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Gene expression pattern

Expression of CRYP-α, LAR, PTP-δ, and PTP-ρ in the developing *Xenopus* visual system

Karl G. Johnson, Christine E. Holt*

Department of Anatomy, University of Cambridge, Downing Street, Cambridge CB2 3DY, UK

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Abstract

Receptor protein tyrosine phosphatases (RPTPs), are involved in axon outgrowth and guidance not only in the *Drosophila* visual system (Garrity et al., 1999. Neuron 22, 707–717) but also in the developing vertebrate retina (Ledig et al., 1999a. J. Cell Biol. 147, 375–388). We have cloned a variety of *Xenopus* RPTPs, including four RPTPs expressed in the developing visual system (LAR, PTP-δ, CRYP-α and PTP-ρ). These four RPTPs are transcribed in the developing optic vesicle during differentiation and in overlapping but distinct patterns in the developing retina during retinal layer formation. LAR, PTP-δ, and CRYP-α are also expressed in retinal ganglion cells during axonogenesis and during axon guidance from the retina to the optic tectum.

Keywords: Receptor protein tyrosine phosphatase; LAR; PTP-δ; CRYP-α; PTP-ρ; Retina; Visual system; *Xenopus*

1. Results and discussion

In *Drosophila*, RPTPs are involved in axon guidance in the CNS (Desai et al., 1997) and may directly link the binding of extracellular ligands to internal changes in tyrosine phosphorylation and actin cytoskeletal rearrangements (Debant et al., 1996; Wills et al., 1999). RPTPs have been cloned in a variety of vertebrate species, and for many of these RPTPs, the expression patterns have been identified (Sap et al., 1990; Yang et al., 1993; Stoker, 1994; Pulido et al., 1995; Stoker et al., 1995; den Hertog et al., 1996; Fuchs et al., 1998). Only one study, however, has focused on the expression patterns of RPTPs in the developing visual system. This study showed the expression patterns for CRYP-α, PTP-μ, CRYP-2, PTP-α and PTP-γ in the developing chicken visual system (Ledig et al., 1999b). We set out to clone and characterize the expression pattern of a variety of other RPTPs in the developing *Xenopus* visual system.

We used degenerate reverse transcriptase–polymerase chain reaction (RT–PCR) and low-stringency hybridization with the cytoplasmic phosphatase domains of *Xenopus* PTP-α to obtain partial clones of several *Xenopus* RPTPs. Full-length cDNAs were cloned and sequenced for *Xenopus* PTP-δ and LAR, and partial clones were obtained for CRYP-α and PTP-ρ. The cloned RPTPs are shown schematically with their putative homologues in Fig. 1.

Whole-mount in situ hybridizations on albino *Xenopus* embryos showed that CRYP-α, LAR, PTP-δ and PTP-ρ are transcribed strongly in the developing CNS (Table 1) (Fig. 2). At stage 19 (Nieuwkoop and Faber, 1967), CRYP-α is expressed in the lateral plate mesoderm (LPM) and weakly in the optic vesicles, while at stage 25, CRYP-α is expressed in the developing somites, spinal cord, and trigeminal complex (Fig. 2A,B). By stage 28, CRYP-α mRNA levels rise in the developing notochord, otic vesicle, spinal cord and subsets of cells in the midbrain and hindbrain (Fig. 2C). LAR mRNA is abundant throughout the developing CNS at stage 19 (Fig. 2D), and is upregulated in the notochord and optic vesicle at stage 24 (Fig. 2E). At stage 28, LAR is expressed in the developing retina, otic vesicle, anterior spinal cord, trigeminal complex, as well as in the posterior notochord (Fig. 2F). PTP-δ is transcribed in the optic vesicles and tailbud of the stage-19 embryo (Fig. 2G), with limited expression in the LPM. PTP-δ expression is restricted to the developing optic vesicles and the developing tail at stage 24 (Fig. 2H). By stage 29/30, PTP-δ is expressed in the developing tail, otic vesicle, dorsal hindbrain and dorsal midbrain (Fig. 2I). PTP-ρ is not expressed in the embryo at stage 19 (Fig. 2J). PTP-ρ is first expressed in the developing optic vesicles and in two subsets of cells (identified by their dorso-ventral position as a subset of

* Corresponding author. Tel.: +44-1223-766-229; fax: +44-1223-333-786.

E-mail address: ceh@mole.bio.cam.ac.uk (C.E. Holt)

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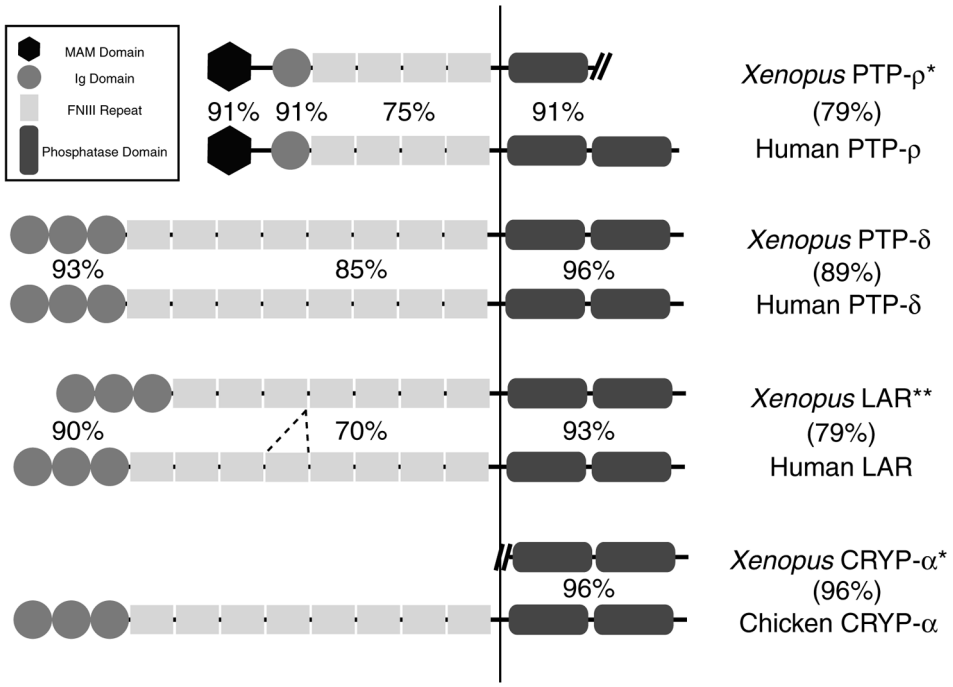

Fig. 1. Schematic diagram of the RPTPs cloned and sequenced in this study, indicating percent amino acid identity between homologous domains in the schematized proteins (with overall identity in parentheses). *Note that *Xenopus* PTP-ρ and *Xenopus* CRYP-α are partial clones of these genes. **The *Xenopus* LAR homologue cloned in this study lacks the fourth FNIII repeat, suggesting that it may not be the full-length LAR, but rather a splice variant. In rats, this splice variant of LAR (lacking the fourth FNIII domain) is preferentially expressed in the brain and spinal cord (Zhang and Longo, 1995).

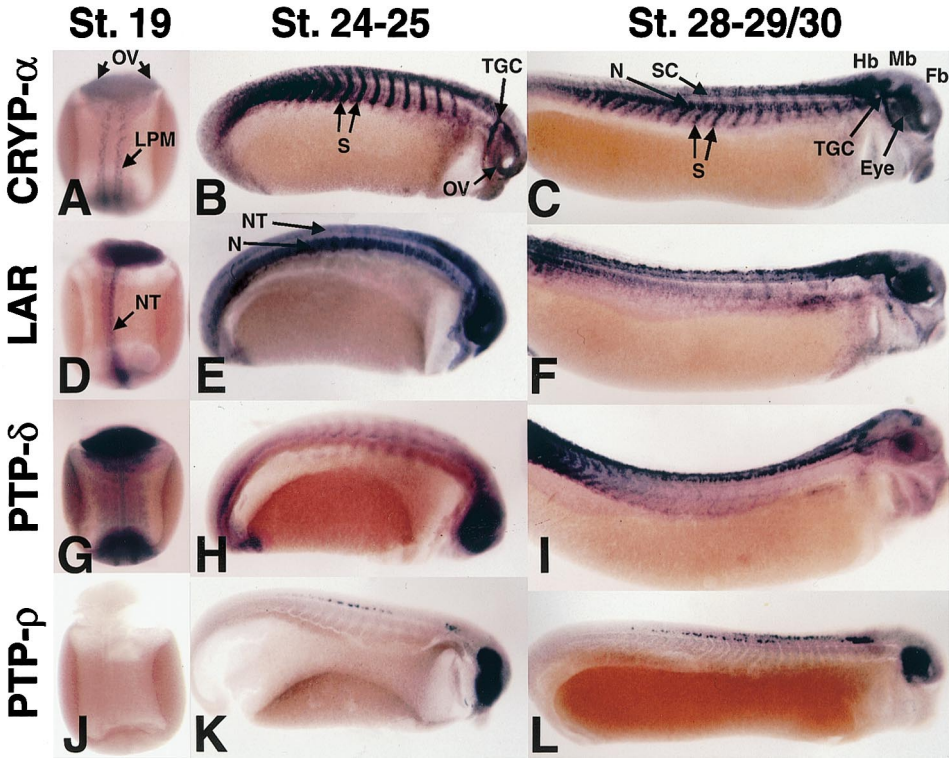

Fig. 2. Whole-mount in situ hybridizations using antisense DIG-labeled mRNA probes for CRYP-α, LAR, PTP-δ, and PTP-ρ in the developing albino *Xenopus* embryo at stages 19 (A,D,G,J); 24 (E,H)–25 (B,K); and stages 28 (C,F,L)–29/30 (I). For the stage-19 embryos, anterior is up (dorsal view). For all other embryos, anterior is right and dorsal is up. OV, optic vesicle; LPM, lateral plate mesoderm; S, somite; TGC, trigeminal complex; N, notochord; SC, spinal cord; Hb, hindbrain; Mb, midbrain; Fb, forebrain; NT, neural tube.

Table 1
RPTP expression during major retinal developmental events in *Xenopus,* correlated with developmental stage

Stage	Major retinal events	RPTPs in the retina	RPTPs in RGCs
19	Proliferation of cells in the optic vesicle	CRYP-α, LAR, PTP-δ	Not available
24–25	Proliferation of cells in the optic vesicle, determination of first RGCs	LAR, PTP-δ, PTP-ρ	Not available
28–29/30	Differentiation of retinal cells, RGC axonogenesis	LAR, PTP-δ, PTP-ρ	Not available
35/36	Retinal lamination begins, RGC axon navigation from retina to optic tract	CRYP-α, LAR, PTP-δ, PTP-ρ	CRYP-α, LAR, PTP-δ
39	Axon guidance in optic tract, target recognition by RGC growth cones, cell division stops in central retina	CRYP-α, LAR, PTP-δ, PTP-ρ	CRYP-α, LAR, PTP-δ
41	Branching and synapse formation by RGC axons in tectum	CRYP-α, LAR, PTP-δ, PTP-ρ	CRYP-α, LAR, PTP-δ

motoneurons and interneurons) along the ventral and medial spinal cord beginning at stage 25 (Fig. 2K). At stage 28, PTP-ρ transcription is maintained in the retinae, and in these rows of cells in the ventral and medial spinal cord (Fig. 2L).

CRYP-α is expressed throughout the retina at stage 35/36. At stage 39, CRYP-α is expressed in all three retinal layers: the ganglion cell layer (GCL), the inner nuclear layer (INL), and the outer nuclear layer (ONL). By stage 41, CRYP-α expression is limited to the GCL and the INL (Fig. 3A–C). LAR is expressed most strongly in deep dorso-medial RGCs at stage 35/36 (arrows), but is present in all retinal layers. At stages 39 and 41, LAR expression is limited to the GCL and INL (Fig. 3D–F). PTP-δ is expressed strongly in the dorsal GCL and INL at stage 35/36, paralleling the dorsal-ventral developmental gradient in the retina (Holt, 1984). At stages 39 and 41, PTP-δ is transcribed throughout the GCL and INL (Fig. 3G–I). PTP-ρ mRNA is transcribed most strongly at stage 35/36 in the ONL, but is also present in the INL. At stage 39, PTP-ρ expression increases in the INL as the level in the ONL decreases. PTP-ρ mRNA is also transcribed by a minority of cells in the GCL (arrowheads) (Fig. 3K). The PTP-ρ transcript at stage 41 is limited to a subset of cells in the INL, but is excluded from the GCL and ONL (Fig. 3L).

In summary, we have identified a variety of RPTPs expressed in the developing *Xenopus* CNS. Within the developing retina, CRYP-α, LAR, PTP-δ and PTP-ρ are transcribed in overlapping but distinct patterns. CRYP-α, LAR, and PTP-δ are all expressed in RGCs during axon elongation, target recognition and synaptogenesis, while PTP-ρ appears to be transcribed in a more dynamic pattern in the photoreceptor and inner nuclear layers.

2. Experimental procedures

2.1. cDNA cloning

PCR was performed on cDNA made from RNA isolated from stage-40 *Xenopus* retinae using the following degenerate primer pairs and an annealing temperature of 45°C: 5′-TCGTCGGGATCCGAARTGYSAYCARTAYTGGCC-3′ and 5′-GTCTCGAATTCTAYTGNTCNTCNGTYTGN-

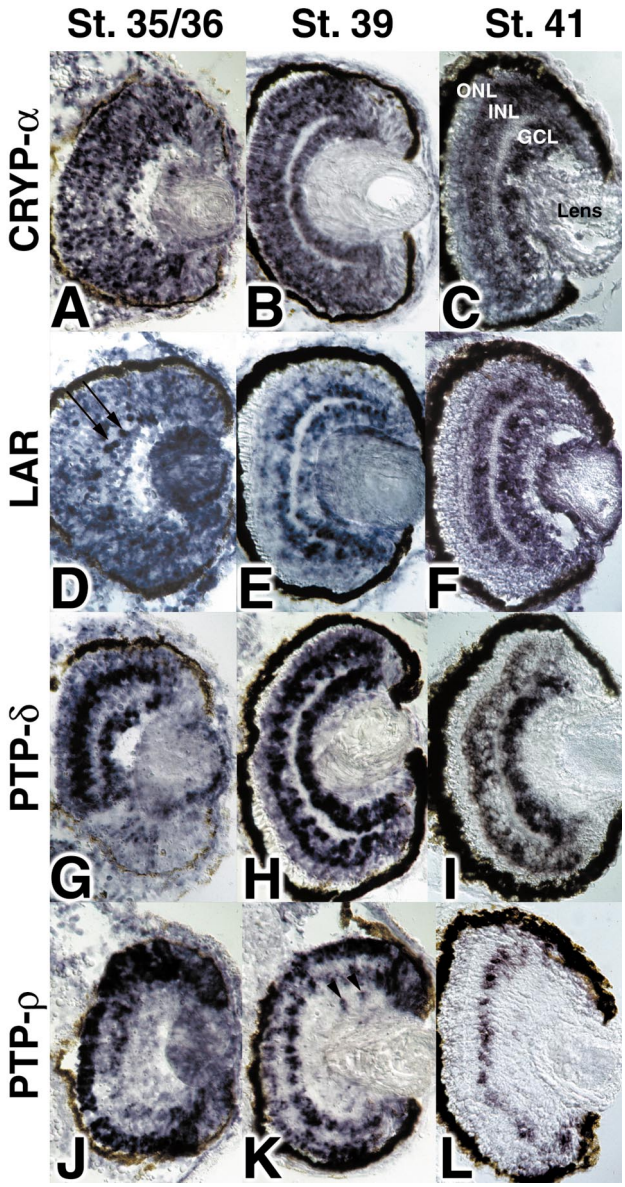

Fig. 3. In situ hybridizations using antisense DIG-labeled mRNA probes for CRYP-α, LAR, PTP-δ, and PTP-ρ on 15-μm paraffin-sectioned pigmented *Xenopus* retinae at stages 35/36, 39 and 41. Sections are near the retinal midline, and dorsal is up. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer.

AC-3′ or 5′-TACTCGGGATCCGAYTTYTGGMGIATG-RTNTGG-3′ and 5′-GTCTCGAATTCCNACNC-CNGC-NGARCARTG-3′.

PCR products were cloned into pGEMT-easy cloning vector (Promega) and sequenced. A stage-28 *Xenopus* head library was screened with the PCR products, yielding partial clones of LAR, PTP-δ, CRYP-α and PTP-ζ. The 5′-ends of these clones were used to repeatedly screen the Battey stage 50 eye library. This resulted in partial clones of LAR, PTP-δ, and CRYP-α. 5′-RACE was used to clone the 5′-end of LAR and PTP-δ. The Battey library was also screened via low-stringency hybridization using the phosphatase domains of *Xenopus* PTP-α which resulted in partial clones of PTP-ϵ, PTP-ρ, CD45 and PTP-γ. GenBank accession numbers are as follows: LAR, AF197945; PTP-δ, AF197944; PTP-ρ, AF173857; CRYP-α, AF198450.

2.2. In situ hybridization

Digoxigenin (DIG)-labeled antisense RNA probes were generated for LAR, PTP-δ, CRYP-α and PTP-ρ (Harland, 1991). Whole-mount in situ hybridization was performed on albino and pigmented *Xenopus* embryos using the procedure described in Harland (1991). These embryos were cleared in 2:1 benzyl benzoate/benzyl alcohol prior to imaging. In situ hybridizations on 15-μm paraffin sections of *Xenopus* embryos were done as described in (Kanekar et al., 1997).

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