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Gene expression pattern

Expression of receptor protein–tyrosine phosphatase alpha, sigma and LAR during development of the zebrafish embryo

Astrid van der Sar, Marco Betist, Jaco de Fockert, John Overvoorde, Danica Živković, Jeroen den Hertog*

Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands

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Abstract

Receptor protein–tyrosine phosphatases (RPTPs) are key players in *Drosophila* development. To study the role of RPTPs in vertebrate development, we have cloned zebrafish (zf) RPTPs, including RPTP alpha (RPTPα), RPTP sigma (RPTPσ) and LAR. These three RPTPs are broadly transcribed in early development. At 24 h post fertilisation (hpf), all three genes are expressed in the nervous system in partially overlapping patterns. At 3 days post fertilisation zf-RPTPα and zf-LAR show similar expression patterns in the central nervous system (CNS), the pharyngeal arches, the pectoral fins and the spinal cord. Interestingly, zf-LAR is uniquely expressed in the neuromast cells, whereas zf-RPTPσ expression is confined to the central nervous system.

Keywords: Protein–tyrosine phosphatase; Expression; Zebrafish; Development; Embryo

1. Results

In *Drosophila*, receptor protein–tyrosine phosphatases (RPTPs) are involved in axon guidance in the central nervous system (CNS) (Van Vactor, 1998). RPTPs of a variety of vertebrates have been cloned and many of these RPTPs are expressed in the developing brain (reviewed by Stoker and Dutta, 1998; Van Vactor, 1998; den Hertog, 1999), indicating their roles in histogenesis of the nervous system. Here, we describe the whole-mount expression patterns of RPTPα, RPTPσ and LAR mRNAs, providing an overview of their overlapping and distinct expression domains during zebrafish development.

Using a polymerase chain reaction- (PCR)-based strategy (see Section 2), we cloned several PTPs, including partial clones of RPTPσ and LAR, from a 1–48 hpf cDNA library. Full length RPTPα was identified in a separate library screen (van der Sar et al., submitted for publication). The cloned zf-RPTPs are shown schematically with their putative homologues in Fig. 1.

Northern blot analysis of zf-RPTPα revealed a transcript of approximately 8 kb (Fig. 2).zf-RPTPα transcript was maternally present and after its absence until mid/late gastrulation it was transcribed from 8 h post fertilisation (hpf) to 7 days post fertilisation (dpf) (Fig. 2). Its highest expression level was found from 12 hpf to 3 dpf. At 4 dpf, expression levels decreased and remained low until 7 dpf (Fig. 2).

Northern blot analysis of zf-LAR showed that the zf-LAR transcript was approximately 8 kb. zf-LAR mRNA was expressed from 12 hpf, when it was faintly present, till 5 dpf. At 4 dpf hardly any zf-LAR mRNA was detected. zf-RPTPσ was approximately 9 kb (Fig. 2), demonstrating that the probes for RPTPσ and LAR do not cross-react with each other. Like zf-LAR, zf-RPTPσ mRNA was first detected at 12 hpf and was present till 3 dpf. Although hardly any zf-RPTPσ transcript was detected at 4 dpf, zf-RPTPσ was detectable again at 5 dpf. Interestingly, the zf-LAR bands were much broader than those of zf-RPTPσ or zf-RPTPα, suggesting that several splice variants of zf-LAR were expressed. Both zf-LAR and zf-RPTPσ were not maternally expressed in contrast to zf-RPTPα.

Whole-mount in situ hybridisation was performed with digoxigenin-labelled riboprobes. zf-RPTPα mRNA was detected as a maternal transcript in the zygote and during cleavage period (Fig. 2, 0–2.2 hpf). Consistently, blastomeres were specifically and homogeneously labelled (Fig. 3A–C).No labelling was observed at 6 hpf (Fig. 3D), which showed together with Northern blotting that zf-RPTPα was not expressed during early and mid gastrulation. At three-somite stage (12 hpf), when zf-RPTPσ and zf-LAR transcripts were first detected, consistent with the results of

* Corresponding author. Tel.: +31-30-212-1800; fax: +31-30-251-6464.
E-mail address: hertog@niob.knaw.nl (J. den Hertog).

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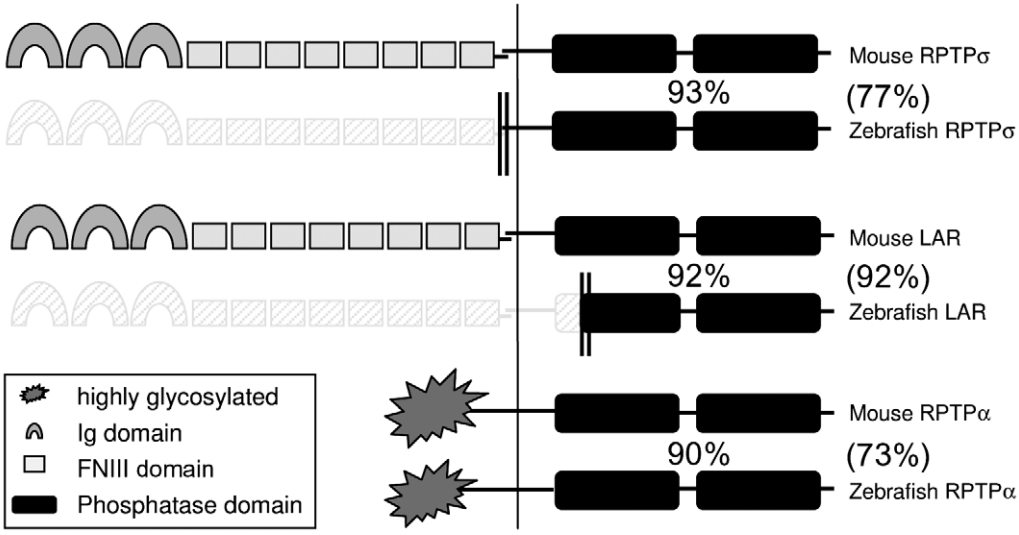

Fig. 1. Diagram of the RPTPs cloned and sequenced in this study, indicating percent amino acid identity between homologous domains in the schematised proteins (with overall identity in parentheses). Note that zf-LAR and zf-RPTPσ are partial clones and unknown sequences are hatched.

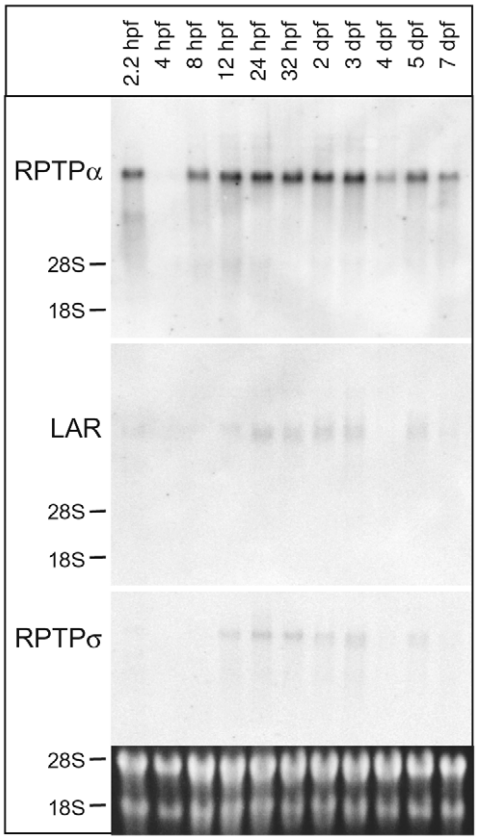

Fig. 2. RPTPα, LAR and RPTPσ expression in time. Total RNA was isolated from embryos of different developmental stages (staging in hpf was done according to Kimmel et al., 1995) and 15 μg of RNA per stage was loaded per lane. Developmental stages are indicated at the top and as a loading control the ethidium bromide stained gel is shown at the bottom. The sizes of the 28S and 18S transcripts are indicated at the left. RPTPα transcript was approximately 8 kb. Note the broad band of LAR transcript around 8 kb. RPTPσ was approximately 9 kb.

Northern blotting (Fig. 2), all three RPTP transcripts were found throughout the embryo, although RPTPσ labelling was more intense in the anterior half (Fig. 3E–G). Expression of zf-RPTPα mRNA at 24 hpf was restricted to the head (Fig. 3I) and was most pronounced in the midbrain, the midbrain/hindbrain boundary and the hindbrain. At 24 hpf, zf-LAR mRNA was present throughout the brain and in the somites (Fig. 3J) and zf-RPTPσ mRNA was detected around the ventricles (Fig. 3H,K). At 3 dpf, zf-RPTPα and zf-LAR expression were confined to the brain and the pharyngeal arches (Fig. 3L,M). RPTPα and LAR were both expressed in the pectoral fin (Fig. 3L,M, white arrow; RPTPα expression was hardly visible because of labelling in surrounding tissue). Finally, zf-LAR, but not RPTPσ (Fig. 3O), was expressed in the spinal cord and in neuromast cells (Fig. 3P,Q).

Taken together, zf-RPTPα is maternally expressed and shows highest expression levels in the brain later in development, consistent with the expression pattern in the mouse (den Hertog et al., 1996), chick (Ledig et al., 1999) and *Xenopus* (Yang and Friesel, 1998). Interestingly, the highly homologous zf-RPTPσ and zf-LAR show distinct expression patterns, wherein zf-RPTPσ is restricted to CNS and zf-LAR is more widely expressed, similar to expression of RPTPσ and LAR in mouse (Schaapveld et al., 1998) and *Xenopus* (Johnson and Holt, 2000). Unique is the expression of zf-LAR in neuromast cells of the lateral line of zebrafish.

2. Materials and methods

2.1. Zebrafish embryos

Zebrafish were kept at 27.5°C on a 14:10 h light/dark cycle. Embryos were obtained by natural matings and

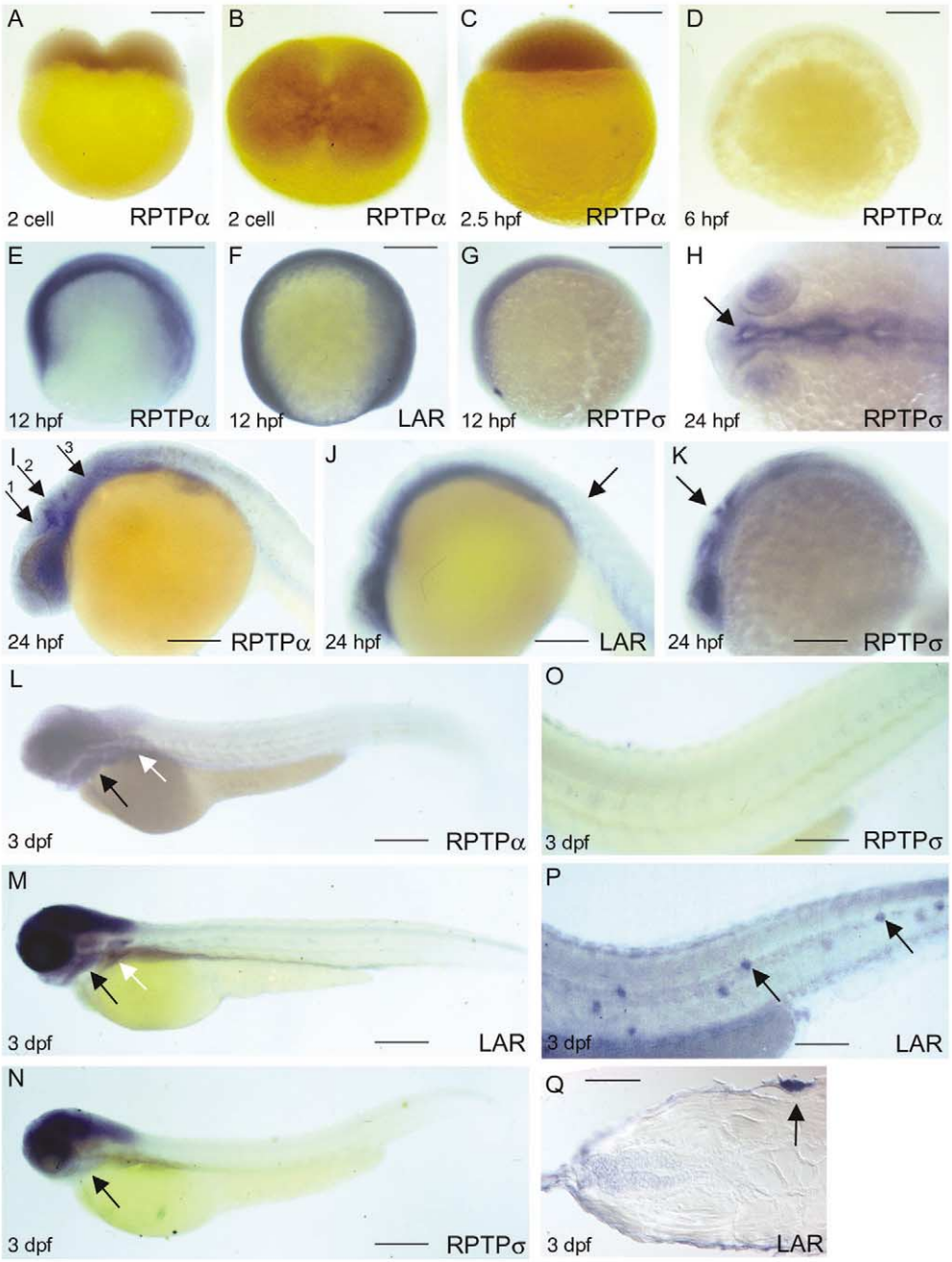

Fig. 3. Whole-mount in situ staining with digoxigenin-labelled antisense probes for RPTPα, RPTPσ and LAR mRNA. (A,B) Two-cell stage, the entire blastomeres are stained for RPTPα transcript. (C) 2.5 hpf, RPTPα transcript is found throughout the embryo. (D) Mid-gastrulation, 6 hpf embryo probed with RPTPα. No transcript was detected. (E–G) Three-somite stage, 12 hpf old embryos. RPTPα (E), LAR (F) and RPTPσ (G) transcripts were detected throughout the embryo, although more intense RPTPσ labelling was found in the anterior half of the embryo. (I) Expression of RPTPα mRNA at 24 hpf. Transcript was detected in the entire head and was most pronounced in the midbrain (arrow 1) the midbrain/hindbrain boundary (arrow 2) and the hindbrain (arrow 3). (J) Expression of LAR mRNA at 24 hpf. Transcript was detected throughout the brain, the eyes and in the somites (arrow). (K, H) Expression of RPTPσ mRNA was detected in the entire head and was most pronounced in the ventricular zones (arrows in H, K) and the eyes. (L–Q) 3 dpf old embryos. RPTPα and LAR mRNA expression was detected in the entire brain, the eyes, the visceral arches (black arrow in L, M) and the pectoral fin (white arrow in L, M). (N) Expression of RPTPσ mRNA was detected in the brain and eyes. No transcript was found in the visceral arches (arrow). (O–Q) Tail of 3 dpf old embryos. (O) No expression of RPTPσ was found in the tail. (P) Overstained embryo to clearly show the LAR transcript in the neuromast cells (arrows). (Q) Transversal section of a 3 dpf zebrafish probed with LAR. Arrow indicates neuromast cells of the lateral line. Dorsal is to the left. (A, C–G, I–P) Anterior to the left, dorsal to the top. (B, H) Dorsal view and anterior to the left. Bars indicate: (A–D, I) 200 μm; (E–G) 180 μm; (H, O–P) 100 μm; (I) 200 μm; (J–K) 140 μm; (L–N) 300 μm and (Q) 40 μm.

cultured in embryo medium at 28.5°C. Staging of the embryos was done according to morphological criteria (Kimmel et al., 1995).

2.2. Cloning of RPTP orthologs from zebrafish

Cloning of zf-RPTPα (accession no. Y15874) has been described elsewhere (van der Sar et al., submitted for publication). zf-LAR (accession no. AJ311885) and zf-RPTPσ (accession no. AJ311886) were cloned in a library screen described in van der Sar et al. (1999).

2.3. Northern blotting and whole-mount in situ hybridisation

Northern blotting was done according to standard protocols, using 15 μg total RNA per lane, formaldehyde-containing 0.8% agarose gels and multiprime (Amersham) labelled specific probes for RPTPα, RPTPσ and LAR. The RPTPα probe was a 1644 bp Eco R1–Cla 1 fragment of zf-RPTPα coding sequence. Fragments encoding the membrane-distal PTP domain of zf-LAR (encoding the C-terminal 260 residues) and zf-RPTPσ (encoding the C-terminal 262 residues) were used as probes. For whole-mount in situ hybridisation, we used the same fragments as the probes for Northern blotting. Digoxigenin-labelled antisense probes were prepared by in vitro transcription, using appropriate polymerases and a nuleotide mix (Roche). Whole-mount in situ hybridisation was done essentially as described by Thisse et al. (1993).

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