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Irpap1 as a specific marker of proximal pronephric kidney tubuli in *Xenopus laevis* embryos

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ABSTRACT LRPAP1, also known as receptor associated protein (RAP) is a small protein of 40 kDa associated with six of the seven members of the evolutionary conserved family of LDL receptors. Numerous studies showed that LRPAP1 has a dual function, initially as a chaperone insuring proper formation of intermolecular disulfide bonds during biogenesis of low density lipoprotein (LDL) receptors and later as an escort protein during trafficking through the endoplasmic reticulum and the early Golgi compartment, preventing premature interaction of receptor and ligand. Because of the general influence of LRPAP1 protein on lipid metabolism, we analyzed the temporal and spatial expression of the *Xenopus laevis* ortholog of *Irpap1*. Here, we show that *Irpap1* was expressed in the developing neural system, the eye and ear anlagen, the branchial arches, the developing skin and the pronephric kidney. The very high expression level of *Irpap1* specifically in the proximal tubules of the developing pronephros establishes this gene as a novel marker for the analysis of pronephros formation.

KEY WORDS: *early embryogenesis, pronephros, neurogenesis, kidney, Xenopus*

Originally, LRPAP1, also known as receptor associated protein (RAP), was identified as a small protein of 40 kDa that was co-purified with low-density-lipoprotein receptor-related protein1 (LRP1) (Strickland *et al.*, 1990). LRP1 belongs to an evolutionary conserved family of seven LDL receptors, the low-density-lipoprotein (LDL) receptor, very-low-density-lipoprotein receptor (VLDLR), Megalin, MEGF7/LRP4, Apolipoprotein E receptor-2 (ApoER2), LRP1, and LRP1b (Herz, 2006). Intracellular localization studies showed that 70% of the protein is retained in the ER, 24% in the early Golgi compartment and very little could be found on the cell surface or in endosomes (Bu *et al.*, 1994a). Human LRPAP1 consists of 323 amino acids, carrying a classical signal sequence at the N-terminus that leads the newly synthesized protein into the lumen of the ER and a tetrapeptide (HNEL) at the carboxy-terminus, which is similar to the ER-retention consensus sequence (KDEL). It was shown that LRPAP1 is expressed in all human and rat tissues and cell lines analyzed. Highest expression was detected in kidney and brain (Zheng *et al.*, 1994).

LRPAP1 binds to all receptors of the LDL receptor family with high affinity (K_D of 1-10 nM), with the exception of the LDL receptor that binds RAP relatively weakly (K_D of 250 nM) (Bu and Marzolo, 2000). All members are type 1 single transmembrane receptors with a short cytoplasmic domain. The extracellular domain consists of approximately 40 amino acids long repeats containing six cysteine residues which form 3 disulfide bonds and epidermal growth factor precursor-homology repeats (Fass *et al.*, 1997). Most of these receptors are multifunctional and bind to a wide variety of ligands, which are subsequently endocytosed. Strikingly, binding of LRPAP1 to the various receptors inhibits binding of all known ligands (Bu and Marzolo, 2000).

Since LRPAP1 resides in the ER and the early Golgi compartment, it was speculated that LRPAP1 might function as a chaperone that facilitates the correct folding of LDL receptors, including the formation of the correct intramolecular disulfide bonds. This was supported through functional studies in mice. Although LRPAP1-knockout mice appeared phenotypically normal, a detailed analysis showed that large amounts of LRP were retained as aggregates in the ER and could not be transported to the cell surface (Willnow *et al.*, 1995; Willnow *et al.*, 1996). The formation of these aggregates in the

Abbreviations used in this paper: ER, endoplasmatic reticulum; IT, intermediate tubulus; K_D, dissociation constant; kDa, kilo Dalton; LDL, low densitiy lipoprotein; LRP1, low-density-lipoprotein receptor-related protein1; LRPAP1, LDL receptor related protein associated protein 1; NF stage, Nieuwkoop and Faber stage; nM, nano mol; odc1, ornithine decarboxylase gene 1; ORF, open reading frame; Pax2, paired-box gene 2; PT, proximal tubulus; RAP, receptor associated protein; rt-PCR, reverse transcription polymerase chain reaction; slc, solute carrier gene; VLDLR, very-low-density-lipoprotein receptor.

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absence of LRPAP1 could be explained by premature interaction of the receptor and its ligands, which are synthesized in the same cellular compartment and/or improper folding (Bu and Marzolo, 2000). Later studies demonstrated that LRPAP1 has similar functions in the biogenesis of VLDLR and other members of the LDLR family (Sato *et al.*, 1999; Savonen *et al.*, 1999). The only receptor that does not require co-expression of LRPAP1 for proper folding and secretion is the LDLR, which coincides with the lower affinity of the receptor for LRPAP1 binding (Medh *et al.*, 1995).

Detailed structural analysis of the LRPAP1 protein revealed that the part between the N-terminal signal sequence and the C-terminal ER retention signal consists of three repeats, (1-100, 101-200, and 300-323) (Bu *et al.*, 1995; Warshawsky *et al.*, 1995). Binding studies using each of the three domains, independently showed that each of them bound to LRP (Bu and Rennke, 1996). Further studies using constructs expressing various LRPAP1 and LRP domains, demonstrated that the C-terminal LRPAP1 repeat binds to all three LRP ligand binding sites with high affinity, whereas the second and third repeat bind to individual ligand binding repeats with lower affinity (Bu and Rennke, 1996). These findings explain how LRPAP1 can block the binding of all ligands to the receptor. Recently two studies published by Fisher and Lee, identified a series of hidden histidine residues which regulate pH-dependent unfolding of the LRPAP1-D3 domain leading to the release of LRPAP1 bound receptor protein (Fisher *et al.*, 2006; Lee *et al.*, 2006). The picture emerging from all the data described above,

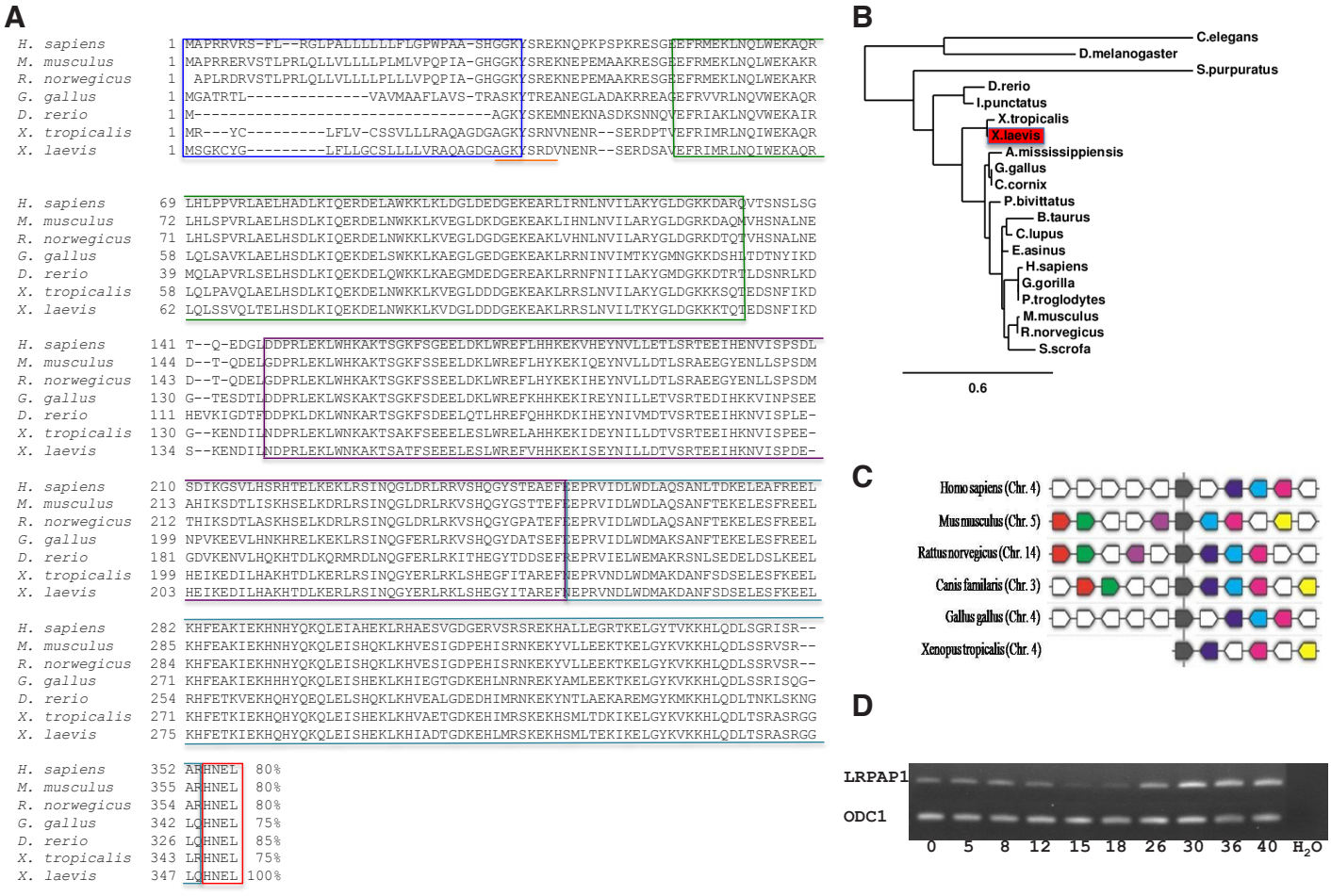

Fig. 1. Identification of *xl-lrpap1* as a true *lrpap1* ortholog. (A) *Comparison oft the deduced LRPAP1 amino acid sequences from* Xenopus laevis *(GenBank Accession no. XP_018083895),* Xenopus tropicalis *(GenBank Accession no. NP_001090847),* Danio rerio *(GenBank Accession no. AAH49517),* Gallus gallus *(GenBank Accession no. NP_990393),* Rattus norvegicus *(GenBank Accession no. AAH98947),* Mus musculus *(GenBank Accession no. NP_038615),* Homo sapiens *(GenBank Accession no. NP_002328). The N-terminal signal peptide is boxed in blue. The processed protein consist of three repeats boxed in green (repeat 1), in purple (repeat 2) and in cyan (repeat 3). An atypical ER retention signal at the C-terminus is boxed in red. The potential proteolytical cleavage site is underlined in orange. Percentage identities are indicated at the end of the aligned sequences.* **(B)** *A phylogenetic tree was constructed based on maximum likelihood. The branch length is proportional to the number of substitutions per site. The bar at the bottom of the phylogram indicates the evolutionary distance, to which the branch lengths are scaled based on the estimated divergence.* **(C)** *Synteny analysis of* lrpap1*: the location and orientation of* lrpap1 *in the respective genomes were compared. Each arrow represents a single gene, the arrow indicates the direction of the ORF. Orthologs are marked with identical colors.* lrpap1 *(black arrow) is present in all species. Upstream,* lrpap1 *is flanked by the same genes, but the exact orientation of these genes to each other varies.* **(D)** *Temporal expression of lrpap1. Semiquantitative rt-PCR analysis revealed maternal presence of* lrpap1 *mRNA until NF stage 12. Zygotic expression of* lrpap1 *starts around NF stage 18 and increased until NF stage 40. Expression of the housekeeping gene* odc1 *was monitored to compare the input of cDNA.*

convincingly suggest a dual function of LRPAP1 as a chaperone insuring the proper formation of intramolecular disulfide bonds during biogenesis of LDL receptors and later as an escort protein during trafficking through the ER and the early Golgi compartment, preventing premature interaction of receptor and ligand. However, as soon as the LRPAP1-receptor complex reaches the medial Golgi compartments, the lower pH in this compartment causes dissociation of the complex, enabling the secretion of proper receptors (Bu and Marzolo, 2000). Because of the general influence of LRPAP1 protein on lipid metabolism we wanted to analyze the potential function of Lrpap1 as the *Xenopus* homolog of LRPAP1 during embryogenesis.

Results

Identification and cloning of the Xenopus laevis lrpap1 ortholog

To analyze the temporal and spatial expression and the function of *lrpap1* during *Xenopus laevis* development, we obtained a plasmid containing the presumptive *Xenopus* ortholog of *lrpap1* from the RZPD. Sequencing of the insert in this plasmid revealed an open reading frame (ORF) of 1081bp encoding a potential Lrpap1 protein of 351 amino acids (Fig. 1). A sequence comparison of the DNA sequence of the ORF with the data base showed that our sequence was 99% identical to the sequence of a predicted *Xenopus laevis* LDL receptor related protein associated protein 1 L homolog (XM_018228406.1). The deduced amino acid sequence starts with a methionine, followed by a 29 aa long signal peptide, which is probably cleaved off at the conserved proteolytic cleavage site KYSR at position 29. The resulting mature protein of 322 amino acid residues consist of three repeats which represent different receptor binding sites. The c-terminal domain contains a coiled-coiled motif (258-292) and the conserved endoplasmic reticulum retention signal HNEL (Strickland *et al.*, 1991) (Fig. 1A).

Comparison of the deduced amino acid sequence of the *Xenopus laevis* cDNA with the LRPAP1 amino acid sequence of other vertebrates showed that *Xenopus* Lrpap1 was highly homologues to all other vertebrate LRPAP1 proteins included in this analysis. Highest homology was found with the Lrpap1 sequence of *Danio rerio* (85%) and lowest homology was found with *Gallus gallus* LRPAP1 sequence (75%) (Fig. 1A). A phylogenetic analysis showed that LRPAP1 is conserved from mammals to insects and annelids (Fig. 1B). Since *Xenopus laevis* has a pseudo tetraploid genome, we analyzed whether a second *lrpap1* locus exists and whether this locus is also transcribed. Searching *Xenopus* genome databases, we could identify two *lrpap1* loci, referred to as *lrpap1*-L and *lrpap1*-S. Transcripts of locus *lrpap1*-L are identical to the cDNA sequence described above, whereas putative transcripts of the second locus, *lrpap1*-S are much shorter (772bp compared to 1081bp). The S locus lacks several exons and the resulting potential mRNA does not contain an ORF. Using specific primer pairs for the putative transcripts of the two different loci, we were able to detect transcripts of locus-L but we could not detect transcripts from locus S, suggesting that the S locus does not encode a functional transcript (data not shown). A synteny analysis using the genomic locus of *Xenopus tropicalis* showed that in all species compared, *lrpap1* is flanked upstream by the same genes, but the orientation of these genes to each other varies (Fig. 1C).

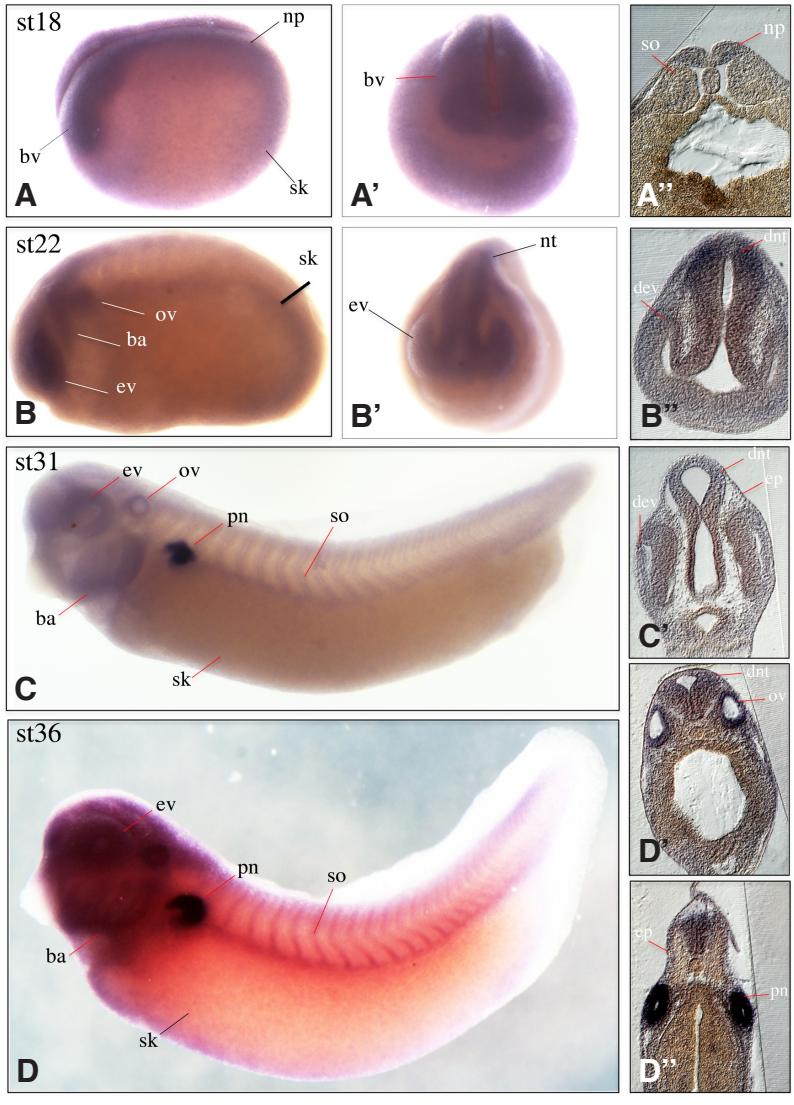

Fig. 2. Spatial analyses of *lrpap1* expression. *Whole-mount and sectioned* in situ *hybridization of wild type embryos at developmental stages 18 to 36. Earliest* lrpap1 *expression was detectable at NF stage 18 when neurulation starts in the neural plate and the developing brain vesicles* **(A,A',A")**. *At NF stage 22 additional expression could be detected in the eye vesicle, the otic vesicle and the branchial arches* **(B,B',B")**. *This expression pattern is maintained until NF stage 36* **(D,D',)**, *but starting at NF stage 31 additional expression was detected in the somites and in distinct cells scattered all over the skin. Highest expression levels were observed in the proximal part of the developing pronephros* **(C,C',D)**. *At NF stage 36* lrpap1 *transcripts are spread through the entire pronephric epithelium and the developing otic vesicles* **(D,D',D")**. *Abbreviations: (bv) brain vesicle, (ba) branchial arches, (dev) distal eye vesicle, (dnt) dorsal neural tube, (ep) epithelium, (ev) eye vesicle, (np) neural plate, (nt) neural tube, (ov) otic vesicle, (pn) pronephros, (sk) skin, (so) somites. A, B, C and D show lateral views of the embryos, A' and B' show frontal views.*

Temporal expression of lrpap1 during Xenopus development and in adult tissues

To analyze the temporal expression of *lrpap1* during embryogenesis we prepared cDNA from mRNA collected from a series of *Xenopus* embryos at different developmental stages. Our rt-PCR analysis showed that maternal *lrpap1* mRNA could be detected from NF stage 0 to stage 12, zygotic expression could be detected as early as NF stage 15. Expression of *lrpap1* increased until stage 30 and was maintained at relatively high levels until stage 40 (Fig. 1D). In adult tissues highest levels of *lrpap1* expression were detected in kidney, brain and liver, weaker mRNA levels were found in heart, lung and gut (data not shown).

Spatial expression of lrpap1 during Xenopus development

Spatial expression of *lrpap1* was analyzed by *in situ* hybridization on whole-mount and sectioned early *Xenopus* embryos (Fig. 2). At the end of neurulation at NF stage 18, first transcripts of *lrpap1* were detectable along the entire anterior-posterior axis within the neural plate, which will later form the brain vesicles. Additional but less abundant expression could be detected in the entire superficial ectoderm of the embryo, which later forms the skin. At NF stage 22 *lrpap1* was still expressed along the now closed neural tube, in the developing brain, in the eye vesicles, the first branchial arch, in the ear anlage and in the skin. This expression pattern was maintained until stages 31 and 36, however, additional domains of expression were observed in somites and most abundantly in the proximal part of the developing pronephros.

The analysis of sectioned embryos allowed a more detailed description of the expression (Fig. 2). At NF stage 18, expression of *lrpap1* in the neural folds was spread over the entire neural plate. In the skin expression was restricted to the epidermis. At NF stage 22, neural expression was restricted to the dorsal part of the neural tube and to the distal part of the eye vesicle. In the head region additional expression was in the ventral mesoderm. In more posterior sections of the anterior trunk region additional *lrpap1* transcripts were detected in the mesoderm overlying the dorsal part of the neural tube. In the branchial arch expression was spread over the whole branchial tissue. At NF stages 31 and 36 neural and mesodermal expression of *lrpap1* in the head region was maintained. Additional domains of expression were detected in the epithelium of the otocysts and strong expression of *lrpap1* was observed in the wall of the developing primitive kidney.

Since *lrpap1* was not expressed throughout the entire kidney anlage, we compared the expression of *lrpap1* at NF stage 36 with the expression of different kidney specific genes known to be expressed in distinct domains of the developing pronephros (Fig. 3). The developing kidney consists of the proximal tubules 1, 2 and 3, intermediate tubular segments 1 and 2, the distal tubules 1 and 2 and the conotruncus. A comparison of the expression of *lrpap1* and *pax2*, which is expressed throughout the entire primitive

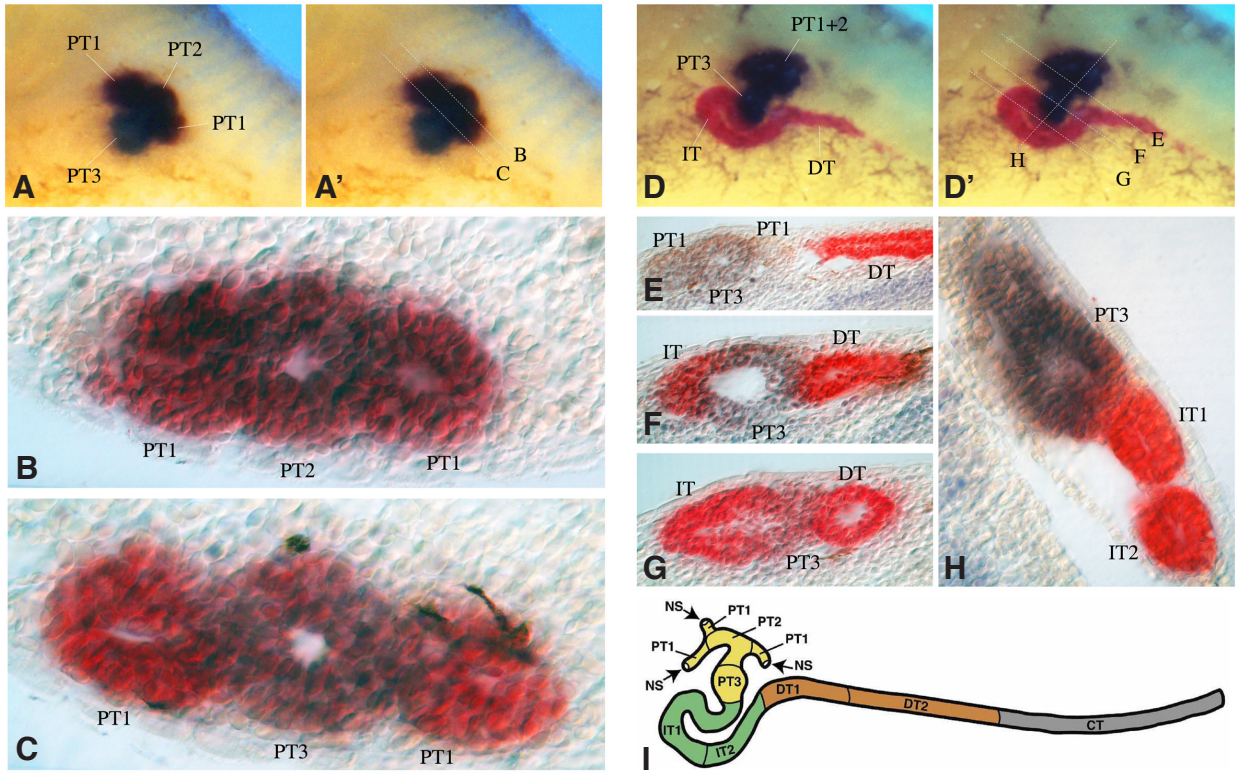

Fig. 3. A detailed comparison of the expression of *lrpap1* and two different solute carrier genes *slc12a1* and *slc5a2*, known to be expressed in distinct domains of the developing pronephros. *Double stained whole mount* in situ *hybridizations on NF stage 36 embryos were performed marking the expression of* lrpap1 *in blue and the expression of either scl5a2* **(A,A')** *or scl12a1* **(D,D')** *in red. Sections of these embryos revealed that* lrpap1 *expression is restricted to the epithelia of the proximal tubules 1,2 and 3* **(B, C, E, F, H)** *whereas* lrpap1 *was not expressed in the intermediate tubules 1 and 2, the distal tubules* **(E,F,G,H)** *and the conotruncus (not shown). The plane of the sections is indicated in* **(A')** *and* **(D')** *respectively. The drawing in I depicts the different parts of the pronephros (taken and modified from (Reggiani* et al.*, 2007). Abbreviations: conotruncus (CT), distal tubule (DT), intermediate tubule (IT), nephrostomy (NS) and pronephric tubule (PT).*

kidney, showed that the *lrpap1* positive domain was restricted to the proximal part of the kidney anlage. Expression of the solute carrier gene *slc12a1* is restricted to the intermediary tubuli 1 and 2 and to the first segment of the distal tubulus (Reggiani *et al.*, 2007). The expression domains of *lrpap1* and *slc12a1* did not overlap but at the anterior border of IT1 between IT1 and PT3 the expression domains of *lrpap1* and *slc12a1* were adjacent to each other, showing that the expression of *lrpap1* was limited to the proximal tubular segment 3. A comparison with *slc5a2*, which is specifically expressed in the proximal tubulus parts 1 and 2 showed that both genes were coexpressed in the proximal tubules 1 and 2 (Reggiani *et al.*, 2007). This analysis clearly showed that *lrpap1* was specifically expressed at relatively high levels in the proximal part of the pronephros.

Discussion

In this report, we analyzed the expression and a potential function of *Xenopus laevis lrpap1* during embryogenesis. The high degree of sequence similarity of LRPAP1 to other vertebrate proteins in combination with the conserved syntenic organization strongly suggests that the *lrpap1* gene we analyzed, is the *lrpap1* ortholog of *Xenopus laevis*. Transcripts of a second copy of the *lrpap1* locus in the pseudotetraploid genome of *Xenopus laevis* was altered so much during evolution, that it does not encode a functional protein and transcripts from this locus could not be detected during the stages we analyzed.

Xenopus lrpap1 is relatively widely expressed with particularly high expression levels in the CNS and the kidney. This is consistent with the expression pattern found in other species (Bu *et al.*, 1994b; Zheng *et al.*, 1994). In the anterior part of the developing central nervous system high expression domains are restricted to the dorsal part of the neural tube and to the distal part of the eye vesicle. This is particularly interesting since the only phenotype that was initially detected in otherwise completely normal LRPAP1 knockout mice is cognitive impairment. As a physiological reason for this observation, a reduced number of somatostatin-expressing neurons was reported (Van Uden *et al.*, 1999).

In the primitive kidney of developing *Xenopus* embryos, *lrpap1* expression is restricted to the proximal tubules. No expression could be detected in other parts of the pronephros.

The very specific expression pattern establishes *lrpap1* as a novel marker for the analysis of pronephros formation.

Methods

Animals

Pigmented and albino *Xenopus laevis* were obtained from Nasco (Ft. Atkinson, WI). Production and rearing of embryos was as described (Hollemann and Pieler, 1999). Staging of embryos was done according to (Nieuwkoop, 1994).

Whole mount in situ hybridization

In general, whole-mount *in situ* hybridization (Wmish) was carried out as described (Hollemann *et al.*, 1998). To generate antisense RNA probes, corresponding plasmids were digested and transcribed as follows: pCMV-Sport6-*lrpap1*, pCMV-Sport6-scl5a2 and pBluescript SK(-)-scl12a1 were linearized with *Sal*I and transcribed using T7. For the analysis of *lrpap1* expression on sections, embryos were embedded in gelatin-albumin embedding medium following Wmish and 30μm section were made using a vibratome (Leica, Germany) and mounted on glass slides.

RNA preparation and reverse transcription

RNA was prepared from whole *Xenopus* embryos and adult *Xenopus* tissues using Trizol or Qiagen RNeasy Kit following the instructions provided by the manufacturer. First strand cDNA was prepared from 500 ng total RNA using oligo–dT- or random primer and reverse transcriptase (Gibco).

RT-PCR

RT-PCR was performed with the following intron spanning primers:

xl-*odc1*-F	5'-GCCATTGTGAAGACTCTCTCCATTC,
xl-*odc1*-R	5'-TTCGGGTGATTCCTTGCCAC,
xl-*lrpap1*-F	5'-GCAAAGAATCTGGCCTTCTG
xl-*lrpap1*-R	5'-TGTCCCAAAACAGCAAAAACA

Microinjections

Morpholino oligonucleotides (Gene Tool, USA) were injected at 0.1 pmol/nl:

Lrpap1-MO-xl: 5'-CCTCTTACCTCAGTTACAATTTATA

Standard-MO: 5'-CCCGTAACACTTCCCTGAATCGTA

For further details see (Wu *et al.*, 2009).

Acknowledgements

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References

BU, G., GEUZE, H.J., STROUS, G.J. and SCHWARTZ, A.L. (1995). 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein. *EMBO J* 14: 2269-2280.

BU, G., MAKSYMOVITCH, E.A., GEUZE, H. and SCHWARTZ, A.L. (1994). Subcellular localization and endocytic function of low density lipoprotein receptor-related protein in human glioblastoma cells. *J Biol Chem* 269: 29874-29882.

BU, G., MAKSYMOVITCH, E.A., NERBONNE, J.M. and SCHWARTZ, A.L. (1994). Expression and function of the low density lipoprotein receptor-related protein (LRP) in mammalian central neurons. *J Biol Chem* 269: 18521-18528.

BU, G. and MARZOLO, M.P. (2000). Role of rap in the biogenesis of lipoprotein receptors. *Trends Cardiovasc Med* 10: 148-155.

BU, G. and RENNKE, S. (1996). Receptor-associated protein is a folding chaperone for low density lipoprotein receptor-related protein. *J Biol Chem* 271: 22218-22224.

FASS, D., BLACKLOW, S., KIM, P.S. and BERGER, J.M. (1997). Molecular basis of familial hypercholesterolaemia from structure of LDL receptor module. *Nature* 388: 691-693.

FISHER, C., BEGLOVA, N. and BLACKLOW, S.C. (2006). Structure of an LDLR-RAP complex reveals a general mode for ligand recognition by lipoprotein receptors. *Mol Cell* 22: 277-283.

HERZ, J. (2006). The switch on the RAPper's necklace. *Mol Cell* 23: 451-5.

HOLLEMANN, T., CHEN, Y., GRUNZ, H. and PIELER, T. (1998). Regionalized metabolic activity establishes boundaries of retinoic acid signalling. *EMBO J* 17: 7361-7372.

HOLLEMANN, T. and PIELER, T. (1999). Xpitx-1: a homeobox gene expressed during pituitary and cement gland formation of *Xenopus* embryos. *Mech Dev* 88: 249-252.

LEE, D., WALSH, J.D., MIKHAILENKO, I., YU, P., MIGLIORINI, M., WU, Y., KRUEGER, S., CURTIS, J.E., HARRIS, B., LOCKETT, S. *et al.,* (2006). RAP uses a histidine switch to regulate its interaction with LRP in the ER and Golgi. *Mol Cell* 22: 423-430.

MEDH, J.D., FRY, G.L., BOWEN, S.L., PLADET, M.W., STRICKLAND, D.K. and CHAPPELL, D.A. (1995). The 39-kDa receptor-associated protein modulates lipoprotein catabolism by binding to LDL receptors. *J Biol Chem* 270: 536-540.

NIEUWKOOP, P.D. and FABER, J. (1994). Normal Table of *Xenopus laevis* (Daudin). Garland Publishing Inc., New York & London.

REGGIANI, L., RACITI, D., AIRIK, R., KISPERT, A. and BRANDLI, A.W. (2007). The prepattern transcription factor Irx3 directs nephron segment identity. *Genes Dev* 21: 2358-2370.

SATO, A., SHIMADA, Y., HERZ, J., YAMAMOTO, T. and JINGAMI, H. (1999). 39-kDa receptor-associated protein (RAP) facilitates secretion and ligand binding of extra-

cellular region of very-low-density-lipoprotein receptor: implications for a distinct pathway from low-density-lipoprotein receptor. *Biochem J* 341 (Pt 2): 377-383.

SAVONEN, R., OBERMOELLER, L.M., TRAUSCH-AZAR, J.S., SCHWARTZ, A.L. and BU, G. (1999). The carboxyl-terminal domain of receptor-associated protein facilitates proper folding and trafficking of the very low density lipoprotein receptor by interaction with the three amino-terminal ligand-binding repeats of the receptor. *J Biol Chem* 274: 25877-25882.

STRICKLAND, D.K., ASHCOM, J.D., WILLIAMS, S., BATTEY, F., BEHRE, E., MCTIGUE, K., BATTEY, J.F. and ARGRAVES, W.S. (1991). Primary structure of alpha 2-macroglobulin receptor-associated protein. Human homologue of a Heymann nephritis antigen. *J Biol Chem* 266: 13364-13369.

STRICKLAND, D.K., ASHCOM, J.D., WILLIAMS, S., BURGESS, W.H., MIGLIORINI, M. and ARGRAVES, W.S. (1990). Sequence identity between the alpha 2-macroglobulin receptor and low density lipoprotein receptor-related protein suggests that this molecule is a multifunctional receptor. *J Biol Chem* 265: 17401-17404.

VAN UDEN, E., VEINBERGS, I., MALLORY, M., ORLANDO, R. and MASLIAH, E. (1999). A novel role for receptor-associated protein in somatostatin modulation: implications for Alzheimer's disease. *Neuroscience* 88: 687-700.

WARSHAWSKY, I., BU, G. and SCHWARTZ, A.L. (1995). Sites within the 39-kDa protein important for regulating ligand binding to the low-density lipoprotein receptor-related protein. *Biochemistry* 34: 3404-3415.

WILLNOW, T.E., ARMSTRONG, S.A., HAMMER, R.E. and HERZ, J. (1995). Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. *Proc Natl Acad Sci USA* 92: 4537-4541.

WILLNOW, T.E., ROHLMANN, A., HORTON, J., OTANI, H., BRAUN, J.R., HAMMER, R.E. and HERZ, J. (1996). RAP, a specialized chaperone, prevents ligand-induced ER retention and degradation of LDL receptor-related endocytic receptors. *EMBO J* 15: 2632-2639.

WU, H.Y., PERRON, M. and HOLLEMANN, T. (2009). The role of *Xenopus* Rx-L in photoreceptor cell determination. *Dev Biol* 327: 352-365.

ZHENG, G., BACHINSKY, D.R., STAMENKOVIC, I., STRICKLAND, D.K., BROWN, D., ANDRES, G. and MCCLUSKEY, R.T. (1994). Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/alpha 2MR, and the receptor-associated protein (RAP). *J Histochem Cytochem* 42: 531-542.

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