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Molecular cloning of *KS*, a novel rat gene expressed exclusively in the kidney

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Molecular cloning of *KS*, a novel rat gene expressed exclusively in the kidney.

Background. We aimed to identify genes with kidney specific, developmentally regulated expression. Here we report the cDNA sequence and expression pattern of *KS*, a novel kidney-specific rat gene.

Methods. A partial cDNA was identified by differential display polymerase chain reaction (PCR) of a renal cell fraction enriched for proximal tubular and renin-expressing cells. Using the partial cDNA as a probe, a rat kidney cDNA library was screened. The full-length *KS* sequence was obtained by PCR amplification of cDNA ends. The expression pattern of *KS* was investigated by Northern blot. RNA was extracted from several organs of newborn and adult rats, as well as from the kidneys of rats with altered tubular function, that is, rats that had undergone unilateral nephrectomy, unilateral ureteral obstruction, neonatal losartan treatment, and the appropriate control animals. The expression of *KS* was also investigated in the kidneys of rats with spontaneous or renovascular hypertension.

Results. The *KS* cDNA (2426 bp) contained one open reading frame encoding a predicted 572 amino acid protein. The derived peptide sequence displayed approximately 70% similarity to the hypertension-related *SA* gene product and approximately 50% similarity to prokaryotic and eukaryotic acetyl-CoA synthases (EC 6.2.1.1). *KS* was expressed in the kidney and not in any other organ assayed. *KS* RNA was not detected in fetal and newborn rat kidney but became apparent after one week of postnatal life. Gene expression was downregulated in rat models of altered tubular function. *KS* expression was decreased in spontaneously hypertensive rats but not in renovascular hypertension.

Conclusion. *KS*, a novel rat gene, exhibits a unique tissue-specific expression exclusively in mature kidneys. The data suggest *KS* may encode an adenosine monophosphate binding enzyme.

The development of mature organs, that is, the differentiation and patterning of cells that leads to organogenesis, is controlled by the coordinated expression of specific genes. Despite progress in recent years, many genes involved in these processes remain unknown. The identification of genes that exhibit highly organ-specific expression should aid the study of mechanisms of organogenesis. Even if such genes are not controllers of cell differentiation, their identification should facilitate the search for "upstream" regulators and may allow selective targeting of organs.

The molecular cloning of genes that are expressed at specific times during organogenesis has been facilitated by the development of polymerase chain reaction (PCR) based techniques to identify differentially expressed genes, namely differential display PCR [1, 2] (DD-PCR) and RNA fingerprinting [3]. In an attempt to identify genes that are differentially expressed in specific renal cell types, we used DD-PCR on RNA derived from a sample of rat kidney cells enriched in proximal tubular and renin-producing cells [4].

This study reports the molecular cloning, cDNA sequence and expression pattern of *KS*, a novel rat gene expressed exclusively in the kidney. This novel gene shows a unique developmental and tissue-specific expression in the mature kidney.

METHODS

Generation of a partial cDNA probe

Kidneys isolated from three-month-old Sprague-Dawley rats (Hilltop Farms, Scottsdale, PA, USA) were minced in saline, and centrifugation through a percoll gradient was performed to isolate a cell fraction enriched in proximal tubular and renin producing cells and one depleted of these cells as described previously [4]. RNA was isolated as described below from this cell fraction sedimenting at 1.067 g/ml, and from a second fraction sedimenting at 1.017 g/ml that contained mostly vascular smooth muscle cells [4]. From each sample, 0.4 μg of RNA were reverse transcribed as described by Liang and Pardee [1, 2], using the RNAmap™ kit (GenHunter Corp., Brookline, MA, USA). DD-PCR was performed using the polyA$^+$ anchored

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primer 5′-TTTTTTTTTTTTMA-3′, arbitrary 10 mer primers provided with the kit and a $^{[35]}$S-deoxy-ATP (DuPont NEN, Boston, MA, USA). The PCR reaction was performed using the cycling parameters described by Liang and Pardee [1, 2] with AmpliTaq DNA Polymerase (Perkin Elmer, Branchburg, NJ, USA) in a Perkin Elmer N801-0150 Thermocycler. Radioactive PCR products were displayed on a sequencing gel (see below). Bands that were differentially expressed in two independent experiments were eluted and reamplified as described previously [2]. The reamplified PCR products were cloned into the pCNTR vector using the General Contractor™ DNA Cloning System (5 Prime - 3 Prime Inc., Boulder, CO, USA) and sequenced (see below). The clone containing the partial *KS* cDNA was selected for further analysis based on its sequence.

Library screening

An adult Sprague-Dawley rat kidney cDNA library in the Uni-ZAP™ XR vector, containing the pBluescript phagemid, was purchased from Stratagene (La Jolla, CA, USA). Phage (10^6 plaque forming units, 10^4 per plate) were screened with the cloned DD-PCR fragment as a probe using protocols recommended by the library manufacturer. Positive plaques were excised *in vivo* to yield cDNAs in the more manageable pBluescript vector. Restriction enzyme digests were electrophoresed, blotted and hybridized with the probe used to screen the library to confirm that the clones contained cDNA homologous to the original probe.

PCR amplification of 5′ and 3′ cDNA ends

Since the cDNA clones isolated from the library did not yield the complete 5′ end and their sequences raised the possibility of alternative splicing, a PCR-based rapid amplification of cDNA ends (RACE) [5] was performed using a commercially available RACE-ready rat kidney cDNA library (Clontech Laboratories Inc., Palo Alto, CA, USA). The gene-specific primers 5′-GCC ACA GCA TCC ACT TCC TGA ACC-3′ for 5′ RACE and 5′-AGA GTG GTG GCT GGT GAC CCT CG-3′ for 3′ RACE, respectively, were derived from the sequence of the clones obtained by library screening (Fig. 2). RACE-PCR was performed with KlenTaq DNA polymerase (Clontech Laboratories, Inc.) and Deep Vent™ DNA polymerase as a proofreading enzyme in a Perkin Elmer N801-0150 Thermocycler according to protocols provided by the manufacturer of the RACE-ready cDNA kit (Clontech Laboratories, Inc.). One third of the RACE reaction was electrophoresed, blotted and hybridized with the partial cDNA probe to confirm that the RACE product contained cDNA homologous to the original probe. The remaining two thirds of the RACE reaction were electrophoresed, and the band of appropriate size was excised, purified and the DNA cloned into the pCNTR vector (5 Prime - 3 Prime Inc.). Six clones of the 5′ RACE product were fully sequenced.

Sequence analysis

Sequencing was performed with the dideoxy chain-termination method [6] using the Sequenase version 2.0 kit (Amersham Corp., Arlington Heights, IL, USA) and a $^{[35]}$S-deoxy-ATP (1500 Ci/mmol; DuPont NEN) according to the manufacturer's protocols. The sequence reactions were separated on a 5% acrylamide-urea sequencing gel. The gel was transferred to blotting paper, dried under vacuum and exposed to Biomax MR film (Kodak, Rochester, NY, USA) for autoradiography. Nested 5′ and 3′ deletions were generated [7] from the cDNAs obtained from library screening using exonuclease III (Life Technologies Inc., Grand Island, NY, USA). Both strands were completely sequenced. To resolve the few remaining ambiguities, parts of the sequence were also sequenced on a Perkin-Elmer ABI Prism™ 377 automated sequencer at the Biomolecular Research Facility of the University of Virginia.

Analysis of the obtained sequence was performed on the local computer network at the University of Virginia, using the Wisconsin software package (Genetics Computer Group, Madison, WI, USA). The BLAST program [8] was used to search for similarities in the GenBank database, and the GAP program, based on the algorithm of Needleman and Wunsch [9], was used to compare full-length protein sequences. The PILEUP program, based on the same algorithm [9], was used to align several full-length protein sequences extracted from GenBank. The PHYLIP software was used to analyze the phylogenetic relationship between these aligned sequences [10]. Parsimony analysis was carried out on 100 random subsets of the alignment to create a phylogenetic "tree," using the *KS* sequence as an outgroup [10]. The MOTIFS program was used to search the predicted peptide sequence for patterns listed in the PROSITE dictionary for sites and patterns in proteins [11].

RNA extraction from treated and untreated rat tissues

Total RNA was extracted by the single step method of Chomczynski [12] using TRI reagent (Molecular Research Center Inc., Cincinnati, OH, USA). To characterize the organ-specific expression of *KS*, RNA was extracted from kidney, heart, lung, total brain, liver, aorta (carefully dissected free from surrounding fat), mesentery, adrenal, stomach, jejunum, ileum, colon, spleen, testes, uterus and ovary of adult (3 months old) Sprague-Dawley rats (Hilltop Farms). To test for developmental differences in the expression of *KS*, RNA was extracted from kidney, brain, lung, heart and liver of newborn Sprague-Dawley rats sacrificed within the first 24 hours after birth, and from the kidneys of 1-, 2-, 3-, 4- and 5-week-old Sprague-Dawley rats. Poly(A^+) RNA was prepared according to a standard protocol [13], using oligo(dT) cellulose spin columns (5 Prime - 3 Prime Inc.).

The expression of *KS* was investigated in genetic and renovascular hypertension. Renal RNA was extracted from four spontaneously hypertensive rats (SHR, 3 months old) and five age-matched Wistar Kyoto control animals (WKY; Charles River, Wilmington, MA, USA). Two-kidney, one-clip (2K1C) hypertension was induced as described previously [14] in eight-week-old Sprague-Dawley rats; 4 and 17 days after renal artery clipping, five 2K1C and five control animals were sacrificed, and RNA was isolated from clipped, contralateral, and control kidneys.

The expression of *KS* was investigated in models of altered tubular function. Renal RNA was extracted two days and seven days after unilateral nephrectomy (UNX). Three-month-old Sprague-Dawley rats underwent UNX after decapsulation under pentobarbital anesthesia (60 mg/kg intraperitoneally), or sham operation (renal decapsulation). Rats were sacrificed two days (5 UNX and 4 controls) or seven days (4 UNX and 4 controls) after operation. RNA was also extracted from four affected and four contralateral kidneys of 28-day-old rats that underwent neonatal unilateral ureteral obstruction (UUO) as described previously [15] and four controls. In addition, neonatal rats were treated from the day of birth with daily subcutaneous injections of the angiotensin II type 1 receptor antagonist losartan (25 mg/kg) or saline ($N = 6$ each) as controls as described previously [16]. Rats were sacrificed on day 13, and renal RNA was extracted.

Northern hybridization

For Northern blot analysis, 10 to 20 μg of total RNA or 2 μg of poly(A^+) RNA were electrophoresed in a 1.2% agarose-formaldehyde gel and transferred to positively charged Nylon membranes (Zeta-Probe; Bio-Rad Laboratories, Hercules, CA, USA) as previously described [17, 18]. Probes were radiolabeled with a $^{[32]}$P-deoxy-CTP (DuPont NEN) either by random prime labeling [19] with a commercially available kit (DECAprime II; Ambion Inc., Austin, TX, USA), or by PCR labeling. For PCR labeling, 15 to 20 ng of the insert of the cloned DD-PCR fragment of *KS* was amplified using the appropriate primers from the DD-PCR kit (T_{12}MA and AP-3 [2]). The final concentrations in the PCR reaction were 1 μM for both primers, 0.83 μM a $^{[32]}$P-deoxy-CTP (3000 Ci/mmol), 1.88 μM deoxy-ATP, -GTP and -TTP, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-Cl pH 8.4. The cycling parameters were 94°C 30 seconds, 40°C two minutes, and 72°C 30 seconds for 40 cycles in a Perkin Elmer N801-0150 Thermocycler. Northern blots were hybridized by a modification of the method of Church and Gilbert [20], as previously described [15, 17]. For autoradiography, membranes were exposed to Kodak XAR films (Kodak), or to a PhosphorImage screen (Molecular Dynamics Inc, Sunnyvale, CA, USA). Signals were quantified after scanning the PhosphorImage screen using the ImageQuant software (Molecular Dynamics Inc.). Blots were rehybridized with a GAPDH cDNA, and the *KS* signal was normalized for GAPDH expression. To test for statistical significance, analysis of variance followed by the Newman-Keuls test for *posthoc* analysis was carried out using the normalized values. Data are expressed as mean ± SEM.

Southern hybridization

Genomic DNA was isolated from Sprague-Dawley rat liver using proteinase K digestion, phenol/chloroform extraction, and ethanol/sodium-acetate precipitation, according to standard protocols [21]. Ten micrograms each of genomic DNA were incubated with 30 U of the restriction enzymes *Acc*I, *Eco*RI, *Hin*dIII, or *Pst*I at 37°C for six hours. Digests and size markers were loaded onto a 0.7% Agarose gel and electrophoresed at 30 milli-Ampere in 0.5 × Tris-Borate-EDTA [21] until an optimal separation was achieved. The gel was submerged in 0.25 M HCl for 15 minutes to depurinate the DNA, soaked twice for 15 minutes in 0.5 M NaOH, 1 M NaCl to denature the DNA, and neutralized for 30 minutes in 0.5 M Tris-HCl pH 7.5, 3 M NaCl. The DNA was transferred by vacuum to Zeta-Probe membranes with 10× standard sodium chloride (SSC; 1 × SSC = 0.15 M NaCl, 0.015 M Na_3 citrate) and the membranes were vacuum dried at 80°C for 30 minutes. Hybridization and detection were carried out as described above for Northern hybridization.

RESULTS

Cloning of the *KS* cDNA

We used DD-PCR to identify mRNAs differentially expressed in rat kidney fractions enriched for proximal tubules and renin-expressing cells. Six differentially expressed cDNAs were identified using one anchored primer and five different arbitrary primers (Fig. 1). Five of these were uniquely expressed in the enriched fraction (Fig. 1B) and one in the depleted fraction (Figure 1A). The differential expression of all six bands was reproducible. The 241 bp cDNA cloned from a DD-PCR fragment 5 was selected for further analysis because its nucleic acid sequence exhibited similarity but not identity to the hypertension-related *SA* gene [22–25]. Screening of an adult Sprague-Dawley rat kidney cDNA library with the 241 bp partial cDNA yielded two positive clones of 1953 and 1759 bp length, respectively (Fig. 2). The shorter clone contained a 285 bp deletion (Fig. 2), but the overlapping parts of the sequence of the two clones were otherwise identical. The 241 bp DD-PCR fragment corresponded not to the 3′ end of the sequence but was found near the 5′ end. A run of seven uninterrupted A's in the *KS* sequence corresponding to the 3′ end of the 241 bp fragment was likely responsible for the annealing of the poly(A^+)-anchored [1, 2] differential display primer. Since neither clone from the cDNA library contained the 5′ end of the gene, 5′ RACE was performed to obtain additional sequence information. The

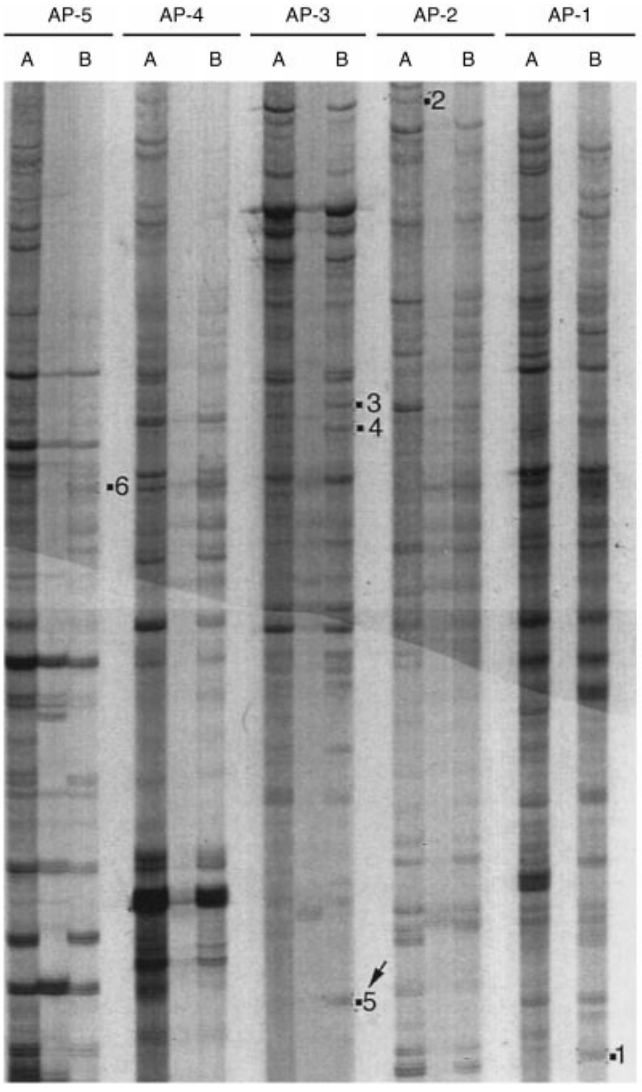

Fig. 1. Differential display of mRNA from rat kidney cell fractions. Numbers on this autoradiogram of the differential display gel indicate cDNA fragments expressed differentially in either the fraction enriched for proximal tubules and renin expressing cells (*B*) or fraction containing vascular smooth muscle cells (*A*). cDNA #5 is described in this study. AP 1-5 are arbitrary primers from the RNAmap kit (detailed in the Methods section). The autoradiogram is paler on the lower half due to the gel having been covered with plastic wrap when the film was exposed.

part of the sequence of the 5′ RACE products that overlapped was identical to the sequence of the cDNA library clones (Fig. 2).

The final nucleic acid sequence of *KS* was derived from the 1953 bp cDNA not containing the deletion and the 5′ RACE products (Fig. 2). The *KS* cDNA contained a single open reading frame of 572 amino acids (Fig. 3). The sequence is available at GenBank accession # AF062389. Southern blotting with the PCR fragment yielded only one band in several restriction digests (Fig. 4), indicating that *KS* is a single-copy gene. One of the cDNA clones contained a 285 bp deletion (Fig. 2), but neither Northern hybridization (Fig. 5) nor 3′ RACE PCR yielded any evidence for a shorter (approximately 2.1 kb long) mRNA transcript corresponding to the 1759 bp clone.

Expression of *KS*

Northern blotting showed a unique tissue-specific expression of *KS*. The mRNA was detected only in the kidney and not in any other organ (Fig. 5). Hybridization of poly(A^+)-purified mRNA from adult and newborn brain, lung, heart, liver and kidney confirmed the selective expression exclusively in the adult kidney. In addition to the organs shown on Figure 5, *KS* was not expressed in a number of epithelial organs tested by Northern hybridization, including stomach, jejunum, ileum, and colon. *KS* expression was developmentally regulated: the mRNA was absent in newborn rat kidney (Fig. 5) but detectable beginning from one week of age, with little change after two weeks of age (data not shown).

KS expression was downregulated in models with impaired renal tubular function. Twenty-eight days after neonatal unilateral ureteral obstruction (UUO), *KS* mRNA was decreased by 59% compared to the average of the values for the contralateral and sham control kidneys (42.4 ± 16.7 arbitrary units in UUO vs. 112.8 ± 21.5 in the contralateral kidney and 93.1 ± 5.7 in sham operated controls, $P < 0.05$; Fig. 6). Neonatal losartan treatment decreased *KS* expression by 37% (29.9 ± 2.9 arbitrary units in losartan treated vs. 47.2 ± 3.7 in saline treated controls, $P < 0.05$). *KS* was downregulated early in the course after unilateral nephrectomy (UNX; Fig. 6). Two days after UNX, *KS* was decreased by 40% (24.6 ± 3.6 vs. 41.1 ± 4.4 arbitrary units in sham operated controls, $P < 0.05$). Seven days after UNX, *KS* expression appeared normal in three of the four rats (Fig. 6), and there was no statistical difference between UNX (29.6 ± 7.3) and controls (47.3 ± 4.9). The kidney weight/body wt ratio was significantly elevated seven days after UNX (4.85 ± 0.11 vs. 3.85 ± 0.21 g/kg in controls, $P < 0.05$) but not after two days (4.61 ± 0.18 vs. 4.35 ± 0.16 g/kg in controls).

Figure 7 shows that the expression of *KS* was lower by 68% in SHR kidney compared to WKY controls (21.4 ± 2.7 in SHR vs. 66.7 ± 3.8 in WKY, $P < 0.05$). In contrast, there was no significant change in the nongenetic, renin-dependent model of renovascular hypertension (2-kidney, 1-clip hypertension), neither four days after clipping (49.7 ± 5.7 in clipped kidneys vs. 47.1 ± 6.1 in contralateral kidneys vs. 50.6 ± 6.4 in sham controls) nor 17 days after clipping (48.8 ± 6.6 in clipped kidneys vs. 41.1 ± 5.1 in contralateral kidneys vs. 51.0 ± 7.3 in sham controls; Fig. 7).

Sequence analysis and comparison

A GenBank search with the complete 2426 bp sequence revealed that *KS* is a novel gene. The two most closely related genes were the rat *SA* gene [22] with 57.4% identity

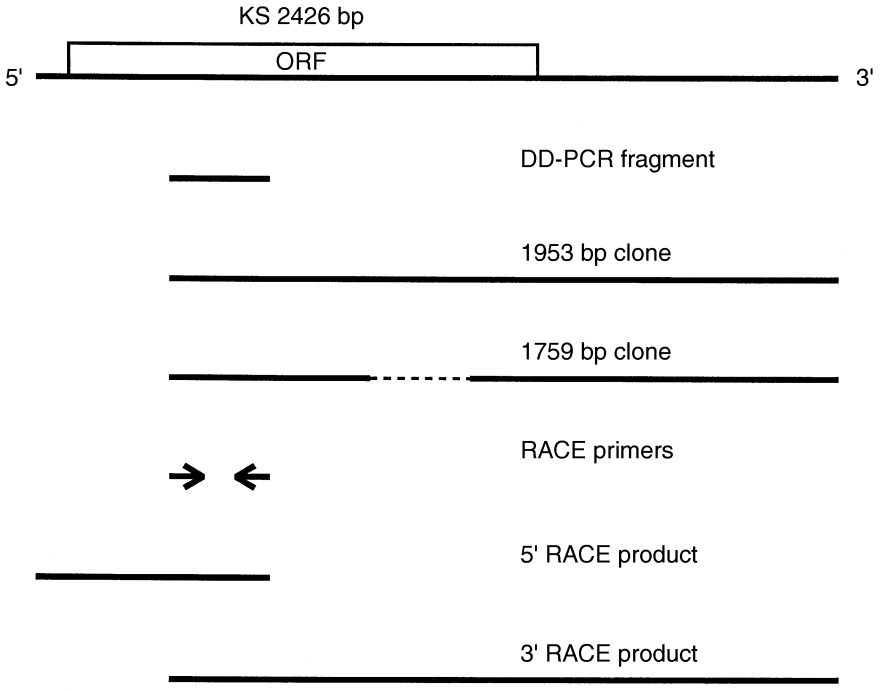

Fig. 2. Schematic representation of the cDNA clones from which the KS sequence was derived. The full sequence with the open reading frame (ORF) is shown on top. DD-PCR indicates differential display, RACE indicates rapid amplification of cDNA ends. The RACE primers are not drawn to scale.

on the nucleotide level and the human *SA* gene [26] with 55.9% nucleotide identity. A search with the derived peptide sequence (Fig. 3) again yielded the rat and human *SA* gene products as the closest relatives, with somewhat higher similarity at the peptide level (Table 1). *KS* exhibited similarity to acetyl-CoA synthase gene products from several species, spanning several kingdoms (Table 1). Comparable values for amino acid identity and similarity were obtained by comparisons between the acetyl-CoA synthases, or by comparing those with the *SA* gene product (data not shown). Figure 8 displays the hypothetical relationship between *KS* and those genes. Like the acetyl-CoA synthase genes and the *SA* gene, *KS* contains the conserved adenosine monophosphate (AMP) binding domain signature (Fig. 3) [27, 28].

DISCUSSION

We identified a novel gene that exhibits a unique tissue-specific expression pattern; *KS* (for kidney specific) is expressed exclusively in the kidney. Its expression in mature kidneys appears to be related to tubular function, as evidenced by its developmental regulation and the lower RNA abundance in models of disturbed tubular function. *KS* is the closest relative of the hypertension-related *SA* gene [22–25] reported so far. The expression of *KS* is downregulated in genetic but not in renovascular hypertension. We speculate that *KS* may play a role in renal tubular function.

The expression of *KS* was restricted to the kidney; we could not detect the message in any other organ. In contrast, the related *SA* gene is more widely expressed, as several reports agree that the *SA* mRNA is detectable in liver and kidney [22, 29, 30]. Some authors reported expression also in brain [29, 31] and testes [29] that was not confirmed by others [30]. The unique tissue-specific expression of *KS* may potentially be useful for several purposes, such as for the identification of *trans*-acting factors specific for renal cell maturation, or for gene targeting to the kidney.

We did not determine the precise cellular localization of *KS* expression by *in situ* hybridization. However, preliminary hybridization experiments with the DD-PCR fragment (data not shown), the *KS* expression pattern in models of altered tubular function, and the expression of the related *SA* gene [30] all suggest that *KS* is expressed in proximal tubules. The cell population from which the DD-PCR fragment was cloned was enriched in both tubular and renin-producing cells [4], but the expression pattern of *KS* showed no relationship to the expression of renin. In high-renin states, *KS* mRNA levels were undetectable (first days of postnatal life [18]), low (losartan treatment and UUO [15–17]) or normal (clipped kidneys of 2K1C [14]); in low-renin states, *KS* mRNA was unaffected (contralateral kidney of 2K1C [14]). Additional experiments will be necessary to define the cell-specific expression of *KS*.

The time course of *KS* expression in ontogeny suggested that the gene is transcribed when nephrogenesis is complete and renal function becomes more mature [32]. The expression pattern of *KS* in several models of altered tubular function supported this notion. The mRNA was less abundant in rats with neonatal UUO [15] and in rats treated with losartan from day 1 after birth in which proper

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   1: catcagtgctgttctctctactggtgcttcgggaactctagaactccttcctgagaggga
  61: agcaaaccaagctattgccagagagagttccataagagctctgaatatgcattggctgtg
   :  ..........................................  M  H  W  L  W
 121: gaaaattccaagactttgcaccttctggggcactgagatgttccaccgttctttccatat
   :    K  I  P  R  L  C  T  F  W  G  T  E  M  F  H  R  S  F  H  M
 181: gaatatcaagaaactaatgcccatacagtggggccaccaagaagtccctgccaagttcaa
   :    N  I  K  K  L  M  P  I  Q  W  G  H  Q  E  V  P  A  K  F  N
 241: cttcgctagtgatgtgatagatcactgggccagcctggagaaggctggcaagagatctcc
   :    F  A  S  D  V  I  D  H  W  A  S  L  E  K  A  G  K  R  S  P
 301: aggtccagccctatggtggatgaatggcagtggggaagaactaaagtggaacttcagaga
   :    G  P  A  L  W  W  M  N  G  S  G  E  E  L  K  W  N  F  R  E
 361: actgagtgaaatcagtaaacagacggccaatgttctcaccggggcctgtggcctgcagcg
   :    L  S  E  I  S  K  Q  T  A  N  V  L  T  G  A  C  G  L  Q  R
 421: tggggaccgtgtagcagtagtgcttcctcgagtcccagagtggtggctggtgaccctcgg
   :    G  D  R  V  A  V  V  L  P  R  V  P  E  W  W  L  V  T  L  G
 481: ctgcatgcgttctggtcttgttttcatgcctggaaccacccagatgaaatccacagacat
   :    C  M  R  S  G  L  V  F  M  P  G  T  T  Q  M  K  S  T  D  I
 541: actctacaggctgcaatcatccaaggccagggccattgtggctggggatgaagtggttca
   :    L  Y  R  L  Q  S  S  K  A  R  A  I  V  A  G  D  E  V  V  Q
 601: ggaagtggatgctgtggcccctgactgctcttttctgaaaatcaagttgctggtgtctga
   :    E  V  D  A  V  A  P  D  C  S  F  L  K  I  K  L  L  V  S  E
 661: aaaaaatcgagaaggatggctaaacttcaaggcactgctaaaggatgcatcccccattca
   :    K  N  R  E  G  W  L  N  F  K  A  L  L  K  D  A  S  P  I  H
 721: tcagtgtgtggagactgtaagccaagaatcagctgccatctatttcactagcgggaccag
   :    Q  C  V  E  T  V  S  Q  E  S  A  A  I  Y  F  T  S  G  T  S
 781: tggccctcccaagatggcagagcactcccactgcagcctgggcctcaaggccaagatgga
   :   G  P  P  K  M  A  E  H  S  H  C  S  L  G  L  K  A  K  M  D
 841: tgctggctggacaggactgggaccttctgacacaatgtggaccatctcagacacaggctg
   :    A  G  W  T  G  L  G  P  S  D  T  M  W  T  I  S  D  T  G  W
 901: gatattaaacattttagggtcatttctggaaccttgggtattgggaacgtgcatatttgt
   :    I  L  N  I  L  G  S  F  L  E  P  W  V  L  G  T  C  I  F  V
 961: ccatcttttgccaaagtttgatccacaaactgttctaaaggtgctttccagctaccccat
   :    H  L  L  P  K  F  D  P  Q  T  V  L  K  V  L  S  S  Y  P  I
1021: caataccctgctgggtgccccccctcatttaccggatgttgctacaacaggatctttccag
   :    N  T  L  L  G  A  P  L  I  Y  R  M  L  L  Q  Q  D  L  S  S
1081: ttacaagttcccacatctgcatagctgcttcagtggaggagagaccctcctcccggagac
   :    Y  K  F  P  H  L  H  S  C  F  S  G  G  E  T  L  L  P  E  T
1141: tctggagagttggaaagccaagacaggactggaaatccgagaaatctatggccagacaga
   :    L  E  S  W  K  A  K  T  G  L  E  I  R  E  I  Y  G  Q  T  E
1201: aacgggaattacctgcagagtttctaggacaatgaaagtcaaaccaggctacctgggaac
   :    T  G  I  T  C  R  V  S  R  T  M  K  V  K  P  G  Y  L  G  T
1261: agccattgtcccttatgatgtccaggtcatagatgagcagggcaatgtcctgccccctgg
   :    A  I  V  P  Y  D  V  Q  V  I  D  E  Q  G  N  V  L  P  P  G
1321: caaggaaggagacatggctctcagggtgaagcccatcaggcctataggcatgttctctgg
   :    K  E  G  D  M  A  L  R  V  K  P  I  R  P  I  G  M  F  S  G
1381: atatgtggacaatcccaagaagacacaggctaatattcgaggagacttttggcttctggg
   :    Y  V  D  N  P  K  K  T  Q  A  N  I  R  G  D  F  W  L  L  G
1441: agaccggggaattaaggatacagaagggtatttccacttcatgggacggacagacgatat
   :    D  R  G  I  K  D  T  E  G  Y  F  H  F  M  G  R  T  D  D  I
1501: cattaattccagtgggtaccgaattggaccttccgaggtggagaatgcactgatggaaca
   :    I  N  S  S  G  Y  R  I  G  P  S  E  V  E  N  A  L  M  E  H
1561: tcctgccgtggttgaaacagctgtgatcagcagcccagaccctatcagaagagaggtggt
   :    P  A  V  V  E  T  A  V  I  S  S  P  D  P  I  R  R  E  V  V
1621: gaaggcatttgtggttctagcccctgagttcctgtcccatgaccaagaccagctcaccaa
   :    K  A  F  V  V  L  A  P  E  F  L  S  H  D  Q  D  Q  L  T  K
1681: ggttcttcaggaacacgtgaagtcagtgacagcaccctacaagtaccccaggaaggtgga
   :    V  L  Q  E  H  V  K  S  V  T  A  P  Y  K  Y  P  R  K  V  E
1741: gtttgtcttagacctgcccaagaccatcacaggaaaaattgagcgagctaaacttcgagc
   :    F  V  L  D  L  P  K  T  I  T  G  K  I  E  R  A  K  L  R  A
1801: caaggaatggaaaacatcaggataagcccaggcccagtgagactcccaaggctcttgctc
   :    K  E  W  K  T  S  G  ..............................
1861: tgtctttcccaatccttttcttatgactccttagtcttcctatagcaatatgaaattatt
1921: catgtagaggcatgtatgtgatttaggtctttgcttggttattgacacagaacaatgaca
1981: cgtttcatgctaaaagaagaaagaaagagggaataagaggtttgaagtcagaaaaggcta
2041: aaacagcaagggaaagaaattgccaattcaagtggtcaggaaggggtagagagaagggga
2101: aagcagaatgaggagagagagagatgagaaggaactggagagaaatagaaaaagggttgt
2161: gtagaaggacactagtgtcttcgccaagttgtgtcaatccttagggacatcatgcccaaa
2221: ggagaaggaaataccacttacagagcatgcccaaggcactgttatacacttgtcatgagt
2281: cctatacaggtcatgagccatcttacttcatctgggtgacatctgcaggctcatcttctg
2341: caccccaaaatgtaccacttcctgtagctcacatttttgttctagctttatttccttga
2401: attaataataaaatcacttcaaaatc
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Fig. 3. The 2426 nucleotide sequence (lowercase) and the predicted 572 amino acid sequence (uppercase) of KS. The putative adenosine monophosphate binding domain is printed underlined and bold. GenBank accession #: AF062389

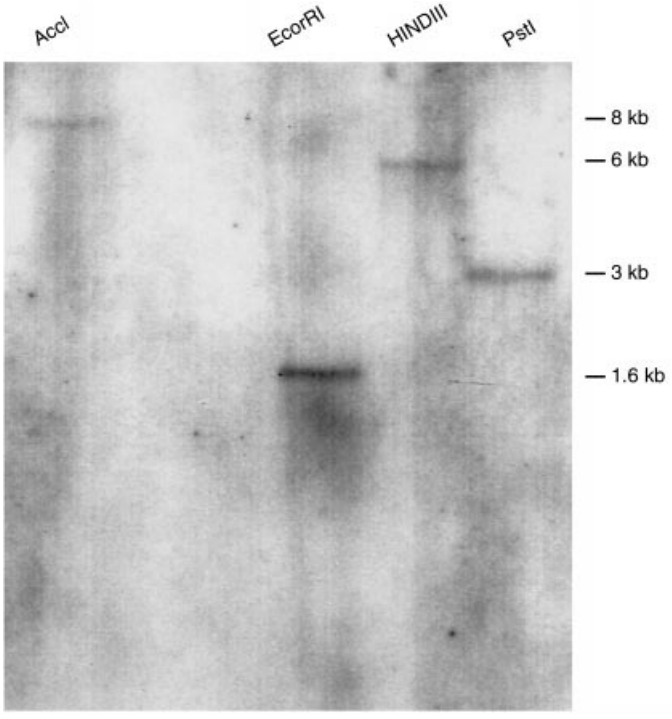

Fig. 4. Southern blot of restriction digests (enzymes indicated on top) of genomic DNA (10 μg per lane) hybridized with the *KS* probe. The approximate size of the bands is indicated.

tubular function does not develop [16, 33]. Our unine-phrectomy experiments demonstrated that the ontogenetic pattern of *KS* expression can to some degree be mimicked in adult rats by inducing tubular hypertrophy. *KS* was downregulated two days after UNX when compensatory renal growth had started, but tended to be normalized seven days after UNX when the growth rate had diminished and tubules were more mature.

The function of *KS* remains unknown and will require further studies. At present, we can only speculate on the possible function of the protein, based on the analysis of its sequence. The similarity to the *SA* gene is not helpful in this regard, as its function is also unknown. However, both *KS* and the *SA* gene exhibit sequence similarity to members of the family of AMP-binding enzymes, notably acetyl-CoA synthases (EC 6.2.1.1.), from several species [27, 34–37]. These enzymes catalyze ATP-consuming reactions that yield acetyl-CoA and pyrophosphate and involve binding to AMP [27, 34–37]. The homology of *KS* and *SA* to those enzymes remains hypothetical in the absence of functional data. Nevertheless, the degree of sequence similarity is rather striking, if one considers the distance of the species involved (spanning several kingdoms). Moreover, both *KS* and *SA* contain the conserved consensus AMP binding domain [28]. Thus, we propose that *KS* and the *SA* gene

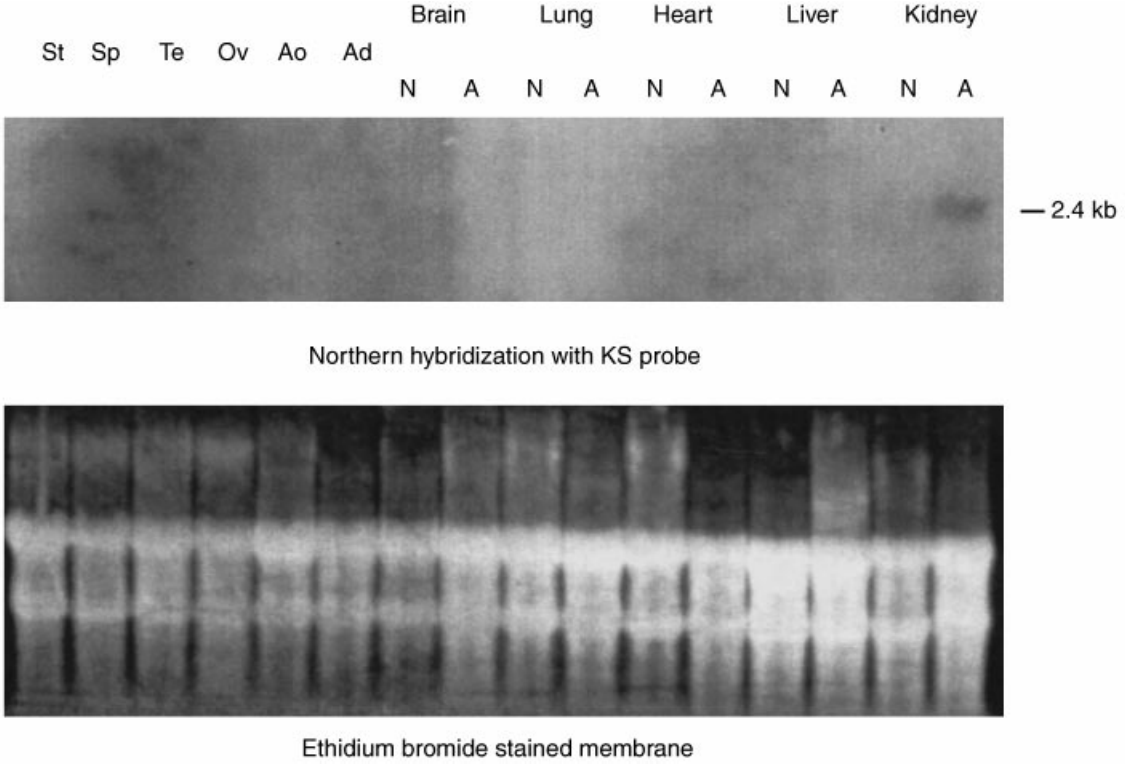

Fig. 5. Northern hybridization of organ-specific and developmentally regulated expression of *KS*. Abbreviations are: St, stomach; Sp, spleen; Te, testis; Ov, ovary; Ao, aorta; Ad, adrenal (all from adult rats); N indicates an organ sampled from newborn, A from adult rat. Twenty micrograms of total RNA were loaded per lane. Note that a hybridization signal with the *KS* probe was observed only in the adult rat kidney.

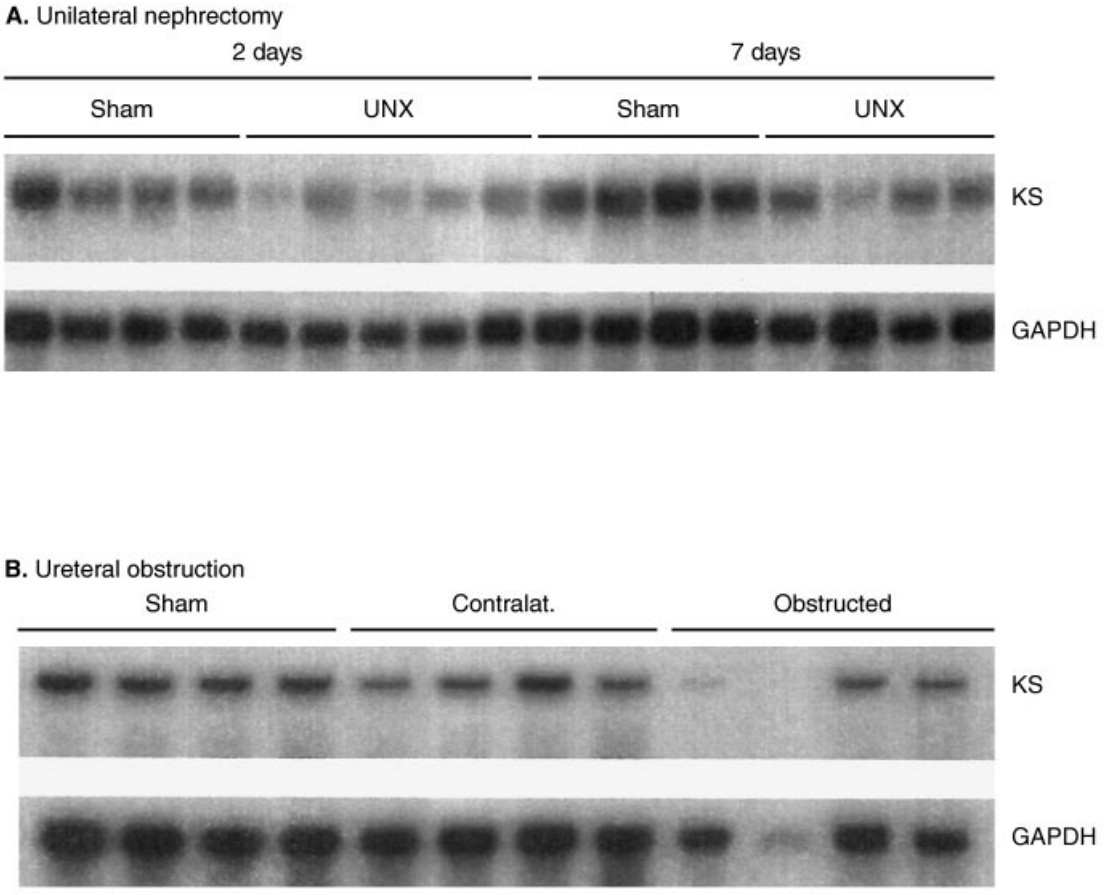

Fig. 6. Expression of *KS* in the kidneys with altered tubular function. (*A*) Unilateral nephrectomy (UNX) was performed in adult rats and RNA extracted from the remaining kidney two or seven days later. (*B*) Unilateral ureteral obstruction was performed on neonates and kidney RNA extracted 28 days later. The abbreviation Contralat. indicates the contralateral, unaffected kidney. Ten micrograms of total RNA were loaded per lane.

define a family of AMP-binding enzymes in mammals. Future experiments will be necessary to test this hypothesis.

The *SA* gene was isolated because of its higher expression in the SHR kidney [22]. In recent years, this finding has been confirmed by several authors [23, 29]. Moreover, the *SA* gene locus cosegregated with hypertension in linkage studies of different animal models [21, 23, 24, 31, 38]. In addition, the *SA* genotype appeared to determine the level of RNA expression [23]. The relationship between human essential hypertension and the *SA* gene is controversial [26, 39]. In spontaneously hypertensive rats, the increased renal *SA* expression was localized to the proximal tubule [30, 40]. In light of these findings, the marked downregulation of *KS* in SHR kidney appears very interesting. As the function of both proteins is unknown, it is not possible to delineate whether decreased or increased expression might contribute to high blood pressure. The renal tubular oxidative metabolism has been reported to be altered in SHR [41]. It is tempting to speculate that downregulation of *KS* might compensate for increased *SA* expression. Decreased *KS* expression was specific for the SHR and not a consequence of high blood pressure itself, as shown by its unaltered expression in renovascular hypertension. However, we cannot exclude the possibility that *KS* downregulation in the SHR might be an epiphenomenon unrelated to hypertension. Cosegregation studies would be necessary to address that question.

From a cDNA library, we obtained one clone with a 285 bp deletion in the open reading frame; this deletion would have resulted in a shorter protein (477 amino acids) without interruption of the reading frame. However, no transcript of corresponding size was found on Northern blots with total or poly(A^+)-purified RNA. Alternative splicing products have been reported for the *SA* gene of rat [24] and human [39]. We performed 3′ RACE PCR with a primer 5′ of the deletion to facilitate detection of putative alternative splicing products. No such product was detected, however, even after hybridization of blotted PCR products with a *KS* probe. Thus, the deletion may have been a cloning artifact.

In conclusion, *KS* is a novel gene with unique tissue-specific expression in the mature kidney. We speculate that

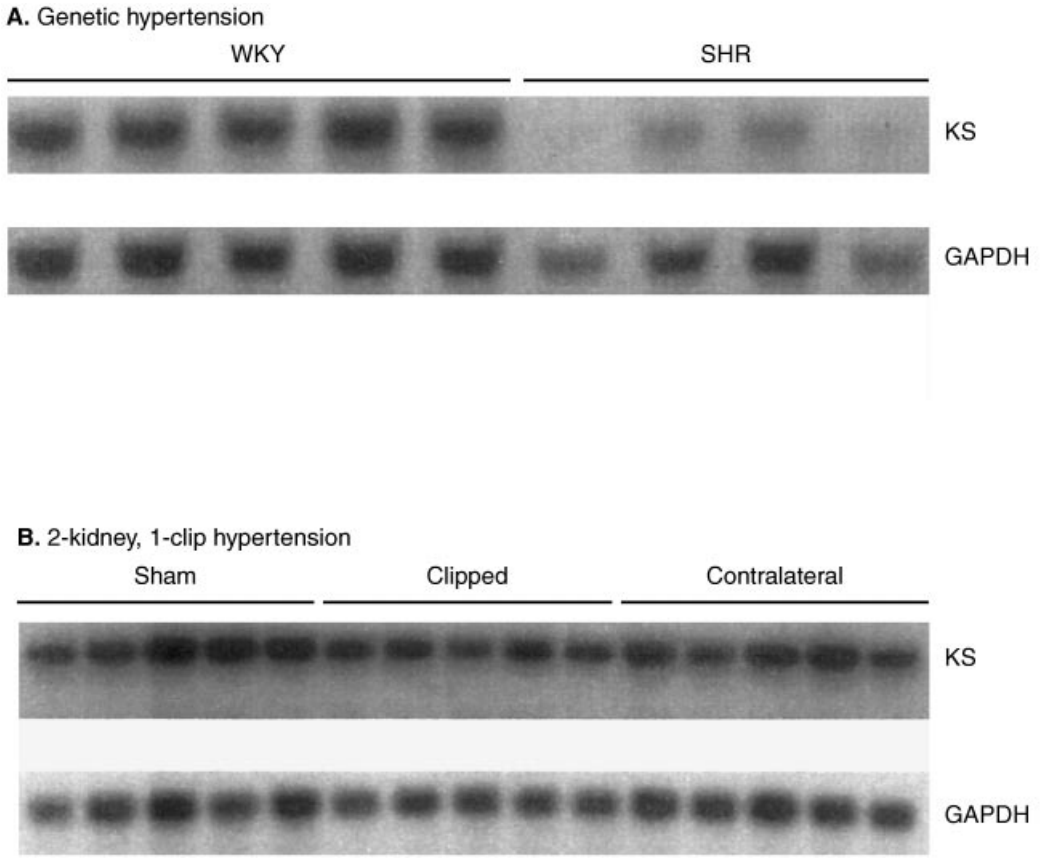

Fig. 7. Expression of *KS* in the kidney of rats with genetic (*A*) or nongenetic (*B*) hypertension. Abbreviations are: SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto control animals. Ten micrograms of total RNA were loaded per lane.

Table 1. Similarity and identity of peptide sequences with the predicted KS amino acid sequence calculated by the algorithm of Needleman and Wunsch [9]

Gene	Identity	Similarity	Gaps	Accession #	Reference
Rat SA gene	51.3%	69.9%	3	A 61209[a]	Iwai and Inagami [22], Harris et al [24]
Human SA gene	53.6%	70.2%	3	D 16350	Iwai et al [26]
Acetyl-CoA synthase (EC 6.2.1.1) from					
Bacterium *(Bacillus subtilis)*	29.2%	51.3%	11	P 39062	Grundy et al [34]
Bacterium *(Escherichia coli)*	28.8%	52.2%	16	P 29212	Fulda et al [27]
Archaebacterium *(Methanothrix soehngenii)*	27.9%	52.4%	20	P 27095	Eggen et al [35]
Yeast *(Saccharomyces cerevisiae)*	27.0%	48.1%	16	Q 01574	De Virgilio et al [37]
Aspergillus *(Emericella nidulans)*	26.1%	46.6%	16	P 16928	Connerton et al [36]
Insect *(Drosophila melanogaster)*	26.1%	50.7%	20	S 52154	(no printed reference available)

[a] We used the longer rat SA sequence according to Harris et al [24] for alignment, which produces a better similarity. The databases contain only the shorter sequence according to Iwai et al [22], accession A 61209.

KS and the previously cloned *SA* gene [22] may define a family of mammalian AMP-binding enzymes with as yet unknown function(s). The expression pattern of *KS* suggests that the gene product may play a role in tubular maturation and function, and, possibly, in genetic hypertension.

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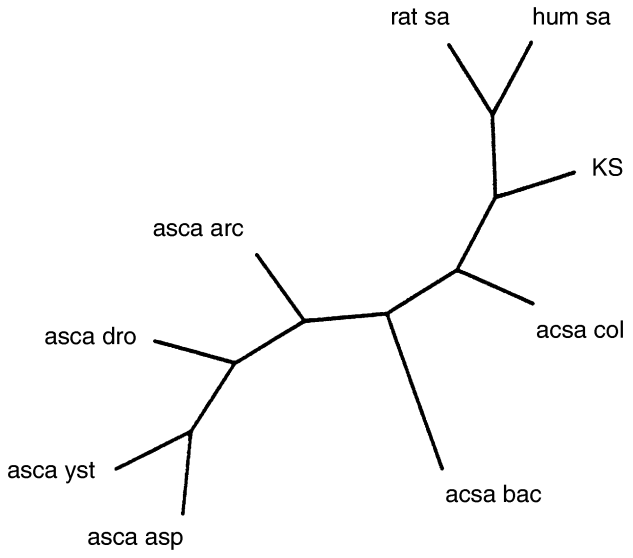

Fig. 8. Hypothetical consensus tree derived from parsimony analysis of 100 random subsets of the peptide sequences of *KS*, rat and human (hum) *SA* gene, and acetyl-CoA synthases (acsa) from *E.coli* (col), *B. subtilis* (bac), archaebacterium (arc), drosophila (dro), *S. cerevisiae* (yst), and *E. nidulans* (asp). Table 1 and text contain further explanation.

APPENDIX

Abbreviations used in this article are: DD-differential display-PCR; GAPDH, glyceraldehyde phosphate dehydrogenase; 2K1C, two kidney, one clip hypertension model; *KS*, kidney specific rat gene; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; *SA*, hypertension-related rat gene; SHR, spontaneously hypertensive rat; UNX, uninephrectomy; UUO, unilateral ureteral obstruction; WKY, Wistar Kyoto rat.

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