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Embryonic expression of *Xenopus* SGLT-1L, a novel member of the solute carrier family 5 (SLC5), is confined to tubules of the pronephric kidney

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ABSTRACT Plasma membrane proteins of the solute carrier family 5 (SLC5) are responsible for sodium-coupled uptake of ions, sugars and nutrients in the vertebrate body. Mutations in SLC5 genes are the cause of several inherited human disorders. We have recently reported the cloning and transport properties of SGLT-1L, a *Xenopus* homologue of the sodium-dependent glucose cotransporter 1 (SGLT-1) [Nagata *et al.* (1999) *Am. J. Physiol.* 276: G1251-G1259]. Here, we describe the phylogenetic relationship of SGLT-1L with other members of the SLC5 family and characterize its expression during *Xenopus* embryogenesis and in organ cultures. Sequence comparisons and phylogenetic analyses of all known vertebrate SLC5 sequences indicated that *Xenopus* SGLT-1L encodes a novel SLC5 member, which shares highest amino acid identity with mammalian ST-1 proteins. Temporal and spatial expression of SGLT-1L during *Xenopus* embryogenesis was examined by whole mount *in situ* hybridization. Initiation of SGLT-1L expression occurred in the late tailbud embryo. Remarkably, expression was restricted to the developing pronephric kidney. SGLT-1L was highly expressed in tubular epithelia, but completely absent from the epithelia of the duct. Analysis of growth factor-treated animal caps indicated that expression of SGLT-1L could also be induced in organ cultures. Taken together, our findings indicate that the expression of sodium-dependent solute cotransporter genes in early segments of the excretory system appears to be conserved between pronephric and metanephric kidneys. Furthermore, we establish SGLT-1L as a novel, highly specific molecular marker for pronephric tubule epithelia undergoing maturation and terminal differentiation in *Xenopus*.

KEY WORDS: *Sodium-dependent solute carrier family, SLC5 family, SGLT-1L, pronephric kidney, Xenopus embryogenesis*

Introduction

The up-take of small molecular-weight solutes (*i.e.*, glucose, amino acids, vitamins) is required for essential metabolic processes in all vertebrate cells, and therefore intrinsic mechanisms have evolved for active accumulation of essential solutes. These transport systems are comprised of specific plasma membrane proteins, which also maintain solute homeostasis inside and outside of the cells, and their defects can lead to fatal diseases. Tissues that are important for the absorption of solutes, such as the placenta, the small intestine, and the kidney, possess specialized transport mechanisms that mediate transcellular transfer of essential nutrients. Transport of solutes can occur via passive and active transport systems (Berger *et al.*, 2000). Passive transporters allow diffusion of solutes across membranes down their electrochemical gradient. Active transporters utilize various energy-coupling mechanisms to create solute gradients. For example, ATP-dependent transporters, which include members of the ABC (ATP-binding cassette) transporter family, are directly energized by ATP hydrolysis. In contrast, ion-dependent solute transporters use primarily electrochemical sodium gradients as a driving force for solute transport.

A first cDNA encoding the intestinal sodium-glucose cotransporter SGLT-1 was isolated by expression cloning and sequencing revealed that the encoded protein represented a novel class of transport proteins distinct from channels and pumps (Hediger *et al.*,

Abbreviations used in this paper: ABC, ATP-binding cassette; NIS, sodium-iodide symporter; SGLT, sodium-dependent glucose cotransporter; SLC, solute carrier; SMIT, sodium/myo-inositol transporter; SMVT, sodium-dependent multivitamin transporter; TSH, thyroid stimulating hormone.

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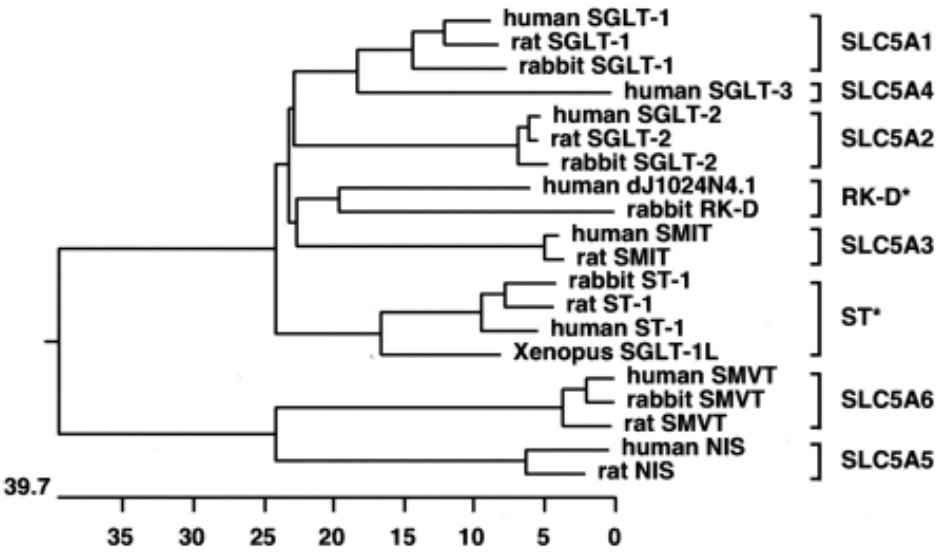

Fig. 1. Phylogenetic tree relating *Xenopus* SGLT-1L to other members of the vertebrate SLC5 family. *The phylogenic tree was constructed by comparison of amino acid residues homologous to transmembrane segments 2 to 6 of human SGLT-1 (Turk and Wright, 1997). The evolutionary distance between any two sequences is the sum of the horizontal branch length separating them. Units indicate the number of necessary substitution events. Vertical distances are for illustration purposes only. Brackets indicate sequences representing orthologues of human SLC5 genes. Asterisks denote SLC5-related genes for which gene symbols have not been assigned yet, as it is currently unclear whether the sequences shown represent orthologues or paralogues of the human genes. GenBank accession numbers and a definition of the amino acids sequences used in the alignment are given in the Materials and Methods section.*

1987). Subsequently, cDNA cloning and more recently genome sequencing has led to the identification of more than 60 structurally related homologues of SGLT-1 from bacteria to vertebrates (Turk and Wright, 1997). The proteins vary in size from about 400 to about 700 residues and possess twelve to fifteen putative transmembrane domains. They generally share a common core transmembrane topology with thirteen putative transmembrane domains, and extracellular amino- and intracellular carboxy-termini. The vertebrate SGLT-1 related genes constitute a distinct family (SLC5) of the solute carrier (SLC) superfamily, which is currently comprised of 37 different transporter families (Human Gene Nomenclature Database; http://www.gene.ucl.ac.uk/nomenclature/). The SLC5 family includes the high-affinity sodium-glucose cotransporter SGLT-1 (SLC5A1), two low-affinity sodium-glucose cotransporters SGLT-2 (SLC5A2) and SGLT-3 (SLC5A4; originally thought to be a neutral amino acid transporter and called SAAT-1), the sodium/*myo*-inositol transporter SMIT (SLC5A3), the sodium-iodide symporter NIS (SLC5A5), and the sodium-dependent multivitamin transporter SMVT (SLC5A6) (Berger *et al.*, 2000). More recently, the sodium-dependent choline transporter (SLC5A7) was identified as a distant member of the SLC5 family (Okuda *et al.*, 2000). Two cDNAs isolated from rabbit kidney ST-1 (Hitomi and Tsukagoshi, 1994) and RK-D (Pajor, 1994) encode putative SLC5 family members of unknown function.

Despite the fact that SLC5 family members exhibit considerable structural similarities, the transported solutes may range from sugars to inorganic anions and expression of each gene occurs in a characteristic tissue-specific manner. Sodium-dependent glucose cotransporters (SGLT-1, -2, -3) are expressed primarily in kidney and intestine, where they mediate the active transport of glucose (and also galactose, in the case of SGLT-1) across apical membranes of renal and intestinal epithelia (Hediger and Rhoads, 1994; Wright, 2001). The *myo*-inositol transporter SMIT plays an important role in mammalian osmoregulation, allowing cells to maintain cell volume but not at the expense of perturbing cellular ion concentrations (Burg *et al.*, 1997). The transporter is expressed in many tissues including brain, kidney, and placenta, where *myo*-inositol is an important osmolyte, serving to protect cells exposed to hypertonic conditions. The multivitamin transporter SMVT, which is expressed in all tissues tested, mediates the uptake of the vitamins pantothenate and biotin and the essential metabolite lipoate (Prasad and Ganapathy, 2000). The iodide symporter NIS is required for iodide transport across the plasma membrane of thyroid follicular cells, lactating mammary gland, stomach, and salivary glands (Riedel *et al.*, 2001). Collectively, these findings demonstrate that SLC5 family members fulfill diverse physiological functions in many tissue and organs of the vertebrate body.

Compelling genetic evidence further supports the importance of several SLC5 proteins for physiological functions of the intestine, kidney, and thyroid gland. Glucose-galactose malabsorption (OMIN 182380; Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov), an autosomal recessive disorder of the intestine, is caused by missense mutations and complete loss of function in the SGLT-1 gene (Turk *et al.*, 1991). Interestingly, affected patients have only mild renal glucosuria indicating that renal SGLT-1 functions are largely dispensable. SGLT-2 has been implicated in renal glucosuria (OMIN 233100), a physiological defect in renal glucose reabsorption (Kanai *et al.*, 1994). More recently, analysis of HNF1α-deficient mice, an animal model for maturity onset diabetes of the young type 3 (MODY3, OMIN 600496), revealed that the renal glucose reabsorption defects were caused by a selective reduction in the expression of SGLT-2 (Pontoglio *et al.*, 2000). This suggests a central role for SGLT-2 in renal glucose reabsorption, as the expression levels of SGLT-1 and SGLT-3 were unaffected. Normal function of the thyroid gland is critically dependent on NIS function. Patients with mutations in NIS suffer from genetic defect in thyroid hormonogenesis I or congenital iodide transport defect (OMIN 274400), an autosomal recessive condition of the thyroid gland (Dai *et al.*, 1996). The defect is characterized by a sharp decrease in thyroid hormone biosynthesis resulting in hypothyroidism and higher circulating levels of TSH, which in turn cause goiter (Riedel *et al.*, 2001). Finally, SMIT may play a role in the pathogenesis of Down syndrome (Berry *et al.*, 1995). It is therefore conceivable that mutations in other SLC5 genes may underlie human disorders manifesting with altered solute homeostasis.

We have recently reported the cloning of a *Xenopus* cDNA, termed SGLT-1L, which encodes an integral membrane protein with high similarity to mammalian SGLT proteins (Nagata *et al.*, 1999). In the adult, SGLT-1L is abundantly expressed in the small intestine

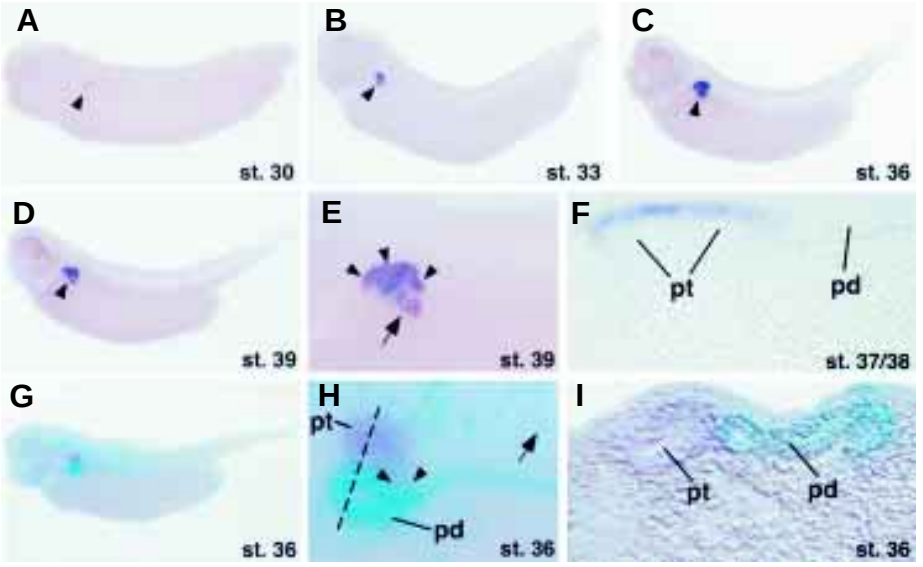

FIG. 2. Expression of *SGLT-1L* is confined to tubules of the pronephric kidney. SGLT-1L *and* CLC-K *transcripts were detected by whole-mount* in situ *hybridization. Lateral views of* Xenopus *embryos with anterior to the left are shown in (A-E, G, H). Transverse sections (F, I) are oriented with dorsal to the left. (A-D) Embryonic* SGLT-1L *expression started at stage 30* **(A)** *and remained exclusively associated with the developing pronephric tubules throughout tadpole stages* **(B-D)**. **(E)** *Enlargement of the pronephric region of the embryo shown in (D) illustrating* SGLT-1L *expression in connecting tubules (arrowheads) and the common tubule (arrow).* **(F)** *Transverse section of stage 37/38 embryo hybridized in whole mount for* SGLT-1L *expression. Embryos were embedded in plastic and 3 μm sections were cut at the level of the pronephros.* SGLT-1L *transcripts were detected in tubular epithelia (pt), but not in the duct (pd).* **(G)** *Double* in situ *hybridization of a stage 36 embryo demonstrating* SGLT-1L *(magenta) and* CLC-K *(blue) expression.* **(H)** *A close-up view of the pronephric region of the embryo shown in (G).* SGLT-1L *expression is present in pronephric tubules (pt), whereas* CLC-K *transcripts are found in the pronephric duct (pd). Arrowheads indicate the junction between the common tubule and the duct. Selected epidermal cells express* CLC-K *(arrow).* **(I)** *Transverse section through the pronephric kidney of a stage 36 embryo hybridized in whole mount for* SGLT-1L *and* CLC-K *expression. The plane of section is indicated by the dotted line in (H).* SGLT-1L *and* CLC-K *transcripts were associated with distinct tubular (pt) and ductal (pd) epithelia, respectively.*

and kidney. SGLT-1L transports preferentially *myo*-inositol and glucose, while galactose is a poor substrate. *Xenopus* SGLT-1L therefore shares properties with both mammalian SGLT proteins and SMIT. In the present study, we established the phylogenetic relationship between *Xenopus* SGLT-1L and the mammalian SLC5 gene family members, determined the expression of SGLT-1L during *Xenopus* embryogenesis, and investigated the regulation of SGLT-1L expression in explant cultures. We found that *Xenopus* SGLT-1L encodes a ST-1 related protein that is expressed exclusively in tubules of the pronephric kidney undergoing terminal differentiation.

Results

Xenopus *SGLT-1L Encodes a Novel Member of the SLC5 Family and is Closely Related to Mammalian ST-1 Proteins*

The evolutionary relationship of *Xenopus* SGLT-1L to the other members of the vertebrate SLC5 family has remained unresolved to date. We therefore performed distance and phylogenetic analyses using human, rabbit and rat sequences encoding various proteins of the SLC5 family. As full-length sequences were not available for all proteins present in the GenBank database, the sequence alignments were performed with amino acid sequences homologous to transmembrane segments 2 to 6 of human SGLT-1 (see Material and Methods) and phylogenetic trees were constructed. *Xenopus* SGLT-1L shared highest amino acid homology with mammalian ST-1 proteins. The degree of similarity between *Xenopus* SGLT-1L and the mammalian ST-1 proteins, as judged by the percentage of amino acid identities was: 80% for both human and rat ST-1, and 78% for rabbit ST-1. In contrast, *Xenopus* SGLT-1L shared only 68% amino acid identity with human SGLT-1. A comparison of the full-length amino acid sequences revealed identities of 66% between *Xenopus* SGLT-1L and rabbit ST-1, but only 49.1% between *Xenopus* SGLT-1L and human SGLT-1. This indicates that *Xenopus* SGLT-1L is most closely related to members of the ST-1 gene family.

A phylogenetic tree constructed from amino acid sequence alignments is shown in Fig. 1. The vertebrate orthologues of SGLT-1, SGLT-2, SMIT, SMVT, ST-1, and NIS form distinct clusters, which is in agreement with Turk and Wright (1997). *Xenopus* SGLT-1L segregates unequivocally with human, rabbit, and rat ST-1. The branching order of the ST-1 sequences is however partly inverted with human ST-1 basal to rat and rabbit ST-1. The reasons for this inversion are unknown, but it could indicate the existence of more than one human ST-1 gene. It should be pointed out that currently only partial sequences are available for human and rat ST-1. A more accurate phylogeny of the ST-1 genes has therefore to await completion of the full-length sequences of these genes. Nonetheless, the present data indicate that *Xenopus* SGLT-1L is most closely related to the mammalian ST-1 genes and thus encodes a novel member of the SLC5 family.

Embryonic Expression of SGLT-1L is Confined to Tubules of the Pronephric Kidney

Temporal and spatial patterns of SGLT-1L expression were visualized in embryos at gastrula, neurula, tailbud, and tadpole stages by whole mount *in situ* hybridization. A control SGLT-1L sense probe did not detect any signal throughout the embryonic stages tested (data not shown). Similarly, no expression could be observed with antisense SGLT-1L probes in gastrula and neurula embryos (data not shown). Transcripts of SGLT-1L were first detectable at late tailbud stages (st. 29/30) as a highly localized staining ventrolaterally to the somites at the posterior end of the hindbrain (Fig. 2A). The position suggested that SGLT-1L expression occurs in an area of the developing pronephric kidney, where cells are undergoing mesenchyme-to-epithelium transition to form pronephric tubules (Nieuwkoop and Faber, 1956). Analysis of tadpole embryos demonstrated that SGLT-1L transcripts were indeed associated with pronephric tubules (Fig. 2 B-D). Up to stage 40, the last embryonic stage analyzed in this study, all other tissues and organs were devoid of SGLT-1L expression. Remarkably, pronephric expression of

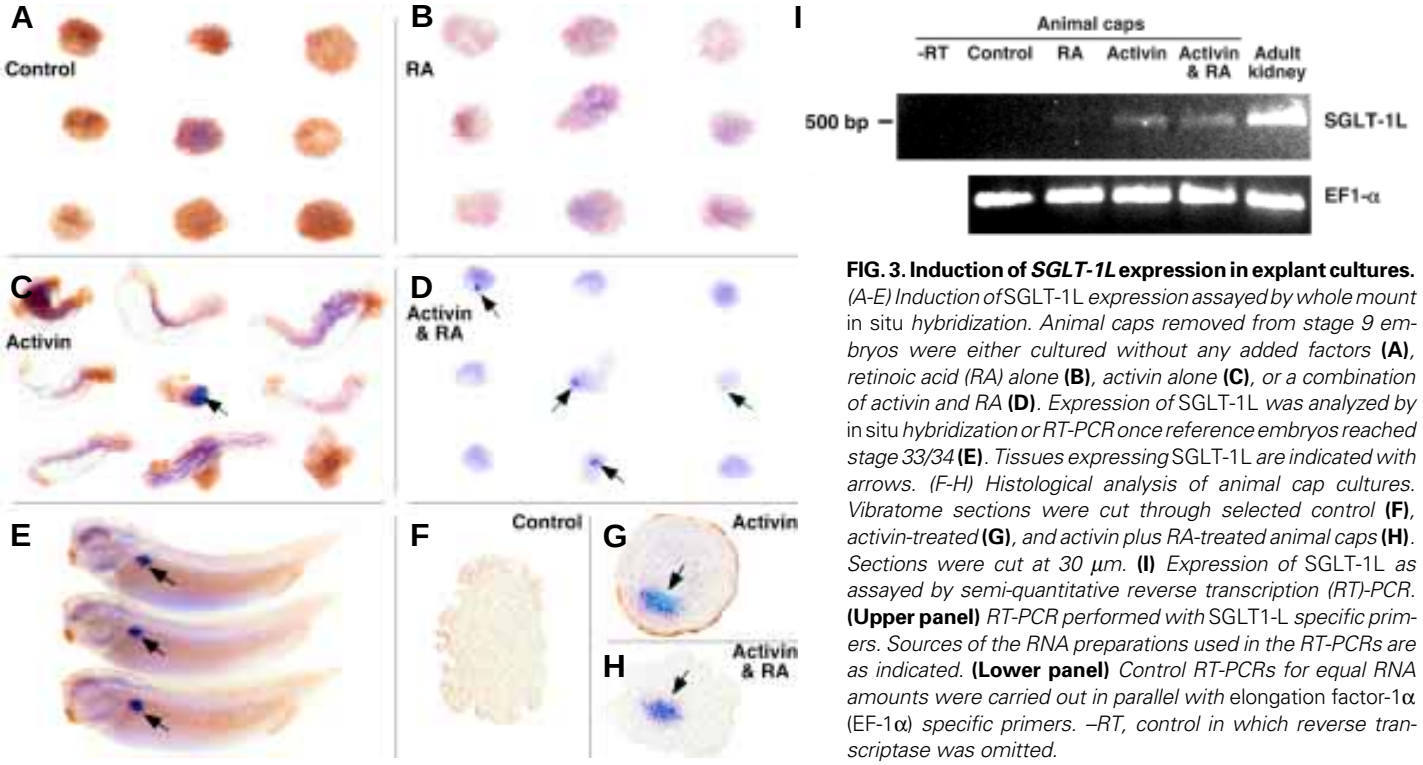

FIG. 3. Induction of *SGLT-1L* expression in explant cultures. *(A-E) Induction of* SGLT-1L *expression assayed by whole mount* in situ *hybridization. Animal caps removed from stage 9 embryos were either cultured without any added factors* **(A)**, *retinoic acid (RA) alone* **(B)**, *activin alone* **(C)**, *or a combination of activin and RA* **(D)**. *Expression of* SGLT-1L *was analyzed by* in situ *hybridization or RT-PCR once reference embryos reached stage 33/34* **(E)**. *Tissues expressing* SGLT-1L *are indicated with arrows. (F-H) Histological analysis of animal cap cultures. Vibratome sections were cut through selected control* **(F)**, *activin-treated* **(G)**, *and activin plus RA-treated animal caps* **(H)**. *Sections were cut at 30 μm.* **(I)** *Expression of* SGLT-1L *as assayed by semi-quantitative reverse transcription (RT)-PCR.* **(Upper panel)** *RT-PCR performed with* SGLT1-L *specific primers. Sources of the RNA preparations used in the RT-PCRs are as indicated.* **(Lower panel)** *Control RT-PCRs for equal RNA amounts were carried out in parallel with* elongation factor-1α (EF-1α) *specific primers. –RT, control in which reverse transcriptase was omitted.*

SGLT-1L was confined to the developing tubules. A detailed inspection of stage 39 pronephroi showed that expression occurred at similar levels in the tubular segments including connecting tubules and the common tubule (Fig. 2E). Transverse sections of st. 37/38 embryos revealed that expression of SGLT-1L was associated with epithelial cells of the pronephric tubules, but not of the duct (Fig. 2F).

We next compared the spatial distribution of SGLT-1L transcripts in the pronephric kidney with the expression of the *Xenopus* CLC-K gene, which encodes a chloride channel of the CLC family (Maulet *et al.*, 1999). Embryonic expression of CLC-K is specific for a subpopulation of epidermal cells and, more importantly, for epithelia of the pronephric duct (S. Eid, Y. Maulet, and A. Brändli, manuscript in preparation). Double *in situ* hybridization experiments demonstrated that SGLT-1L and CLC-K transcripts are indeed associated with separate populations of pronephric epithelia (Fig. 2 G,H). There was no evidence for coexpression of SGLT-1L and CLC-K at the junction between pronephric tubules and duct. Similarly, transversal sections failed to reveal evidence for an overlap in the expression patterns of SGLT-1L and CLC-K (Fig. 2I). In summary, SGLT-1L expression in the early *Xenopus* embryo is highly tissue-specific and confined to epithelial cells of developing pronephric tubules.

Growth Factor-Dependent Induction of SGLT-1L *Transcription in Explant Cultures*

Treatment of animal cap ectoderm with a combination of the growth factor activin A and retinoic acid induces with high frequency the formation of pronephric tubules (Moriya *et al.*, 1993; Uochi and Asashima, 1996). We extended these observations by assaying for SGLT-1L expression in animal caps explanted at late blastula stages and incubated in buffer alone or in the presence of added factors (10 ng/ml activin and/or 100 μM retinoic acid). The animal caps were cultured until control embryos reached stage 33/34 and were then analyzed by reverse transcription-polymerase chain reaction (RT-PCR) for the presence of SGLT-1L transcripts (Fig. 3I). Control cultures and those treated with retinoic acid alone were negative for SGLT-1L expression. On the other hand, strong expression was detected with RNA purified from adult kidneys. Furthermore, analysis of cultures treated with activin or combinations of activin with retinoic acid clearly showed induction of SGLT-1L expression.

Animal cap cultures were also analyzed by whole mount *in situ* hybridization for the presence of tissues expressing SGLT-1L transcripts (Fig. 3 A-H). Consistent with the RT-PCR analysis, no expression of SGLT-1L was found with control cultures or in those treated with retinoic acid alone (Fig. 3 A,B,F). Treatment of animal caps with activin alone resulted in the characteristic extended explants indicating the induction of dorsal mesoderm, but only 1 out of 21 animal caps analyzed revealed localized expression of SGLT-1L (Fig. 3C). Activin in combination with retinoic acid was however more effective in that 14% (n=36) of the explants were positive for SGLT-1L transcripts (Fig. 3D). Explants usually had a single patch of tissue expressing SGLT-1L. Sections of animal caps revealed that SGLT-1L expressing cells were arranged in clusters deep in the explants (Fig. 3 G,H). Taken together, these findings indicate that a significant fraction of pronephric tubules induced in animal caps treated with a combination of activin and retinoic acid matures to express SGLT-1L.

Discussion

Xenopus *SGLT-1L is a Novel ST-1 Related Member of the SLC5 Family*

Based on amino acid sequences comparisons, *Xenopus* SGLT-1L was originally thought to encode a SGLT-1 related protein (Nagata *et al.*, 1999). To date, the SLC5 gene family consists of

seven distinct human genes. Furthermore, the GenBank database contains several entries of mammalian nucleotide sequences encoding proteins with high homology to SLC5 genes, but unknown function (Wright, 2001). We therefore decided to reinvestigate the phylogenetic relationship of *Xenopus* SGLT-1L. Amino acid sequence comparisons and phylogenetic analysis indicated that *Xenopus* SGLT-1L unequivocally segregates with mammalian cDNAs encoding ST-1 homologues. These include a full-length rabbit cDNA from kidney (Hitomi and Tsukagoshi, 1994), and partial cDNAs from rat and human brain (Poppe *et al.*, 1997). The deduced amino acid sequences of *Xenopus* SGLT-1L (673 aa) and rabbit ST-1 (674 aa) differ in length by only one residue and they share an overall amino acid identity of 66%. The phylogenetic analysis could however not conclusively resolve whether *Xenopus* SGLT-1L is an orthologue or a paralogue of rabbit ST-1. *Xenopus* SGLT-1L and rabbit ST-1 differ markedly with respect to their expression in adult tissues as determined by Northern blot analysis. Heart, lung, and liver are devoid of any expression of these genes. Rabbit ST-1 transcripts are specifically present in the kidney and brain, but absent from the intestine (Hitomi and Tsukagoshi, 1994). By contrast, *Xenopus* SGLT-1L expression is detected in the kidney and intestine, but not brain (Nagata *et al.*, 1999). The differences in tissue distribution would favor the hypothesis that *Xenopus* SGLT-1L is a paralogue of rabbit ST-1. Unfortunately, the transport activities or any additional properties of rabbit ST-1 are presently unknown, which precludes further comparisons. The imminent completion of the human and mouse genome projects should however provide conclusive answers to the question about the number of the ST-1 related genes in mammals. For now, we have therefore decided against renaming the *Xenopus* SGLT-1L gene.

Expression of SLC5 *genes During Embryogenesis and Kidney Organogenesis - Functional Implications*

Analysis of the embryonic expression patterns of genes encoding solute transporters may reveal important information about morphogenesis and terminal differentiation of epithelia in the organs of the vertebrate body. Furthermore, it provides a basis to uncover the regulatory relationship between embryonic transcription factors and transporters. At present, information on the embryonic expression of SLC5 genes in the developing embryo is only available for SMIT, SGLT-1, and SGLT-2. As revealed by *in situ* hybridization on rat embryo sections, high expression of rat SMIT is associated with the embryonic brain and spinal cord (Guo *et al.*, 1997). Metanephric expression of SMIT begins at E16 in the nephrogenic zone and becomes gradually more intense in the renal medulla. Renal expression of rat SGLT-1 and SGLT-2 occurs from E18 and E17 onwards, respectively, as demonstrated by Northern blot analysis (You *et al.*, 1995). In the mouse, SGLT-1 transcripts and protein as well as glucose transport activity are detected as early as E16 in tubular segments of the renal cortex and outer medulla (Yang *et al.*, 2000). These findings indicate that expression of SLC5 genes is associated with late phases of metanephric kidney development, when tubules begin to mature and acquire reabsorptive transport properties (Horster, 2000).

Whether mammalian SLC5 genes are expressed earlier during pronephric and mesonephric kidney development is currently unknown. Our analysis of embryonic *Xenopus* SGLT-1L revealed exclusive expression in the developing pronephric kidney. In contrast to the adult, no intestinal SGLT-1L expression could be detected in embryos up to stage 40. This is consistent with histological data indicating that substantial differentiation of intestinal epithelia does not occur before stage 45/46 (Chalmers and Slack, 1998). As observed with other SLC5 gene family members in the developing metanephros, the onset of SGLT-1L expression in the pronephric kidney was a late event associated with terminal differentiation of the organ. Interestingly, pronephric SGLT-1L was first detectable from stage 29/30 onwards, which is seven hours after the onset of pronephric Na,K-ATPase expression at stage 26 (Eid and Brändli, 2001). This suggests that the sodium gradients generated by the activity of Na,K-ATPase, which provide the driving force for sodium-dependent solute transport, are probably present from the onset of SGLT-1L expression.

A second striking observation was the finding that, as the pronephric kidney becomes functional at stage 37/38, SGLT-1L expression was confined to tubules and absent from the excretory duct. Physiological functions of SGLT-1L are therefore associated with the early segments of the pronephric nephron. As shown by Northern blot analysis rabbit ST-1 is prominently expressed in the adult kidney (Hitomi and Tsukagoshi, 1994), but the distribution of ST-1 transcripts along the nephrons of the metanephric kidney is unknown. More detailed information on the renal distribution is however available for several other members of the SLC5 gene family. The NIS protein is most prominently found in the distal tubular system, but also present at lower levels in proximal tubules, and is thought to mediate both transepithelial as well as intracellular iodide transport in the kidney (Spitzweg *et al.*, 2001). SMIT is most abundantly expressed in the medullary thick ascending limbs of Henle's loop and papilla segments, which highlights the important role of this transporter in providing osmoprotection to renal epithelia from the adverse effects of hypertonicity generated during the urinary concentration process (Yamauchi *et al.*, 1995; Wiese *et al.*, 1996; Guo *et al.*, 1997). By contrast, sodium-dependent glucose transporters are expressed in the renal cortex and outer medulla, where they are confined to distinct segments of the proximal tubules. The low affinity/high capacity sodium-dependent glucose transporter SGLT-2, localized in the S1 segment of the early proximal convoluted tubule, is responsible for reabsorption of the bulk of glucose (Kanai *et al.*, 1994; You *et al.*, 1995). The remainder of filtered glucose is captured by SGLT-1, a high-affinity/low capacity glucose transporter expressed in the S3 segment of the late proximal tubule (Lee *et al.*, 1994; You *et al.*, 1995). Although the renal distribution of SGLT-3, the second low affinity glucose transporter (Mackenzie *et al.*, 1994), has not yet been determined, it appears that glucose reabsorption from the urinary filtrate is achieved by the action of several types of SGLTs arranged in series along the proximal tubule.

The physiological roles of *Xenopus* SGLT-1L and the structurally related ST-1 transporters are currently unknown. SGLT-1L prefers *myo*-inositol to glucose as a substrate and thus resembles the transport properties of SMIT (Nagata *et al.*, 1999). On the other hand, a comparison of the renal expression patterns indicates that *Xenopus* SGLT-1L shares expression in early segments of the nephron with mammalian SGLTs. *Xenopus* SGLT-1L may therefore play a role in solute reabsorption from the urinary filtrate rather than in osmoregulation. The expression of *Xenopus* SGLT-1L does however not appear to be restricted to a specific tubular segment. Further studies will be needed to elucidate the physiological functions of both *Xenopus* SGLT-1L and the mammalian ST-1 genes. In particular, the issue of whether these proteins have the ability to transport other solutes than *myo*-inositol and glucose remains to be resolved.

In Vitro Induction of Pronephric Tubules in Animal Cap Explants

The generic vertebrate nephron consists of three principle components: the corpuscle, the tubule, and the collecting duct. All three embryonic kidneys, including its simplest form the pronephros, share this basic organization (Brändli, 1999). The dissection of the molecular events underlying the formation of these structures is the subject of intense experimental investigation. Studies by Asashima and colleagues have demonstrated that the formation of pronephric tubules can be recapitulated *in vitro*. Pronephric tubules are rarely detected by histology in animal caps treated with activin alone, but treatment of animal caps by combination of activin and retinoic acid results in efficient induction of tubules (Moriya *et al.*, 1993; Uochi and Asashima, 1996). The induction of tubules is accompanied by the expression of the molecular marker LIM-1. We have previously confirmed and extended these findings by demonstrating the specific induction of other early pronephric markers, such as Pax-2 and Pax-8, in explant cultures (Heller and Brändli, 1997; Heller and Brändli, 1999). Unlike SGLT-1L, none of these markers are however specific to pronephric tubules at the embryonic stages tested. In the present study, we showed that SGLT-1L could be induced in animal cap cultures by activin plus retinoic acid indicating that the explants differentiate sufficiently enough to express markers of tubules undergoing terminal differentiation. RT-PCR analysis clearly confirmed the presence of SGLT-1L transcripts in activin plus retinoic acid treated explants, but surprisingly also in activin alone treated cultures. Inspection of individual activin-treated explants hybridized for SGLT-1L indicated however that only a small fraction of the explants expresses the marker. The presence of retinoic acid is therefore essential for the induction of SGLT-1L expression in explant cultures.

Conclusions

In the present study, we extended the characterization of *Xenopus* SGLT-1L by investigating its phylogenic relationship within the SLC5 family and determining its expression during embryogenesis and in explant cultures. We found that SGLT-1L shares highest amino acid identity with the orphan sodium-dependent cotransporter ST-1. *In situ* hybridization identified SGLT-1L as a highly specific molecular marker for maturing tubular epithelia of the pronephric kidney. This suggests a role for SGLT-1L in the reabsorption of solutes from the urinary filtrate and underscores at the molecular level that the nephrons of the pronephric kidney are organized into functionally distinct compartments. In a broader sense, our findings demonstrate that the localization of sodium-dependent solute transport activities in early nephron segments has been evolutionary conserved between pronephric and metanephric kidneys.

Materials and Methods

Embryos and Animal Cap Cultures

Xenopus embryos were obtained by *in vitro* fertilizations and staged as described previously (Nieuwkoop and Faber, 1956; Brändli and Kirschner, 1995). Dissection and culture of animal caps were performed according to (Heller and Brändli, 1997). Human recombinant activin A (gift of Danny Huylebroeck) was used at a final concentration of 10 ng/ml. All-*trans* retinoic acid (Sigma, R-2625) was dissolved in ethanol to generate a 0.1 M stock solution and diluted to final concentration of 100 μM (Uochi and Asashima, 1996). Animal caps were cultured in 0.5x MMR/0.1% BSA at 20°C in 24-well dishes until reference embryos reached stage 33/34. Animal caps were either frozen in liquid nitrogen for later isolation of RNA or fixed for whole mount *in situ* hybridization.

In Situ *Hybridization*

Probe synthesis and purification was performed as described previously (Helbling *et al.*, 1998). Following plasmids were used: pXSGLT1L (clone 1802) containing the entire open reading frame (ORF) of *Xenopus* SGLT-1L (Nagata *et al.*, 1999) cloned into the pBluescript II SK(-) vector (Stratagene); and pX6-XCLCK with the ORF of *Xenopus* CLC-K (Maulet *et al.*, 1999) in pBluescript SK(-) (Stratagene). Whole-mount *in situ* hybridizations were performed according to (Harland, 1991) with the following modifications. Ethanol replaced methanol. The RNAse digestion step was omitted. CHAPS was left out of all buffers. Maleic acid buffer (MAB; 100 mM maleic acid, 150 mM NaCl, pH 7.5) with 2% blocking reagent (Roche Diagnostics) and 20% sheep serum (Life Technologies) were used in the blocking and antibody incubation steps. Double *in situ* hybridizations were performed with digoxigenin-labeled CLC-K and fluorescein-labeled SGLT-1L antisense probes. After hybridization, the digoxigenin-labeled probes were detected with an anti-digoxigenin antibody coupled to alkaline phosphatase (Roche Diagnostics). The chromogenic reaction was carried out using 3.5 μl of BCIP (50 mg/ml in DMF; Roche Diagnostics) in 1 ml TKML Buffer (100 mM Tris, pH 8.2; 150 mM KCl; 10 mM $MgCl_2$). Alkaline phosphatase activity was inactivated in MAB containing 10 mM EDTA at 65°C for 10 minutes, followed by dehydration in ethanol for 5 minutes. Subsequently, fluorescein-labeled probes were detected with an alkaline phosphatase-coupled anti-fluorescein antibody (Roche Diagnostics). Chromogenic reactions were performed using 3.5 μl of Magenta phosphate (25 mg/ml in DMF; B-5667; Sigma) in 1 ml TKML buffer. Where necessary, embryos and animal caps were bleached with 0.75% hydrogen peroxide-5% formamide-0.5x SSC. Sense probes were prepared and tested negative by *in situ* hybridizations.

Histological Analyses

For plastic sections, embryos were embedded in Durcupan (Fluka) and sectioned with a glass knife at 3 μm. Individual sections were then transferred to drops of water on microscope slides and allowed to dry. For vibratome sections, the specimens were embedded in 4% agarose (NuSieve 3:1, FMC) and sections (30 μm) were cut with a vibrating blade microtome (Leica VT1000S). Sections were transferred to microscope slides, coverslips were mounted with 85% glycerol, and sealed with nail varnish.

Photography and Computer Graphics

Photographs of embryos and animal caps were taken digitally with a Zeiss SV11 stereoscopic microscope equipped with a Zeiss AxioCam Color camera (Carl Zeiss AG). DIC Normarski images of sections were acquired digitally with a Zeiss Axioskop 2 MOT microscope equipped with an AxioCam Colour camera. Composite figures were organized and labeled using Adobe Photoshop 5.0.2 and Canvas 6.0 software.

RT-PCR Analysis

Total RNA was extracted from animal cap explants and adult *Xenopus* kidneys with TRIzol reagent (Life Technologies) according to the manufacturer's instructions. RNA samples were precipitated with ethanol at room temperature and resuspended in water. Single-stranded cDNAs were synthesized from 5 μg of total RNA in the presence of Superscript II RNase H^- reverse transcriptase (Life Technologies) using antisense primers EF1α-2 and SGLT1L-2 (5'-CCA GGC AAT ACC ATC ATA AAG AGG-3'). Control syntheses were also carried out in the absence of reverse transcriptase. The sense primers EF1α-1 and SGLT1L-1 (5'-GGG CGC TCT TCA TCC AAC A-3') and the antisense primers EF1α-2 and SGLT1L-2, respectively, were used to analyze the cDNAs by PCR. Primers EF1α-1 and EF1α-2 were described previously (Heller and Brändli, 1997). The PCR conditions were as follows: 95°C for 30 seconds (denaturation step), 55°C for 45 seconds (annealing step), and 72°C for

45 seconds (extension step). 40 cycles were carried out, and the extension cycle was at 72°C for 7 minutes. The amplification products were analyzed on a 1.5% agarose gel and visualized by ethidium bromide staining.

Phylogenetic Analyses

Amino acid sequences homologous to transmembrane segments 2 to 6 of human SGLT-1 (Turk and Wright, 1997) were used for the sequence alignments. All sequences started with the conserved SL peptide motif, terminated with a conserved G residue, and ranged between 119-121 residues in length. Sequence alignments were performed with the MegAlign Program (DNASTAR) using the Clustal method and a PAM250 residue weight table. The aligned sequences were used to construct a phylogenetic tree with the Neighbor-Joining algorithm (Saitou and Nei, 1987). The GenBank accession numbers of the sequences used in the alignment are: human NIS, U66088; human RKD, AL109659; human SGLT-1, M24847; human SGLT-2, M95549; human SGLT-3, AJ133127; human SMIT, L38500; human SMVT, AF069307; human ST-1, U41898; rabbit SGLT-1, X06419; rabbit SGLT-2, M84020; rabbit RKD, U08813; rabbit SMVT, AF080067; rabbit ST-1, D16226; rat NIS, U60282; rat SGLT-1, U03120; rat SGLT-2, U29881; rat SMIT, AJ001290; rat SMVT, AF026554; rat ST-1, U47673; and *Xenopus* SGLT-1L, AB008225.

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