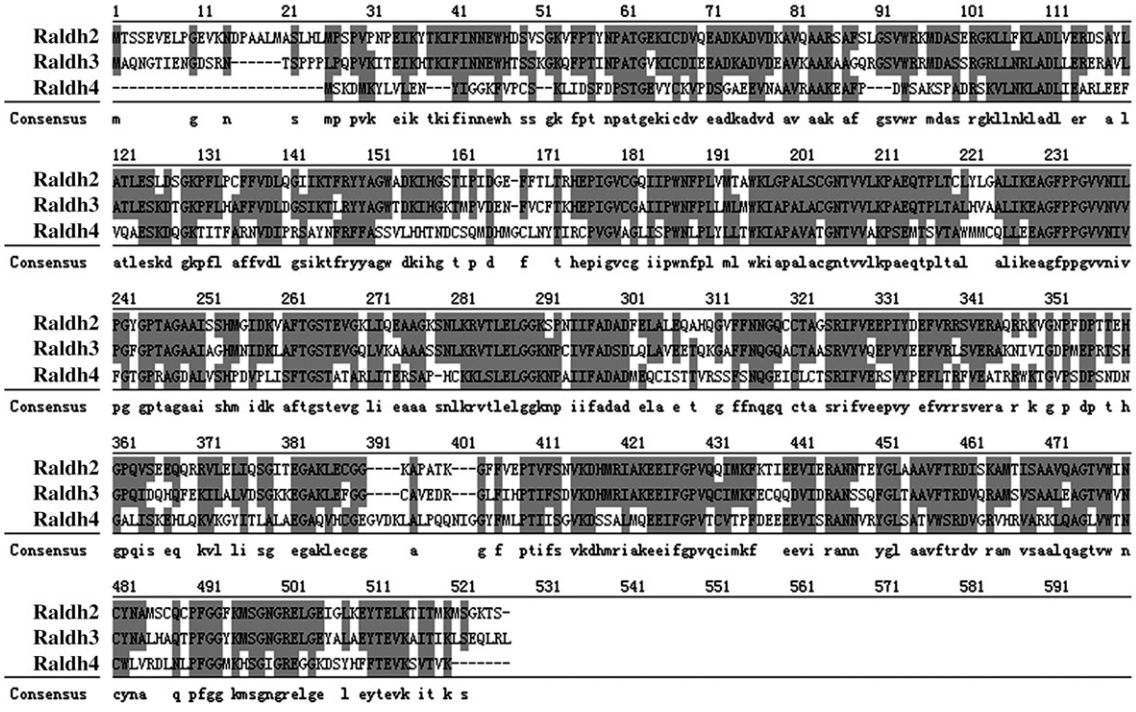

Fig. 1. Amino acid sequence alignment of zebrafish Raldh2, Raldh3 and Raldh4. The amino acid identities and similarities of zebrafish Raldh2 with zebrafish Raldh3 and Raldh4 are determined using Jellyfish software (www.biowire.com; version 1.4). Identical amino acid residues are crosshatched, and dashes represent gaps for alignment purposes.