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Amino acid cotransporter SLC3A2 is selectively expressed in the early proximal segment of *Xenopus* pronephric kidney nephrons

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Abstract

The transport of amino acids across membranes is critical to all cells. As amino acids freely pass through the glomerular filtration barrier of the kidney, they must be efficiently resorbed to avoid depletion of circulating amino acid reserves. Not only do defects in amino acid resorption lead to costly wastage, they also cause congenital aminoacidurias. A clone encoding *Xenopus* SLC3A2 was identified and shown to be expressed at high levels in the early segment of the pronephric proximal tubules in developing tadpoles. The type II membrane glycoprotein encoded by this gene can associate with a wide variety of protein partners and participates in a broad spectrum of biological processes. In this report, the first whole-mount analysis of SLC3A2 during early embryonic development is presented. The expression pattern of SLC3A2 in the early proximal segment of the *Xenopus* pronephros is analogous to that of a previously described SLC7A8/XAA2 amino acid transporter. In mammals, SLC3A2 and SLC7A8/XAA2 associate to form a functional neutral amino acid transporter complex and coexpression of these two genes in a small domain within the pronephric tubules indicates that this is also the situation in the developing *Xenopus* kidney.

Keywords: Solute carrier; 4F2hc; Pronephros; CD98; SLC7A8; Neutral amino acid transporter; Lat2

The heteromeric amino acid transporters (HATs) contain SLC3 heavy chains and SLC7 light chains (reviewed by Hediger et al., 2004). The light chains encode multipass membrane transporter subunits while the heavy chains have a single large extracellular C-terminal domain that has ~30% amino acid identity to bacterial α-glucosidases (Palacin and Kanai, 2004). In the example of SLC3A2, the HAT heavy chain was first identified as a surface antigen on lymphocytes, and is also known as lymphocyte activation antigen 4F2/4F2hc and as the heavy chain of CD98 (Hemler and Strominger, 1982). In terms of amino acid transport, association of the light chain to a SLC3 heavy chain is essential for correct translocation to the cell surface (Chillaron et al., 2001; Wagner et al., 2001). SLC3A2 is a multifunctional protein that dimerizes with a range of amino acid transporters, including those of the L, y^+L, x_c^- and asc systems and also participates in a variety of other cell functions such as cell adhesion (Palacin and Kanai, 2004). The phenotype of SLC3A2 mutants is predicted to be lethal, while mutation of its protein partners can cause a range of congenital defects including lysinuric protein intolerance.

We have previously described the distribution of two other amino acid transporter genes, SLC6A14/XAA1 and SLC7A8/XAA2/Lat2 (Zhou and Vize, 2004). Of these, only SLC7A8/XAA2 is known to associate with the SLC3A2 heavy chain in mammalian cells (Bauch et al., 2003). In this report, we characterize the expression of SLC3A2 in *Xenopus* embryos and show that it is identical to that of its potential protein partner, SLC7A8/XAA2. Furthermore, these data indicate the recently identified early proximal domain of pronephroi (Zhou and Vize, 2004) is functionally analogous to the S1 segment of mammalian nephrons which is the major site of SLC3A2 and SLC7A8/XAA2 expression (Bauch et al., 2003; Rossier et al., 1999). This report is the first whole-mount analysis of SLC3A2 expression during early embryogenesis and contributes to the segmental mapping of the simple embryonic nephrons.

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1. Results and discussion

A targeted bioinformatics screen has identified a number of solute carriers and carrier ancillary genes that are present in a *Xenopus laevis* adult kidney cDNA library (Zhou and Vize, 2004). Clone XAA3 (GenBank BU905366, IMAGE: 4033093, protein MGC53951) corresponds to SLC family 3 member 2 (SLC3A2), activators of dibasic and neutral amino acid transport. The *X. laevis* predicted protein is 48% identical to human SLC3A2 (Fig. 1).

The expression of *Xenopus* SLC3A2/XAA3 during early development was characterized with a combination of colorimetric and fluorescent in situ hybridization. This gene is expressed at high levels within a very restricted portion of the forming pronephric kidney, first beginning at NF stage 28 (Nieuwkoop and Faber, 1994). No other regions of expression are detected between stages 28 and 40, although low-level ubiquitous expression is very possible. At stages 28–30 of development the pronephric expression resembles, and could be mistaken for, expression in the pronephric glomus (Fig. 2). However, as development proceeds, the expression domain clearly corresponds to the most dorso-anterior portion of the pronephric tubules—the early proximal domain (Fig. 2E,G). To further refine where within the pronephric tubules this expression is occurring embryos were developed for both SLC3A2/XAA3 and for a general marker of the pronephric tubules, Na^+K^+ATPase. Both fluorescent colorimetric (FCIS) and double fluorescent in situ hybridization (FISH) techniques were used. These data show that early SLC3A2/XAA3 expression occurs in just the dorsal most margin of the pronephric tubule primordium. In FCIS staining this appears as dark blue, and in double FISH as yellow (Fig. 2). In later stages of development, the strongest expression remains in the early proximal domain, but faint expression also appears to occur in the late proximal domain. This restriction is maintained until the latest stage examined, stage 40.5.

The coexpression of two genes, the products of which are known to interact in the mammalian kidney and intestine, in simple *Xenopus* embryonic kidneys is very interesting.

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Xenopus         MTQDTALDMKDVELNELEQEKVPMADGAGDSP-TGGGEKNGVVKVKLDDDDDMPAKSQKF 59
human           MSQDTEVDMKEVELNELEPEKQPMNAASGAAMSLAGAEKNGLVKIKVAEDEAEAAAAAKF 60
                *:*** :***:******* ** **  .:* :   .*.****:**:*: :*:  .* : **

Xenopus         TGLSKEELLRVAGTPTWVRVRWALLILFWLGWAGMLAGAVVIIVQAPRCRPLPAMEWWNK 119
human           TGLSKEELLKVAGSPGWVRTRWALLLLFWLGWLGMLAGAVVIIVRAPRCRELPAQKWWHT 120
                *********:***:* ***.*****:****** **********:***** *** :**:.

Xenopus         GPLYQVGDPATFQEDGAGNIQSIEKRLESLTSLKVKGLIIGPIHVTKKDQIGETELTDID 179
human           GALYRIGDLQAFQGHGAGNLAGLKGRLDYLSSLKVKGLVLGPIHKNQKDDVAQTDLLQID 180
                *.**::**  :** .****: .:: **: *:*******::**** .:**::.:*:* :**

Xenopus         PNYGTMEQFTSLLEAARKKSIQIILDLTPNYRSENSWFEKAERENNIFFEKVKEAVNVWL 239
human           PNFGSKEDFDSLLQSAKKKSIRVILDLTPNYRGENSWFFT---QVDTVATKVKDALEFWL 237
                **:*: *:* ***::*:****::*********.***** .   : : .  ***:*::.**

Xenopus         EHGVGGIYFGDSENFPNANSFIYEWGNMTANYSKEGKPRVLLLSTSSAQNNLTGGFNETI 299
human           QAGVDGFQVRDIENLKDASSFLAEWQNITKGFSED---RLLIAGTNSSDLQQILSLLESN 294
                : **.*: . * **: :*.**: ** *:* .:*::   *:*: .*.*:: :   .: *:

Xenopus         DGTLFYRFLGAENKKSFGSLGESIKQYVEETGIQGNSWMIGAPQMRHMASLVNEKLLRVY 359
human           KDLLLTSSYLSDSGSTGEHTKSLVTQYLNATGNRWCSWSL--SQARLLTSFLPAQLLRLY 352
                .. *:     ::. .:     . :.**:: ** :  ** :  .* * ::*::  :***:*

Xenopus         QLLLFTLPGTPISLYGDEIGLKD--LPGQPAQSSRPNMQWEEVSVSNNSPQIASDVNANI 417
human           QLMLFTLPGTPVFSYGDEIGLDAAALPGQPMEA--PVMLWDESSF----PDIPGAVSANM 406
                **:*********:  *******.   ***** ::  * * *:* *.    *:*.. *.**:

Xenopus         TFKAQDADKGSFLNVYRKLSDLRGKERSLLHGEFTLLYNSEEAISFLRSWDQNERYVTAL 477
human           TVKGQSEDPGSLLSLFRRLSDQRSKERSLLHGDFHAFSAGPELFSYIRHWDQNERFLVVL 466
                *.*.*. * **:*.::*:*** *.********:*  :  . * :*::* ******::..*

Xenopus         NFNYEGEVELFLKKD--GGEELPEQGTVVLSSNPQRKDGETVSLKSFKLGAGEALLLKYP 535
human           NFGDVGLSAGLQASDLPASASLPAKADLLLSTQPGREEGSPLELERLKLEPHEGLLLRFP 526
                **.  *    :  .*  .. .** :. ::**::* *::*..:.*: :** . *.***::*

Xenopus         YSG 538
human           YAA 529
                *:.
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Fig. 1. Alignment between *Xenopus* and human SLC3A2. *Xenopus* (GenBank BU905366) and human (Genbank AB018010) predicted proteins were aligned with clustalw. Identical amino acids are indicated with an asterisk, strongly similar substitutions with a colon, and weakly similar substitutions with a period.

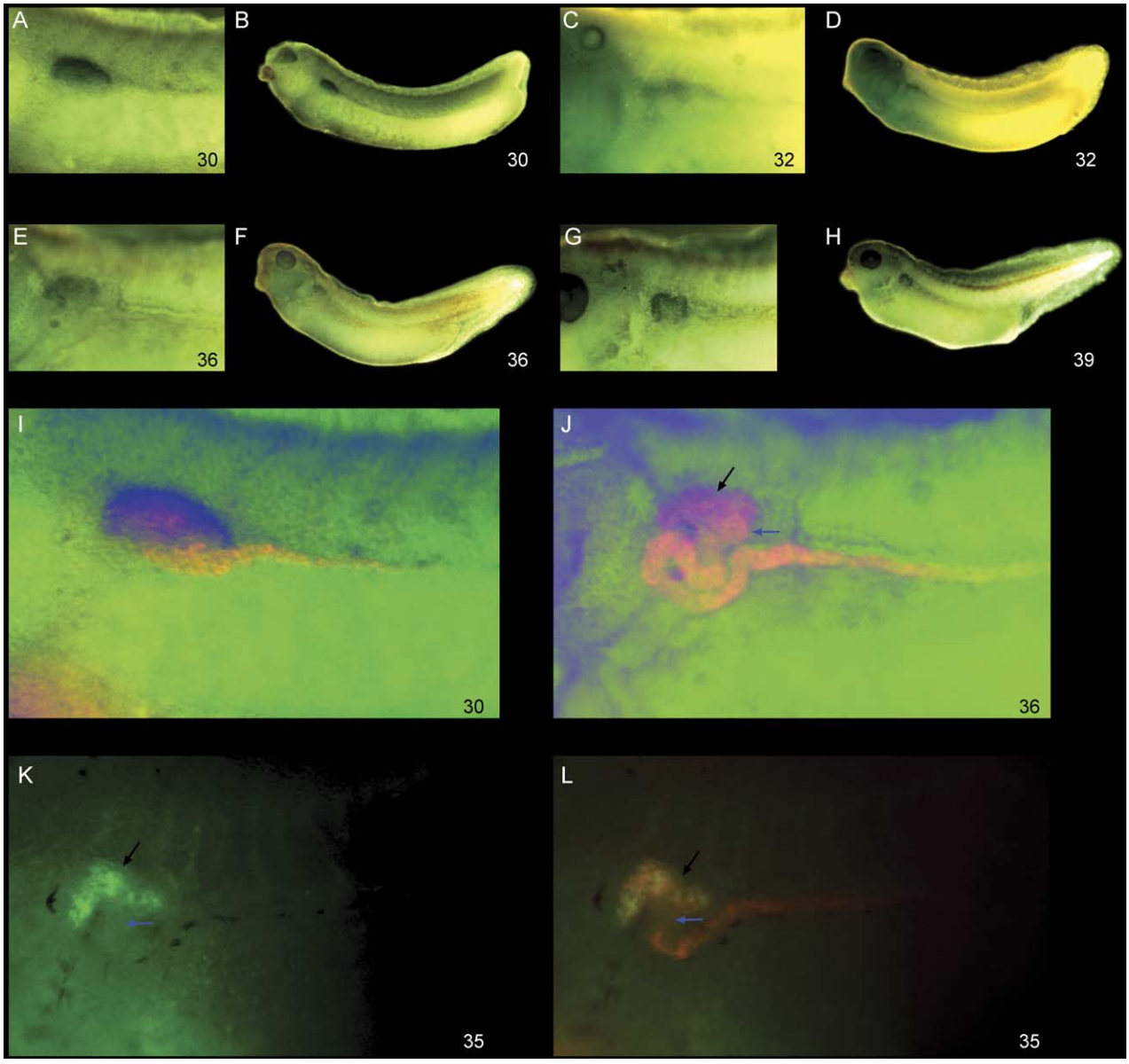

Fig. 2. Expression of *Xenopus* SLC3A2 in the pronephros. Panels A–H illustrate SLC3A2 expression in *Xenopus laevis* embryos of various stages, which are indicated in the lower right of each panel. Detection is via colorimetic in situ hybridization. Panels I and J are FCIS overlays, where colorimetic SLC3A2 expression (blue) has been counter-stained for Na^+K^+ATPase expression via FISH. Good overlap appears blue or blue/purple (black arrow), weak overlap appears pink (blue arrow), and no overlap appears orange. Panels K and L illustrate FISH data. K is SLC3A2 expression alone developed with a green substrate, while L is an overlay illustrating SLC3A2 expression as green, Na^+K^+ATPase expression as red, and coexpression as yellow. Most overlap occurs in the early proximal domain (black arrow), but a low level of coexpression is also observed in the late proximal domain (blue arrow).

The major site of expression is the early proximal domain, with lighter expression in the late proximal domain. This pattern strongly resembles that of the mammalian SLC3A2/SLC7A8 genes where expression is highest in the most proximal segment of the nephron, S1, and lower in the more distal S2 segment (Verrey et al., 2004). This observation lends support to the observation that the simple pronephric nephrons have a similar segmental organization of solute recovery and transporter function as do the more complex nephrons of mammalian metanephroi (Zhou and Vize, 2004). Not only do these disparate kidney forms utilize similar genetic networks during their early embryonic patterning (Vize et al., 1997), they also have a very similar functional organization.

2. Experimental procedures

IMAGE clone 4033093 (GenBank BU905366) was obtained from OpenBiosystems. This clone contains the SLC3A2 cDNA inserted into pCMV-SPORT6 and this plasmid named pXAA3. In situ probes were generated by cutting pXAA3 with *Sma*I and transcribing with T7 polymerase. FCIS was performed according to the protocol

of Zhou and Vize (2004) and double FISH via the protocols of Gerth et al. (in press).

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References

Bauch, C., Forster, N., Loffing-Cueni, D., Summa, V., Verrey, F., 2003. Functional cooperation of epithelial heteromeric amino acid transporters expressed in madin-darby canine kidney cells. J. Biol. Chem. 278, 1316–1322.

Chillaron, J., Roca, R., Valencia, A., Zorzano, A., Palacin, M., 2001. Heteromeric amino acid transporters: biochemistry, genetics, and physiology. Am. J. Physiol. Renal Physiol. 281, F995–1018.

Gerth, V.E., Zhou, X., Vize, P.D., 2005. Nephrin expression and three-dimensional morphogenesis of the Xenopus pronephric glomus. Dev. Dyn. in press.

Hediger, M.A., Romero, M.F., Peng, J.B., Rolfs, A., Takanaga, H., Bruford, E.A., 2004. The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Introduction. Pflugers Arch. 447, 465–468.

Hemler, M.E., Strominger, J.L., 1982. Characterization of antigen recognized by the monoclonal antibody (4F2): different molecular forms on human T and B lymphoblastoid cell lines. J. Immunol. 129, 623–628.

Nieuwkoop, P.D., Faber, J., 1994. Normal table of *Xenopus laevis* (Daudin). Garland, New York.

Palacin, M., Kanai, Y., 2004. The ancillary proteins of HATs: SLC3 family of amino acid transporters. Eur. J. Physiol. 447, 490–494.

Rossier, G., Meier, C., Bauch, C., Summa, V., Sordat, B., Verrey, F., Kuhn, L.C., 1999. LAT2, a new basolateral 4F2hc/CD98-associated amino acid transporter of kidney and intestine. J. Biol. Chem. 274, 34948–34954.

Verrey, F., Closs, E.I., Wagner, C.A., Palacin, M., Endou, H., Kanai, Y., 2004. CATs and HATs: the SLC7 family of amino acid transporters. Pflugers Arch. 447, 532–542.

Vize, P.D., Seufert, D.W., Carroll, T.J., Wallingford, J.B., 1997. Model systems for the study of kidney development: use of the pronephros in the analysis of organ induction and patterning. Dev. Biol. 188, 189–204.

Wagner, C.A., Lang, F., Broer, S., 2001. Function and structure of heterodimeric amino acid transporters. Am. J. Physiol. Cell Physiol. 281, C1077–C1093.

Zhou, X., Vize, P.D., 2004. Proximo-distal specialization of epithelial transport processes within the Xenopus pronephric kidney tubules. Dev. Biol. 271, 322–338.