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# Renal osmotic stress-induced cotransporter: Expression in the newborn, adult and post-ischemic rat kidney

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**Renal osmotic stress-induced cotransporter: Expression in the newborn, adult and post-ischemic rat kidney.** The renal osmotic stress-induced cotransporter (ROSIT), a new putative member of a family of organic solute transporters, is highly expressed in the kidney. Our *in situ* hybridization data now reveal that large amounts of ROSIT mRNA can be found in the S3 segment of the proximal tubule. In the developing kidney, ROSIT mRNA is expressed after the S-shaped body stage. Because the S3 segment is the major site of damage in the post-ischemic kidney, we evaluated alterations in ROSIT mRNA expression after ischemic acute tubular necrosis. Renal osmotic stress-induced cotransporter mRNA levels were already decreased eight hours post-ischemia. At seven days post-ischemia, ROSIT mRNA reappeared in a mosaic pattern in the regenerating S3 segment, being fully expressed three weeks after the insult except for focal areas. The exact localization of this putative osmolyte transporter in the kidney, together with that of other known osmolyte transporter will contribute to a better understanding of the mechanism of medullary osmolyte accumulation and its vectorial transport.

Accumulation of organic osmolytes is a critical component of the adaptive response to volume changes and hypertonicity in renal tubular cells [recently reviewed in 1, 2]. These processes depend critically on specific transport proteins, several members of which have already been identified and cloned [3–6]. The osmolyte transporters belong to a larger family of transporters that additionally includes transporters for amino acids and several neurotransmitters. One important feature of at least some of these transporters is their regulation by changes in extracellular tonicity (such as [7, 8]). *In vitro* experiments in MDCK cells have shown that extracellular hypertonicity leads to an increased expression of osmolyte transporter mRNAs or increased osmolyte transport activity in these cells [5, 9–12]. These findings could be extended by demonstrating that increased osmolyte transport *in vivo* also is accompanied by the upregulation of the mRNAs coding for the corresponding transporters [13–15].

Recently, Wasserman and colleagues identified a new putative member of the cotransporter family [16]. The corresponding mRNA is highly expressed in the normal adult kidney and is structurally related to recently cloned cDNAs encoding neurotransmitter transporters in the brain [17, 18]. Because the expression of this new renal transporter is up-regulated in hypernatriemic rats, it was termed renal osmotic stress-induced cotransporter (ROSIT). The original report describing the location of ROSIT mRNA expression made use of Northern hybridization, so that the exact site of ROSIT expression in the kidney could not be precisely demonstrated [16].

The nephron-specific localization as well as the stage-dependent onset of ROSIT mRNA expression during nephrogenesis is of particular interest because of the expression of other osmolyte transporters expressed in the different nephron segments. In this report we describe the expression of ROSIT mRNA in the normal adult and developing rat kidney. In addition, we studied ROSIT mRNA expression by *in situ* hybridization in the post-ischemic kidney, a situation characterized by both cellular regeneration and profound volume and electrolyte changes [19]. The expression pattern of ROSIT mRNA after acute tubular necrosis contributes to a better understanding of both the pathophysiological events in the kidney and to the regulation of ROSIT itself.

## METHODS

### Animals

Male adult Sprague-Dawley (SD) rats (70 to 100 days old) as well as female, pregnant SD rats (for obtaining newborn animals) were kept under standard laboratory conditions in the Animal Care Facility in Mannheim, Germany. Animals had free access to rat chow, containing 19% protein, and tap water.

### Induction of ischemic acute renal failure

Rats were anesthetized by the intramuscular injection of ketamin (75 mg/kg) and xylazin (6 mg/kg). After a midline incision, the left and right renal pedicles were carefully located. Following an injection of 100 IU heparin (dissolved in 1 ml 0.9% NaCl) into the tail vein, both renal arteries were occluded with a microaneurysm clamp for a period of 45 minutes. In order to prevent fluid loss by evaporation, 1 ml of 0.9% NaCl was administered to the peritoneal cavity and the operation field was covered with humidified, sterile gauze tampons during the ischemic period. Sham-operated rats were treated similarly to the ischemic animals except for the fact that the renal pedicles were not clamped but only briefly manipulated (touching of both renal hila). After removal of both clamps, abdominal incisions were sutured up. The animals were subsequently subjected to perfusion-fixation after

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definite periods of reperfusion (3 and 8 hr as well as 1, 2, 7, 10, 14 and 21 days post-ischemia). Blood samples were obtained from the animals three days before as well as 24 hours post-ischemia, and at the end of the different reperfusion periods in order to assess the transient rise of plasma creatinine and urea levels in post-ischemic animals. Twenty-four hours post-ischemia the serum urea rose to 133.8 ± 60.06 mg/dl and serum creatinine to 1.48 ± 0.68 mg/dl, whereas in the sham-operated animals the values were 35.2 ± 2.64 mg/dl for serum urea and 0.26 ± 0.02 mg/dl for serum creatinine 24 hours after the sham operation (mean ± SE). In the post-ischemic animals, serum urea and serum creatinine levels returned to baseline seven days after the ischemic insult.

**Perfusion-fixation**

For optimal preservation of tissue morphology and tissue RNA content, adult animals were perfused retrogradely through the distal abdominal aorta. Perfusion was conducted at a pressure level of 210 to 220 mm Hg with 2% freshly dissolved paraformaldehyde (PFA) in PBS, pH 7.4, for three minutes and subsequently with a 18% sucrose solution in PBS, adjusted to 800 mOsm/kg, for another three minutes at the same pressure level. After removal, kidneys were cut into slices, mounted qickly onto small pieces of styrofoam and then snap-frozen in liquid nitrogen-cooled isopentane.

Newborn (Po)-animals were perfused anterogradely through the left ventricle for two minutes with 2% PFA in PBS at a pressure level of 180 to 200 mm Hg. Thereafter the kidneys were carefully removed and soaked for two hours in 18% sucrose-PBS solution at room temperature before being snap-frozen as described above. All tissues were stored at −80°C until further use.

**Molecular cloning and preparation of riboprobes**

To prepare a specific cDNA encoding ROSIT, total rat kidney RNA was reverse transcribed and amplified by PCR using specific 5'- and 3'-primers. The following oligonucleotides were used: 5'-CAT CTG TCC TCA TGT ACC TG-3' (sense primer, nucleotides 1067 to 1086 according to [16]) and 5'-TGG TCT CAG TCG TAG ACA GA-3' (antisense primer, nucleotides 1945-1964). The obtained PCR fragment with the expected length of 898 bp was then subcloned into the pCRII vector (Invitrogen, NV Leek, The Netherlands). The resulting clone was sequenced from both ends to confirm the identity of the PCR product with the previously published sequence.

To generate sense and antisense RNA probes for *in situ* hybridization experiments, the recombinant plasmid was restricted appropriately. *In vitro* transcription was carried out according to the protocol supplied by the manufacturer (Boehringer Mannheim, Mannheim, Germany). The lengths of the digoxigenin-labeled riboprobes were checked on a denaturing formaldehyde agarose gel stained with ethidium bromide. Transcripts were finally subjected to partial alkaline hydrolysis to obtain fragments of a calculated average length of 250 nucleotides.

***In situ* hybridization**

*In situ* hybridization was essentially carried out as described previously [20]. Cryostat sections (5 to 7 μm thick) were transferrred onto silane-coated glass slides. Sections were postfixed in 4% paraformaldehyde (in PBS pH 7.4) for 20 minutes, rinsed three times in PBS and washed in DEPC-treated, bidestilled water for 10 minutes. In order to improve permeabilization, a mild deproteinization step was performed by immersing slides in 0.1 M HCl for 10 minutes, followed by two short rinses (5 min each) in PBS. To reduce background, slides were acetylated for 20 minutes in 0.1 M triethanolamine pH 8.0, containing 0.25% acetic anhydride, added immediately before starting this step. After rinsing in PBS, slides were dehydrated for five minutes each in 70%, 80%, and 95% ethanol and air-dried for 20 minutes. A prehybridization step was carried out by incubating sections with prehybridization solution (50% deionized formamide, 50 mM Tris HCl pH 7.6, 25 mM EDTA pH 8.0, 20 mM NaCl, 0.25 mg/ml tRNA from yeast, 2.5× Denhardt's solution). Sections were placed in a moist chamber and incubated at 42°C for two hours. Meanwhile, hydrolyzed riboprobes were diluted in deionized formamide, boiled for two minutes, and chilled on ice. Subsequently the individual components of the hybridzdiation mixture were added and mixed vigorously (final concentrations were: 50% deionized formamide, 20 mM Tris HCl pH 7.6, 1 mM EDTA pH 8.0, 0.33 M NaCl, 0.2 M DTT, 0.5 mg/ml tRNA, 0.1 mg/ml sonicated, denatured DNA from fish sperm, 1× Denhardt's solution, 10% dextran sulfate). Concentration of labeled sense or antisense probe was 5 to 10 ng per μl hybridization mixture. After removal of prehybridization solution, 25 μl of hybridization mixture were applied to each section and carefully covered with a siliconized coverslip. Hybridization was performed at 42°C in a moist chamber for 16 hours. Washing procedures included a first washing step in 2 × SSC (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) at room temperature for 20 minutes, followed by three stringent washes at 49°C (1 hr each) in 1 × SSC, 0.5 × SSC and 0.1 × SSC, each containing 50% formamide. Slides were then rinsed at room temperature in 0.5 × SSC for 15 minutes, again rinsed in 0.2 × SSC for 10 minutes and equilibrated twice for five minutes in buffer I (100 mM Tris HCl, 150 mM NaCl, pH 7.4).

Sections were now covered with blocking medium (buffer I containing 1% blocking reagent (Boehringer Mannheim, Mannheim, Germany) and 0.5% bovine serum albumin (BSA), and incubated for 30 minutes at room temperature in a moist chamber. Blocking solution was then drained from the slides and a polyclonal alkaline phosphatase-coupled sheep anti-digoxigenin antibody (diluted 1:500 in blocking medium) was applied to the sections. After an incubation of two hours at room temperature the sections were incubated overnight at 4°C. The next morning sections were washed twice for 15 minutes in buffer I and equilibrated for two minutes in buffer II (100 mM Tris-HCl pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$). For signal development a substrate solution [buffer II containing 0.417 mM nitro blue tetrazolium chloride, NBT (predissolved in 70% dimethylformamide); 0.406 mM 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt; BCIP or X-phosphate (predissolved in 100% dimethylformamide) and 1 mM levamisol (to inhibit remaining endogenous alkaline phosphatase activity)] was freshly prepared. After covering sections with large amounts of substrate solution, slides were kept at 4°C in a moist chamber in the dark. Color reaction was controlled under the microscope and terminated (usually after 12 to 24 hr) by immersing the slides twice for five minutes in buffer III (100 mM Tris-HCl, 1 mM EDTA, pH 8.0). After rinsing in PBS, sections were mounted in bicarbonate-buffered glycerol pH 8.6.

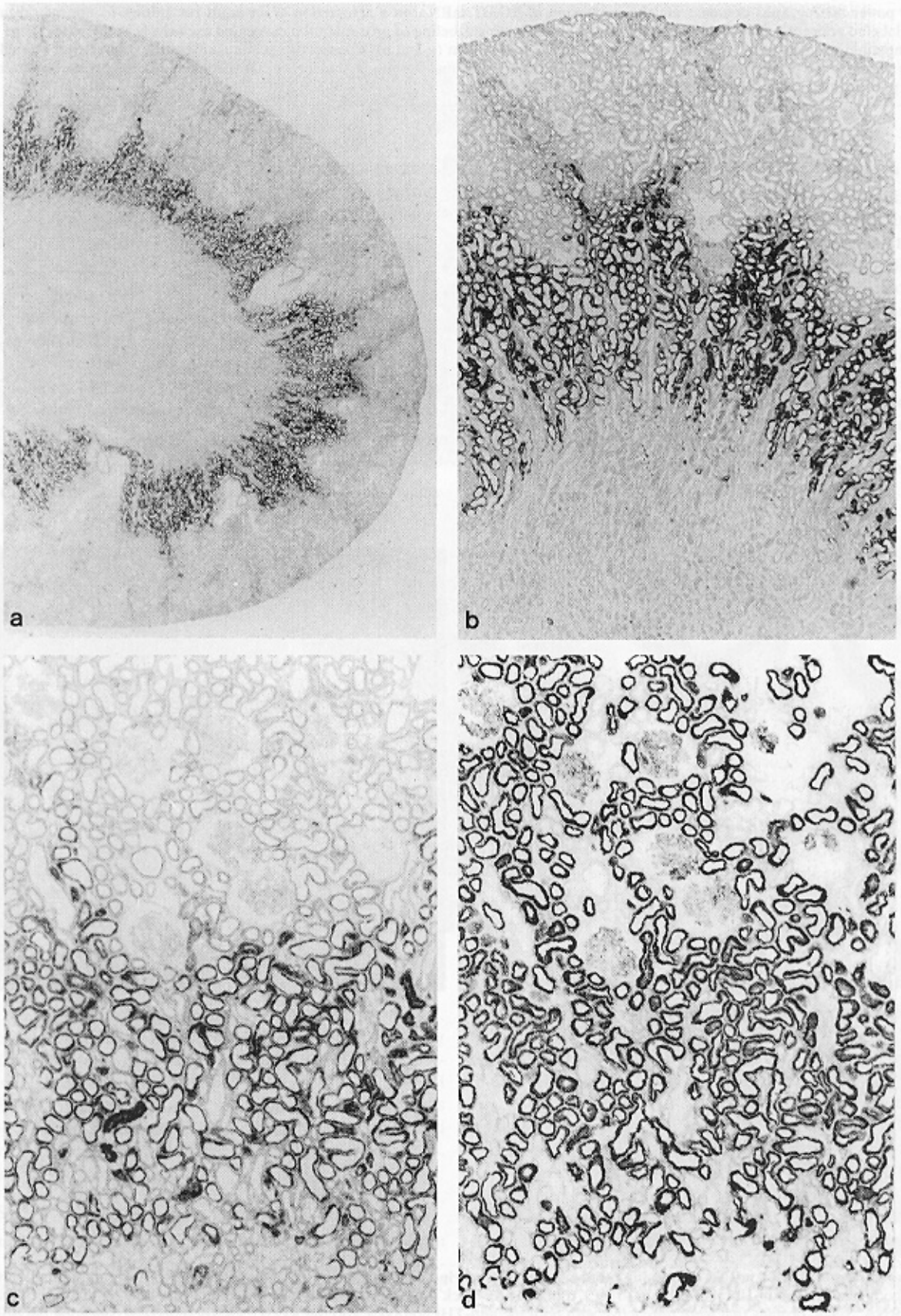
a
b
c
d

**Fig. 1. Low power micrographs demonstrate the expression of ROSIT mRNA on a cryosection of an adult rat kidney.** *In situ* hybridization using a digoxigenin labeled antisense riboprobe yields strong and homogeneous labeling of proximal tubules located exclusively in the outer stripe of the outer medulla compatible with the topographical arrangement of the S3 segment (a and b). Consecutive sections reveal coexpression of ROSIT mRNA (*c*) and alkaline phosphatase (*d*) in tubules of the outer stripe, whereas proximal tubules in the cortical labyrinth (S1 and S2 segments) are only positive for alkaline phosphatase (magnification, a ×14, b ×41, c and d ×62).

### Combined *in situ* hybridization and immunocytochemistry

To identify thick ascending limb (TAL) profiles, *in situ* hybridization with the ROSIT-specific riboprobe and immunohistochemical staining with a polyclonal anti-Tamm Horsfall protein (THP) antibody was performed on the same tissue section (Tamm Horsfall protein is expressed exclusively in thick ascending limbs [21]). After post-hybridization washes, a mixture of the anti-digoxigenin antibody (diluted 1:500 in blocking medium) and the anti-THP antibody (diluted 1:30 in blocking medium) was applied. This mixture was administered to the sections for two hours at room temperature, followed by an overnight incubation at 4°C in a moist chamber. Slides were then rinsed twice for five minutes in buffer I, and subsequently the anti-THP antibody was detected with a Cy3-coupled secondary antibody (Dianova, Hamburg, Germany), diluted 1:300 in buffer I. After incubating for one hour at room temperature, slides were washed twice for five minutes in buffer I and subsequently processed as described in the normal *in situ* hybridization protocol.

### Controls

The specifity of the obtained *in situ* hybridization signal was verified by parallel incubation with antisense and sense riboprobes on alternate sections. Throughout all experiments sense probes did not produce any detectable signal. As further negative controls, some sections were hybridized without antisense probe, while others were processed by omission of anti-DIG antibody. Both controls yielded completely negative results.

### Alkaline phosphatase histochemistry

To identify all segments of the proximal tubule, the high enzymatic activity of alkaline phosphatase present in the brush

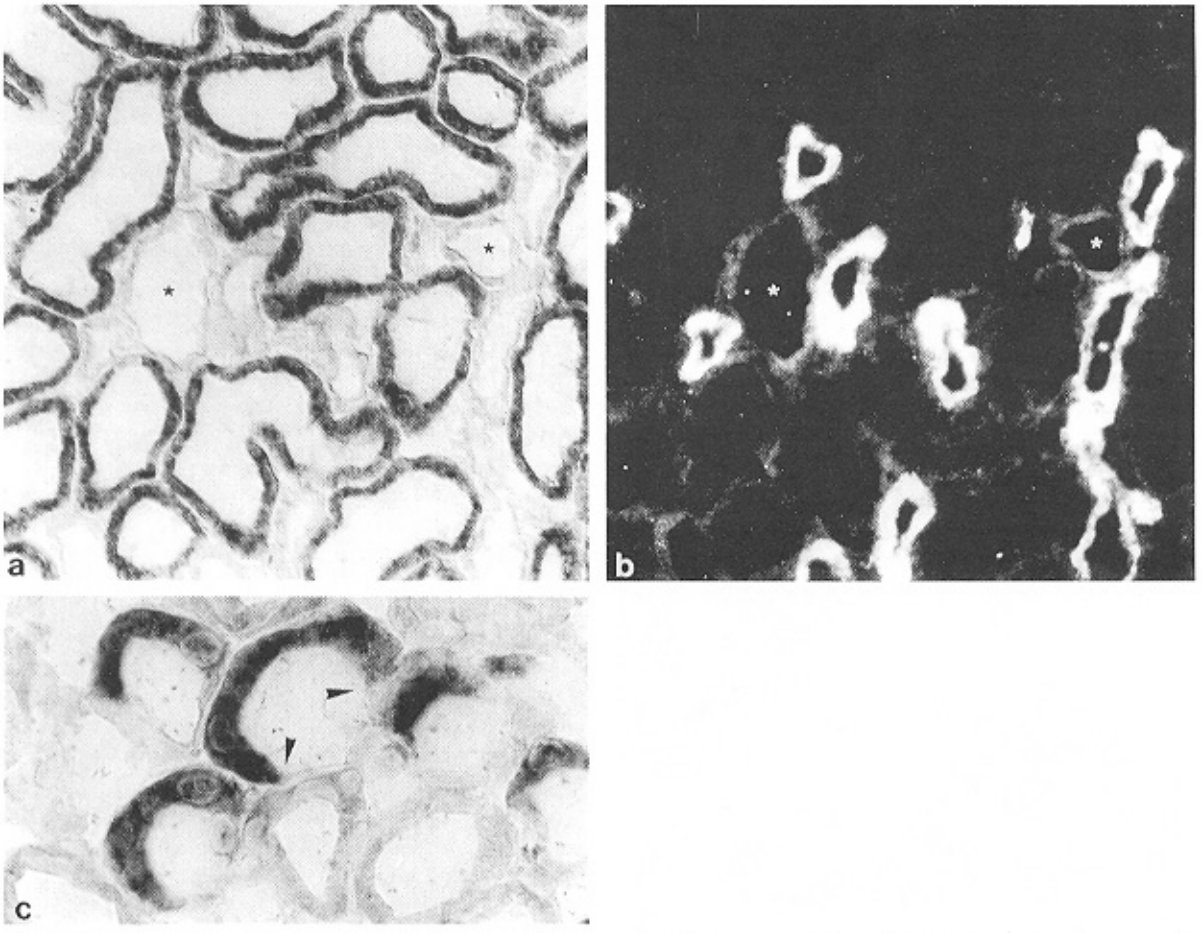


**Fig. 2. Detailed view of ROSIT mRNA expressing tubules in the outer stripe.** Double labeling with the ROSIT cRNA (*a*) and immunohistochemistry with an antibody against Tamm-Horsfall protein (THP), a marker for thick ascending limb profiles (*b*), shows that only proximal tubules are labeled by ROSIT cRNA. Single profiles that were unreactive with both the anti-THP antibody and the ROSIT cRNA represent collecting ducts (indicated by asterisks in a and b). (*c*) The abrupt distal end of ROSIT mRNA expression in proximal tubule cells can be seen at the transition to flat thin descending limb cells (arrowheads) (magnification, a and b ×246, c ×615).

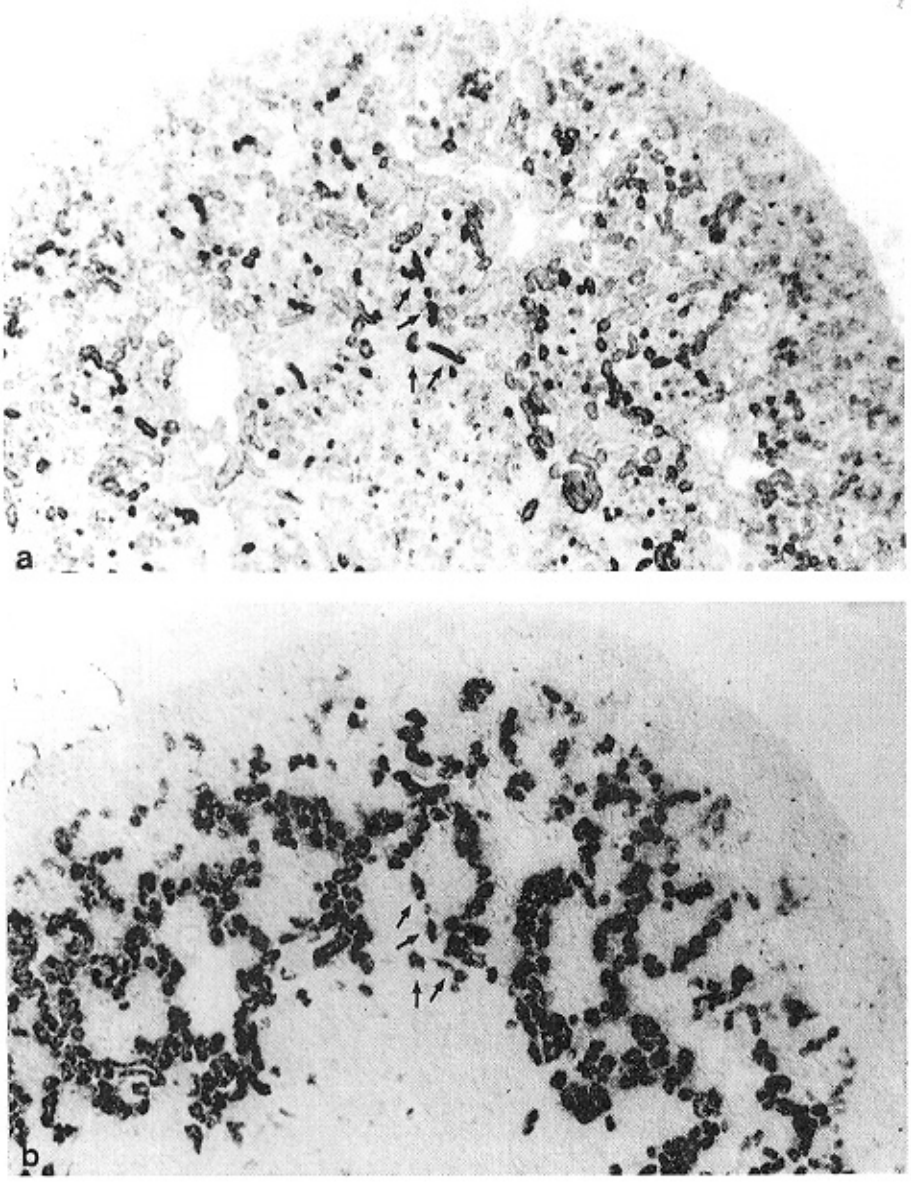


**Fig. 3. Expression of ROSIT mRNA in the neonatal rat kidney.** As seen in the overview, a strong hybridization signal can be found in numerous tubular profiles (*a*), which represent only a portion of all proximal tubules as demonstrated by alkaline phosphatase histochemistry on a consecutive section (*b*) (some profiles, which express both alkaline phosphatase and ROSIT mRNA, are indicated by arrows; all magnifications, ×62).

border of the proximal tubule was demonstrated histochemically. In brief, cryostat sections were covered with a solution containing 0.3 mM nitro blue tetrazolium chloride and 0.3 mM 5-bromo-4-chloro-3-indolyl-phosphate (4-toluidine salt) in 0.2 M Tris-HCl buffer, pH 9.5. After five minutes or when blue reaction products were visible, sections were immersed for 10 minutes in bidistilled water, fixed in 4% paraformaldehyde (in PBS) for 10 minutes, rinsed again in water and mounted.

## RESULTS

Using non-radioactive *in situ* hybridization on sections from adult rat kidneys, we were able to show that ROSIT mRNA-positive tubules were concentrated in the outer stripe of the outer medulla and in the deep portions of medullary rays (Fig. 1 A, B). The cortical labyrinth as well as the inner stripe and the inner medulla were devoid of a hybridization signal. Apparently, the ROSIT mRNA signal was exclusively located in the S3 segments of the proximal tubule. As shown in Figure 1 B and C, neither S2 segments (which are situated in the cortical labyrinth and in the middle and upper portions of the medullary rays) nor S1 segments expressed ROSIT mRNA. Serial sections were used for *in situ* hybridization with ROSIT antisense RNA and for alkaline phosphatase histochemistry in order to unequivocally demonstrate that ROSIT mRNA is expressed in the S3 segment of the proximal tubule. Tubular profiles in the outer stripe with strong alkaline phosphatase activity also contained an *in situ* hybridization signal (Fig. 1 C, D). Other tubular structures transversing the outer stripe of the outer medulla did not express ROSIT mRNA. Double labeling with an anti-THP antibody that recognized TAL profiles did not reveal colocalization of THP and ROSIT mRNA. The remaining tubular profiles unreactive with ROSIT cRNA and with the anti-THP antibody represented collecting ducts (Fig. 2 A,

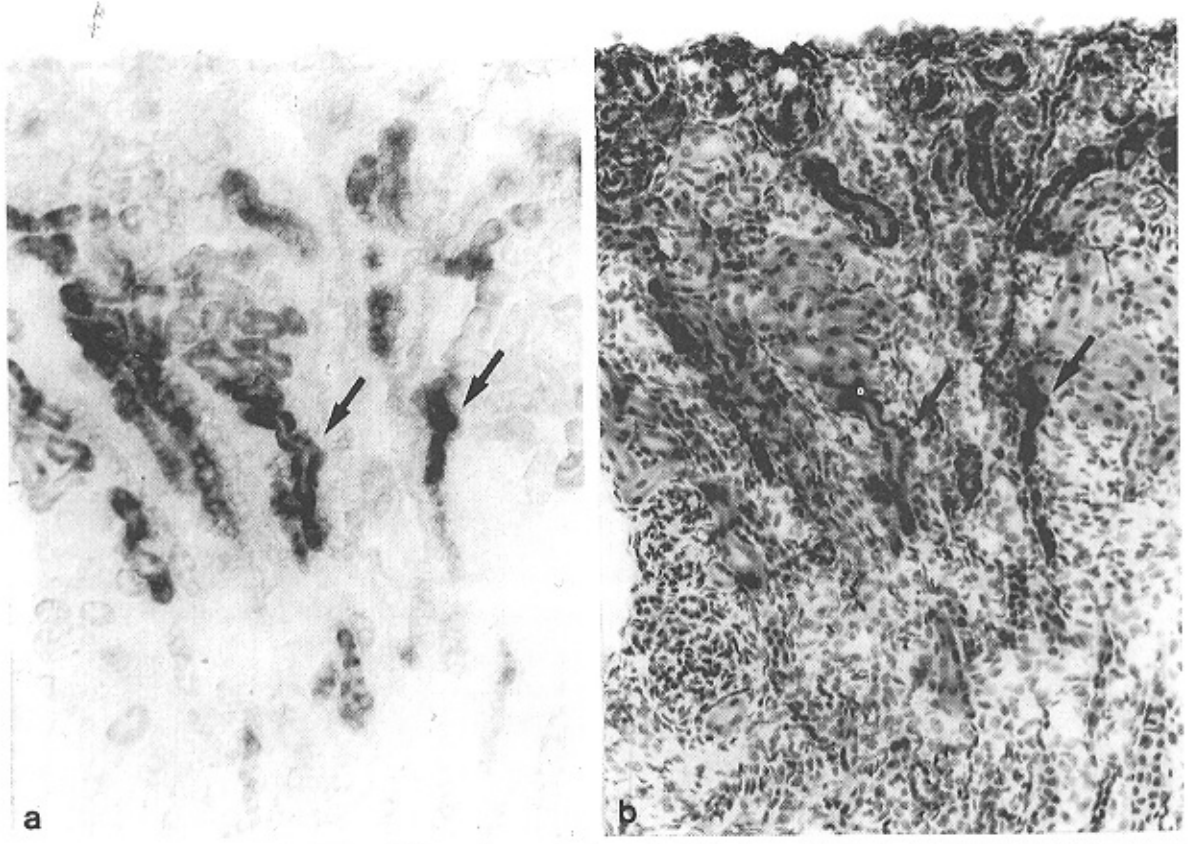


**Fig. 4. Detailed analysis reveals that in the kidney of the newborn rat only proximal tubular profiles but not S-shaped bodies exhibit a hybridization signal for ROSIT mRNA.** The straight proximal tubular profiles, which will later comprise the S3 segments, show the strongest hybridization signal (two examples in a and b are marked by arrows). For a comprehensive analysis, the same section which was first probed with ROSIT cRNA (*a*), was then subjected to staining with H & E (*b*) (all magnifications, ×154).

B). The distal end of the tubular staining for ROSIT mRNA was located sharply at the transition point between the S3 segment and the thin descending limb (Fig. 2C). These data clearly show that ROSIT mRNA was strongly expressed in the S3 segment.

In the neonatal rat kidney ROSIT mRNA is not expressed immediately below the renal capsule, but rather in tubular profiles some distance below the capsule, indicating that during the earliest stages of nephron development ROSIT mRNA is not expressed (Fig. 3A and 4A). When comparing consecutive sections from neonatal kidneys either probed with ROSIT antisense RNA (Fig. 3A) or stained for alkaline phosphatase activity (Fig. 3B), only a portion of alkaline phosphatase positive profiles strongly expressed ROSIT mRNA. On closer examination the S-shaped bodies did not express ROSIT mRNA, but tubular profiles close to S-shaped bodies did (Fig. 4).

Evaluation of ROSIT mRNA expression was also performed after acute tubular necrosis induced by bilateral ischemia. Three hours after the ischemic insult, ROSIT mRNA is still present in the S3 segment (Fig. 5A), although the labeling appeared somewhat reduced when compared to a kidney section from sham-operated animals (data not shown). In contrast, on sections of kidneys eight hours after ischemia, the ROSIT mRNA signal in the S3 segment had already disappeared (Fig. 5B). ROSIT mRNA was still absent two days after ischemia (Fig. 5C) and began to reappear seven days after ischemia, at which time the regenerative processes in the proximal tubules were accompanied by cystic enlargement of the tubular profiles (Fig. 5D). Higher magnification of these enlarged proximal tubules showed that ROSIT mRNA was present in a mosaic pattern in proximal epithelial cells at seven days post-ischemia (Fig. 6A). In kidneys 21 days after ischemia ROSIT mRNA was found to be expressed in the S3 segment at a strength comparable to sham-operated animals (Fig. 6B). Although at this time point the S3 segments were almost fully regenerated, focal areas in the outer stripe showed enlarged, ROSIT mRNA-negative tubules (Fig. 6B). We also evaluated ROSIT mRNA expression in kidneys obtained 10 and 14 days after ischemia (data not shown). Compared to the situation in kidneys seven days after ischemia, the proportion of regenerated, intact S3 segments (and the concomitant decrease of cystically enlarged tubules) was accompanied by a stronger and more frequent tubular labeling for ROSIT mRNA. The expression of ROSIT mRNA on kidney sections from sham-operated animals at the various reperfusion time points yielded the expected strong labeling of S3 segments identical to that seen in Figures 1 and 2 (data not shown).

## DISCUSSION

The sequence analysis of the recently cloned renal osmotic stress-induced cotransporter ROSIT revealed remarkable homologies to other renal organic osmolyte transporters [16]. ROSIT

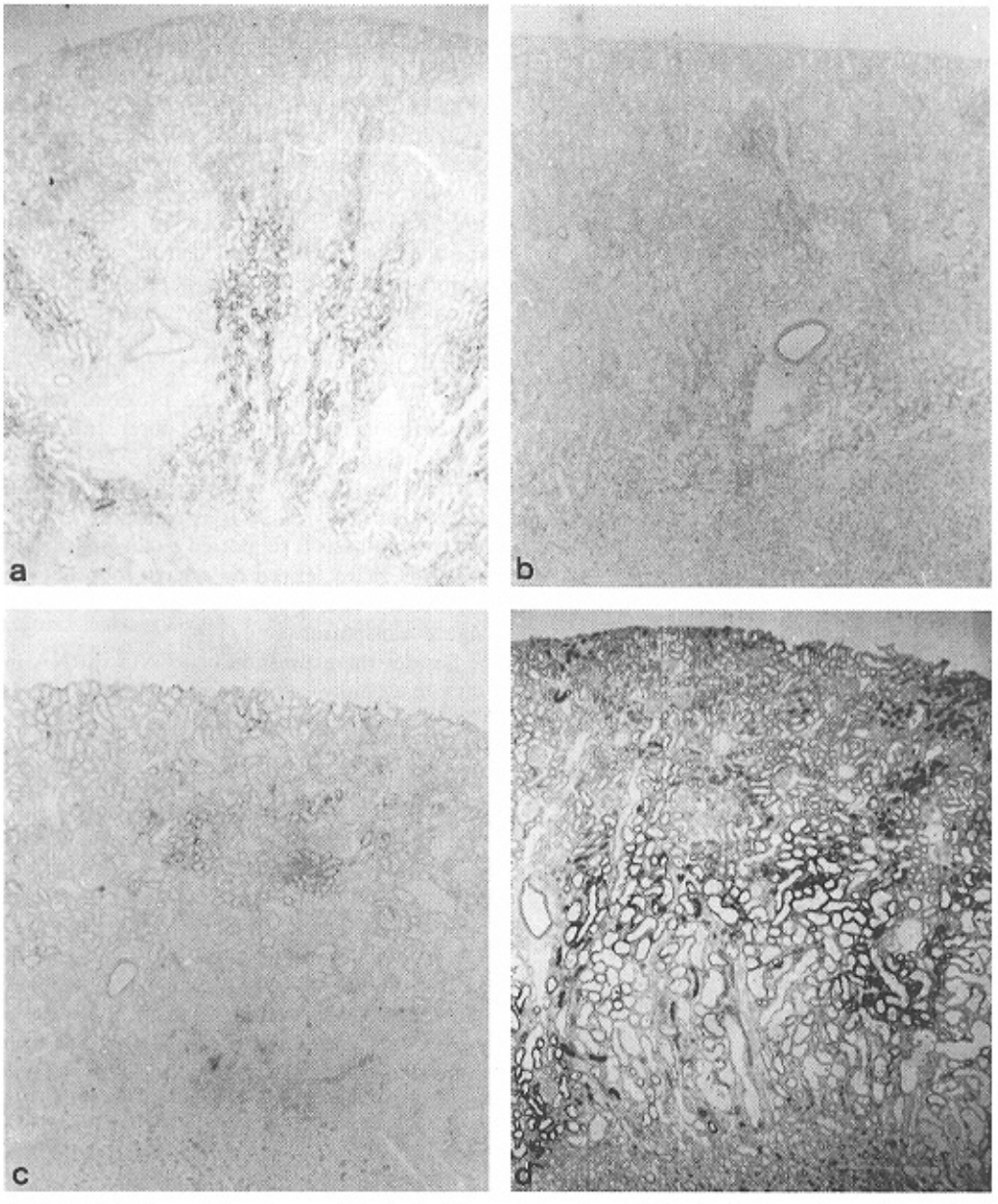


**Fig. 5. Expression of ROSIT mRNA on sections from post-ischemic kidneys.** Overviews which include the renal capsule and the beginning of the inner stripe show that ROSIT mRNA expression has become faint but is still expressed in the S3 segment three hours after ischemia (*a*) and virtually absent 8 and also 48 hours after ischemia (*b and c*). One week after the ischemic insult ROSIT mRNA can be found in some but not all tubules of the S3 segment, which often show cystic enlargement (*d*) (all magnifications, ×22).

shares a high degree of sequence similarity with other members of the organic solute cotransporter family (such as betaine, *myo*-inositol or taurine transporter), and it can therefore be assumed that ROSIT has a related function in the kidney, although the precise role and the substrate specificity of ROSIT are still unknown. The ROSIT mRNA encodes a protein with 12 membrane-spanning regions. Between transmembrane domains 7 and 8, however, ROSIT contains an extracellular loop that is significantly larger than the corresponding loop in other solute transporters. This prominent feature has previously only been described for two other orphan transporters detected in the brain that show the highest homologies to ROSIT (about 43%) [17, 18]. Northern blot analysis had indicated that under normal conditions ROSIT mRNA is present in the renal cortex [16]. The data presented above clearly demonstrate that ROSIT mRNA is expressed in the S3 segment of the proximal tubule in the adult rat kidney. Very recent RT-PCR data also suggest that the S3 segment is the main site of ROSIT mRNA expression [22]. We therefore assume that the cortical preparation used in the original description of ROSIT was contaminated with outer stripe [16].

Our study precisely shows that ROSIT mRNA is highly and constitutively expressed in the S3 segment of the normal adult kidney. From these data it can be concluded that the S3 segment is the nephron portion to which a functional role of ROSIT can be attributed. The site of expression of ROSIT seems interesting when compared to the medullary location of the known renal

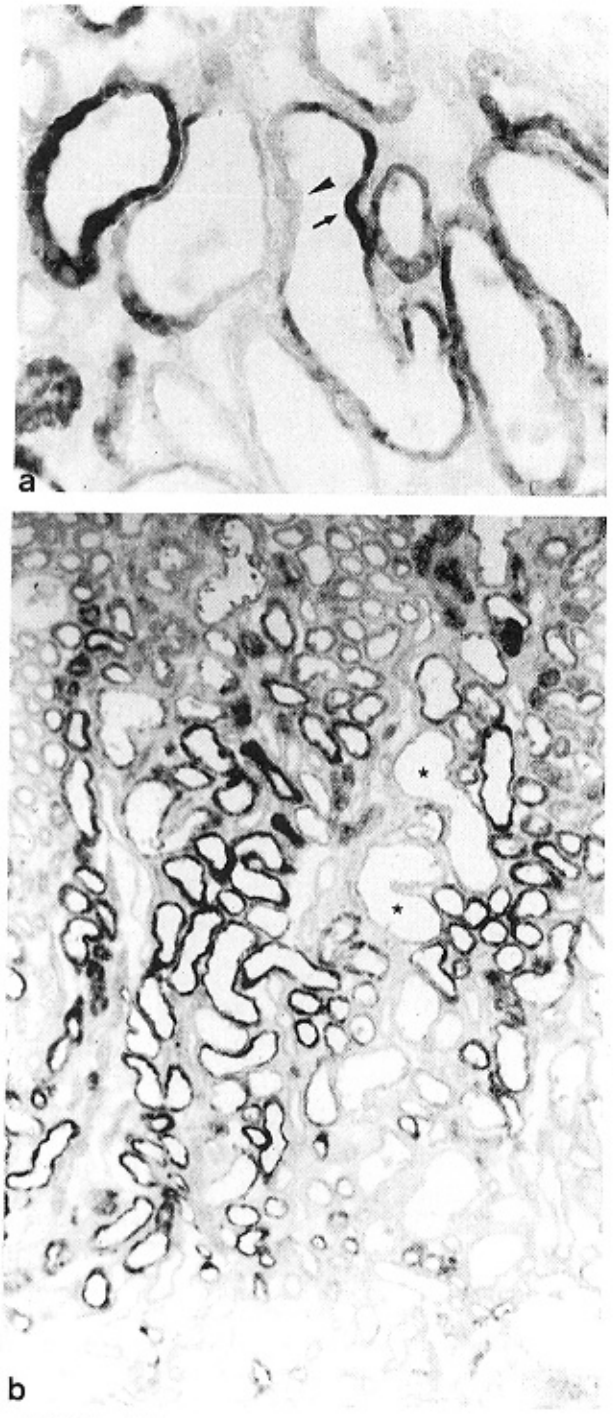


**Fig. 6. ROSIT mRNA expression seven and 21 days post ischemia.** (*a*) Higher magnification of ROSIT mRNA expression seven days post-ischemia points to a characteristic situation in the outer stripe where only some cells in a given proximal tubule express ROSIT mRNA (arrow), whereas other cells do not (arrowhead). (*b*) The representative expression pattern of ROSIT mRNA three weeks post-ischemia in the outer stripe. Regenerated tubules show strong and homogeneous labeling with ROSIT cRNA, whereas the epithelium of some cystically enlarged tubular profiles (marked by asterisks) does not express ROSIT mRNA (magnification, *a* ×210, *b* ×154).

osmolyte transporters. The betaine transporter mRNA is strongly expressed in the medullary thick ascending limbs of Henle's loop (MTAL) and in inner medullary collecting ducts (IMCD) [13]. Thick ascending limbs and possibly IMCD cells are the sites of expression of the $Na^+$/*myo*-inositol cotransporter [15, 23]. Very recent data demonstrate that the taurine transporter, besides its expression in the thin descending limb (DTL) and glomerular epithelial cells, is expressed and regulated in the S3 segment [24]. Similar to what has been described for the taurine, $Na^+$/*myo*-inositol and betaine transporters, ROSIT mRNA levels were increased by hypertonic stress [16]. This observation together with the fact that ROSIT and taurine transporter mRNA are both expressed in the S3 segment suggests that both transporters serve a similar or complementary function. The border between the cortex and the outer stripe marks the transition to an environment with increasing osmolality [25]. In such a setting, ROSIT would help the S3 segment of the proximal tubule to adapt to the hyperosmolar condition in the outer stripe. Thus far, however, no substrate could be identified for ROSIT. We therefore cannot exclude, that ROSIT serves a function unrelated to the accumulation of osmolytes, such as transport of toxic substances. This latter hypothesis is supported by the unusual structure of ROSIT, which is characterized by a large loop between transmembrane segments 7 and 8, a feature not found in other members of the solute transporter family [16].

Besides the expression of ROSIT mRNA in the normal adult rat kidney, our results reveal that ROSIT mRNA is already clearly detectable in proximal tubules of the neonatal kidney but not in the S-shaped bodies. The stage-dependent expression of ROSIT during nephrogenesis correlates well with that of other transporters located in the S3 segment of the proximale tubule such as the $Na^+$/glucose cotransporter 1 (SGLT1) and rBAT, an amino acid transporter, which all start to be expressed in the late fetal period as demonstrated by Northern blot analysis [26, 27]. Already in the neonatal rat kidney, ROSIT is confined to the straight portion of the proximal tubule, indicating that the differentiation of the proximal tubule into the S1, S2 and S3 segments occurs very early in nephron development. Only after the substrate of ROSIT has been identified will we be able to determine whether ROSIT also is functional at this early stage and when it begins to contribute to renal function, that is the establishment of and adaptation to a hyperosmotic environment.

Our analyis of ROSIT mRNA expression in the recovery phase of bilaterally-induced ischemic acute tubular necrosis revealed that ROSIT mRNA completely disappears within the first eight hours post-ischemia and reappears approximately one week after the ischemic insult. The S3 segment of the proximal tubule is the major site of damage after an ischemic injury [28]. The expression of PCNA, a marker for the S-phase of the cell cycle, peaks at approximately 48 hours after the ischemic insult, which is a sign that the surviving cells have left the $G_0$-phase in order to replace the dead cells [28]. Our observation that ROSIT mRNA disappears after acute tubular necrosis and reappears late in the recovery phase confirms previous data on vimentin expression. Vimentin, an intermediate filament-cytoskeletal protein, is down-regulated during nephron development. In the post-ischemic kidney vimentin expression can again be detected in the S3 segment of the proximal tubule at 24 hours post-ischemia, its expression remains high for at least five days after the ischemic insult [28]. The observation on the reappearance of vimentin and the disappearance of ROSIT indicates that the surviving cells are less differentiated while they are in the cell cycle and for some days thereafter. Even at 21 days after ischemia we were able to

find putative proximal tubular profiles that did not express ROSIT mRNA. Those profiles were cystically enlarged indicating a sustained damage to those nephron segments. The lack of expression of ROSIT, a protein related to osmolyte transporters, may contribute to prolong the time before the kidney has recovered completely, because the cells of the S3 segment cannot respond to their hyperosmotic environment. In addition, a delayed onset of the expression of ROSIT and functionally related proteins may actually play a role in the formation of those cystic structures we found at late stages after ischemia.

In conclusion, this study identifies the S3 segment of the proximal tubule as the site of expression of the renal osmotic stress-induced transporter (ROSIT) in the adult rat kidney. We also describe the expression pattern of ROSIT in the developing rat kidney and in the post-ischemic rat kidney. Together with recent data demonstrating the nephron specific expression of main osmolyte transporters, our data contribute to a better understanding of osmolyte transport in the kidney.

## ACKNOWLEDGMENTS

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## APPENDIX

Abbreviations used in this article are: PBS, phosphate-buffered saline; PFA, paraformaldehyde; ROSIT, renal osmotic stress-induced cotransporter; SD, Sprague-Dawley rats; BSA, bovine serum albumin; NBT, nitro blue tetrazolium chloride; TAL, thick ascending limb of Henle; THP, Tamm Horsfall protein.

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