

## Molecular cloning of the $\alpha$ -subunit of rat endopeptidase-24.18 (endopeptidase-2) and co-localization with endopeptidase-24.11 in rat kidney by in situ hybridization

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Endopeptidase-24.18 (endopeptidase-2, EC 3.4.24.18, E-24.18) is a Zn-ectoenzyme of rat renal and intestinal microvillar membranes exhibiting an oligomeric structure,  $\alpha_2\beta_2$ . The primary structure of the  $\alpha$ -subunit of E-24.18 has been defined by molecular cloning and its expression mapped in rat kidney by in situ hybridization. A 2.9-kb cDNA coding for the  $\alpha$ -subunit was isolated and sequenced. It had an open reading frame of 2,244 base pairs coding for a type I membrane protein of 748 amino acids. The deduced amino acid sequence showed 87% identity with that of meprin A, a mouse metallo-endopeptidase, sharing common properties with the rat enzyme, and 85% identity with the human intestinal enzyme, 'PABA-peptide hydrolase'. Northern blot analysis revealed the  $\alpha$ -subunit to be encoded by a single mRNA species of 3.2-kb. In situ hybridization performed on rat kidney showed a co-localization of E-24.18 with endopeptidase-24.11 in proximal tubules of juxtamedullary nephrons, suggesting that the two enzymes have similar or complementary physiological functions in kidney.

Membrane protein; Endopeptidase-2; cDNA; Rat kidney; In situ hybridization

### 1. INTRODUCTION

The brush border membrane of rat kidney contains two well-defined metallo-endopeptidases: endopeptidase-24.11 (EC 3.4.24.11, E-24.11) and endopeptidase-24.18 (endopeptidase-2, EC 3.4.24.18, E-24.18) [1–3]. These enzymes are major constituents of the membrane, together constituting about 10% of the microvillar proteins [2]. E-24.11 is a 94 kDa ectoenzyme, a type II integral membrane protein. Its cDNA has been cloned and sequenced in rat [4], rabbit [5], mouse [6] and human [7]. E-24.11 appears to be a major enzyme in the inactivation of many peptides, including substance P and natriuretic peptides [8–12]. E-24.18 is a disulphide-linked oligomer composed of two non-identical subunits ( $\alpha$  and  $\beta$ ) [2]. Like E-24.11, it contains Zn at the active site and is strongly inhibited by chelating agents [2]. However, it is not sensitive to phosphoramidon [1] and can thus be distinguished from E-24.11. E-24.18 also hydrolyses a variety of peptides, including sub-

stance P, bradykinin, luliberin (LHRH) [13], neuropeptide Y [14] and transforming growth factor- $\alpha$  [15], the three last named being resistant to attack by E-24.11.

Here we report the cloning and sequencing of a cDNA encoding one of the E-24.18 subunits. The predicted amino acid sequence suggests that this protein is very similar in sequence to the  $\alpha$ -subunit of mouse meprin A [16], which has recently been shown to be a new member of the astacin family of metallo-endopeptidases [17]. Using in situ hybridization techniques, we have compared the expression of E-24.18 and E-24.11 in rat kidney and suggest that they may have similar or complementary functions in the kidney.

### 2. EXPERIMENTAL

#### 2.1. Amino acid sequencing

The sequence at the N-terminus of the papain-released form [2] was determined using an Applied Biosystems 477A protein sequencer.

#### 2.2. Isolation of the cDNA clones and DNA sequencing

The rat kidney  $\lambda$ gt11 cDNA expression library (Clontech Co., Palo Alto, CA, USA) was screened following standard procedures [18] with a rabbit polyclonal antibody (RRt 151) directed against rat E-24.18 [3]. One positive clone was isolated from  $1.0 \times 10^5$  recombinant lambda phages, subcloned as an *Eco*RI fragment in pUC19 plasmid, and sequenced by the dideoxy chain-termination method [19] using T7 DNA polymerase (Pharmacia, Canada) according to the manufacturer's protocol. The sequencing was performed by walking along the

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**Abbreviations:** E-24.18, endopeptidase-2; E-24.11, endopeptidase-24.11; PABA, *N*-benzoyl-L-tyrosyl-D-aminobenzoic acid hydrolase; bp, base pair; kb, kilobase; SDS, sodium dodecyl sulphate; PAGE, polyacrylamide gel electrophoresis.

cDNA with primers. An oligonucleotide (60-mer) was designed according to the determined sequence and used to probe a rat kidney  $\lambda$ gt10 cDNA library (Clontech Co, Palo Alto, CA, USA) by plaque hybridization. Four positive clones were picked in the first round hybridization but only one survived through to second round screening. The purified  $\lambda$ gt10 insert was subcloned into pUC19 and sequenced on both strands as described above. Procedures for the lambda phage purification and cDNA cloning have been described in detail [18]. Protein sequences were analyzed using the Fasta Programme [20].

### 2.3. Northern blot analysis

Total cellular RNA extracted from rat kidney and poly(A)<sup>+</sup>RNA from rat kidney, skeletal muscle and brain (Clontech Co., Palo Alto, CA, USA) were separated on a 1% agarose-formaldehyde gel and transferred to nitrocellulose membranes. The membranes were hybridized under high stringency conditions [18] with the cDNA isolated from a rat kidney  $\lambda$ gt11 cDNA library previously labelled with [<sup>32</sup>P]dCTP (3,000 Ci/mmol; ICN Biomedicals, Canada) by random oligomer priming. Prior to use, the radiolabelled probe was purified on Nensorb 20 cartridges (NEN-Dupont, Boston, MA) to remove excess unincorporated radioactive nucleotides.

### 2.4. cRNA probes

Rat E-24.18 and E-24.11 cDNAs, cloned in PSPT18 plasmids, were used to generate, by *in vitro* transcription, cRNA probes of approximately 1,000 and 1,350 nucleotides, respectively. Probes were labelled with [<sup>32</sup>S]UTP (1,409 Ci/mmol; NEN-Dupont, Boston, MA) to a specific activity of  $1-4 \times 10^6$  cpm/ $\mu$ g. Probes were separated from unincorporated nucleotides on G-50 spin-columns and their sizes verified by denaturing polyacrylamide-urea gel electrophoresis.

### 2.5. *In situ* hybridization

Male Wistar rats (180-200 g) were perfused with freshly prepared 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4). Pieces of kidney and brain were immersed in 4% paraformaldehyde overnight at 4°C, embedded in paraffin and stored at 4°C. 5  $\mu$ m sections were cut and mounted on microscope slides coated with 2% 3-aminopropyltriethoxysilane. Tissues were stored at 4°C until hybridization, which was essentially as described by Jaffe et al. [22]. Hybridized kidney sections were exposed under X-ray film (X-OMAT, Kodak, Toronto, Canada) for 2 days, dipped in liquid emulsion (K5, Ilford, Toronto, Canada) and developed after 2 weeks of exposure.

## 3. RESULTS AND DISCUSSION

### 3.1. Cloning and sequence analysis of the $\alpha$ -subunit of E-24.18 cDNA

Immunological screening of the rat kidney  $\lambda$ gt11 cDNA expression library allowed the isolation and purification of one positive cDNA clone (clone 6.2). The cDNA fragment was excised, subcloned into the plasmid pUC19 and sequenced. The insert of clone 6.2 was 882 nucleotides long and contained an open reading frame of 294 amino acids, but lacked initiator and stop codons. A second rat kidney cDNA library was screened by plaque hybridization using a synthetic oligonucleotide probe based on the sequence of clone 6.2. This yielded clone 4 of 2,827 bp, including the 3' region, the stop codon and the poly(A) tail but still lacking the initiator codon (Fig. 1). The polymerase chain reaction, using the RACE (rapid amplification of cDNA ends) protocol [23] yielded a cDNA which overlapped with the sequence of clone 4 and extended the 5' end by 101

nucleotides (Fig. 1). An in-frame putative initiator methionine was found at nucleotide-33. The nucleotide sequence of the  $\alpha$ -subunit of E-24.18 cDNA consists of 2,928 bp with an open reading frame of 2,244 bp ending with a TGA stop codon at nucleotide 2,277 (Fig. 2). The cDNA contains 32 nucleotide residues in the 5' non-coding region and a long 3' untranslated region of 652 nucleotide residues which includes a polyadenylation consensus sequence, AATAAA, located at 2,901, followed by a tract of 9 consecutive adenines (Fig. 2). The initiator Met codon at nucleotide 33 was found in a eukaryotic consensus sequence for initiation of translation with an adenine residue in position 3 relative to the AUG [24]. The length of the cDNA (2,928 bp) was consistent with the size of the mRNA (about 3.2 kb) as measured by Northern blot hybridization (Fig. 3, lanes 1 and 3). The small difference in size might be attributed to either a longer 5' non-coding sequence, or a longer poly(A) tail in the mRNA, as compared to the cDNA isolated here. No signal was detected in poly(A)<sup>+</sup> RNA from skeletal muscle and brain (Fig. 3, lanes 2, 4 and 5). When a similar blot was probed with the cDNA fragment of E-24.11, a 3.6-kb mRNA was detected in kidney (results not shown).

### 3.2. Amino acid sequence of the $\alpha$ -subunit and comparison with other metallo-endopeptidases

Translation of the open reading frame predicts a primary translation product of 748 amino acids (Fig. 2). Hydropathy plot analysis of this sequence identified a single putative hydrophobic membrane-spanning domain of 20 amino acids located near the C-terminus of the protein. Another hydrophobic sequence, located near the N-terminus, most likely corresponds to a cleavable signal peptide as already suggested by Jiang et al. for the mouse enzyme [16]. However, the initiator methionine found in our sequence is different from the one proposed by Jiang et al. [16]. No other methionine was found upstream from the ATG codon in our cDNA sequence, suggesting a difference between the sequence of the rat and mouse enzymes. Amino acid sequencing of the N-terminal region of the papain-released form of E-24.18 gave the sequence, NALRDPxxRWKPxi-PYILADNLDL, which was also found in both the papain- and detergent-solubilized forms of the mouse enzyme [16]. Taken together, these findings are consistent with the enzyme being a type-I transmembrane protein anchored by a C-terminal transmembrane domain of 20 amino acids, followed by a short, positively charged, cytoplasmic tail of 9 residues at the C-terminus (Fig. 2). The large extracellular domain starts at Asn-67 and results from the removal of the signal peptide and a pro-region by hydrolysis of the Arg-66-Asn-67 bond. The calculated *M<sub>r</sub>* of the resulting 682-residue protein is 77,873. The extracellular domain contains six Asn residues, each located within the consensus sequence for N-glycosylation, (Asn-X-Ser/Thr). Glycosylation of one

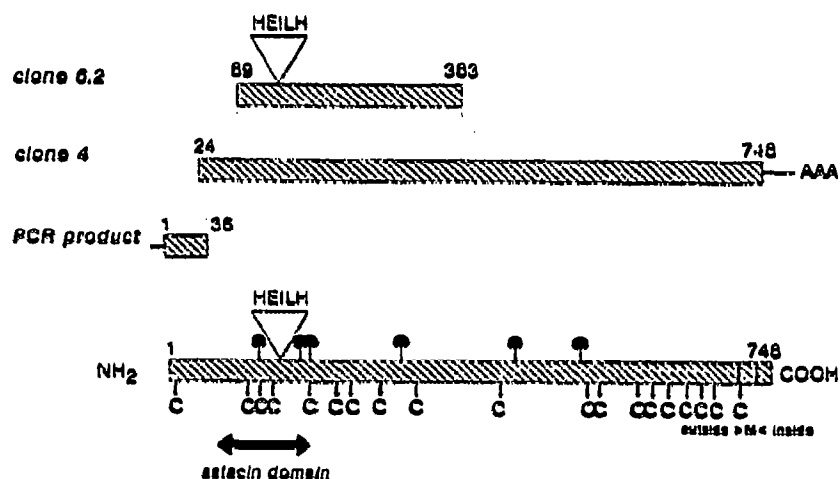


Fig. 1. Schematic model of the primary structure of the  $\alpha$ -subunit of rat E-24.18 and the different cDNA clones isolated. Clones 6.2 and 4 refer to the clones isolated from  $\lambda$ gt11 and  $\lambda$ gt10 libraries, respectively. PCR product was obtained by the polymerase chain reaction according to the rapid amplification of cDNA ends. The boxed areas represent the coding region, while single lines represent the 5' and 3' non-coding regions. Numbers refer to the positions of amino acids starting at the N-terminal Met. The sites of possible *N*-glycosylation are indicated by 'lollipop'. Cysteine residues, C. The membrane spanning region, separating the large extracellular and small cytoplasmic domains is identified by M.

or more of these sites could account for the difference observed between the calculated  $M_r$  and that estimated for the native enzyme by SDS-PAGE under reducing conditions (80 kDa) [2,3]. There are 19 cysteine residues distributed throughout the molecule, of which one is located in the signal peptide and another in the trans-membrane domain.

The His-Glu-Ile-Leu-His sequence, which corresponds to the consensus sequence H-E-X-X-H characteristic of the active site of most zinc metallo-endopeptidases [25], was found in the extracellular domain of the enzyme. Site-directed mutagenesis of E-24.11 has shown that the Glu and the two His residues of this sequence play an essential role in its catalytic activity [26,27]. The His residues act as Zn-binding ligands, while the Glu is involved in acid-base catalysis. It is likely that these residues play a similar role in E-24.18.

Comparison of the amino acid sequence of E-24.18 with that of mouse meprin A revealed 86.8% identity, suggesting a relatively high degree of interspecies conservation, as suggested in previous reports [3,16,28]. The rat enzyme showed 84.4% identity with the partial sequence of the human intestinal brush border 'PABA-peptidase' (*N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid hydrolase, PPH) [17]. Recently, Dumermuth et al. [17] have reported that the partial cDNA sequences of PPH and meprin A showed 30% identity with astacin, a 200 residue zinc metalloproteinase isolated from the crayfish, *Astacus fluviatilis* [29]. Several cysteine residues, which have been shown to participate in the formation of disulphide bridges in astacin [29] were conserved in PPH and meprin A. These authors have suggested that the regions of PPH and meprin A which possess a significant sequence identity with the crayfish protease

be referred to as the 'astacin domain' and propose that these enzymes are members of an 'astacin family' [17]. Our sequence of E-24.18 exhibited 40% identity and 50% similarity (i.e. conservative amino acid substitutions) to astacin in a 129 residue stretch (from 92 to 221) that included the putative active site and several cysteine residues. Like PPH, meprin A and astacin, E-24.18 has an extended zinc binding site (HExxHxxGFxHE; Fig. 2) unique to this family [17]. Thus, E-24.18 is a member of the astacin family. The crystal structure of astacin has been recently solved [30]. The active site is at the bottom of a cleft, the Zn being liganded by His-92, His-96 and His-102 and a water molecule is anchored to Glu-93 and Tyr-149. Our sequence also contains a Tyr at approximately the same position downstream from the Zn-binding motif, possibly serving the same function in catalysis.

### 3.3. Renal expression of E-24.18

We have used in situ hybridization to compare the localization of E-24.18 and E-24.11 mRNA in the kidney. Strong staining was observed in the proximal tubules of the nephrons located in the juxtamedullary region of the cortex (Fig. 4a). The outermost region of the cortex was unstained, but small groups of stained tubules were observed in a radial pattern between the unstained outer and the highly stained juxtamedullary zones. This pattern is identical to that observed by immunohistochemistry using the rabbit polyclonal antibody, RRt151, in rat and mouse kidneys [3]. The same juxtamedullary distribution was obtained using a cRNA probe against E-24.11 mRNA (Fig. 4b). This distribution of the two enzymes may be specific to rodents, since E-24.11 seems to be located in all cortical

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1  GAGGTTCTCTCCAAACACCACTTCACATTCGCAACA  M L N T L F V C L L S L S F S A H I A A I V
22  E I O H L S T G H D H D D V D V G H Q Q H D I S E I H S A A
94  TCC ATT CAG CAT CTT TCG ACT GCA CAT GAC CAC CAT GAT GTA GAC GTT GGT GJA CAA CAG AAG GAC ATT TCA GAA ATC AAC TCA GCT GCA
52  G L N L F Q G D I L L F R T R N A L R D P S R W H P P I P
106  GGC CTG AAC CTT TTT CAA GGG GAC ATC CTT CTG CCC AGA ACC AGA AAT GCC CTG AGA GAT CCG TCA AUC AGA TGG AAG CCC CCC ATT CCA
82  Y I L A D H L D L N A K G A I L N A V E M F R L H S C V D F
276  TAC ATT CTG GCT GAC AAT CTG GAT CTG AAC GCC AAA GGA GCC ATC CTC AAT GCC TTT GAG ATG TTC CGC CTC AAG TCT TGT GTC GAC TTC
112  K F Y E G R S E Y I I F O O F S C W S H V G D O H V G O A A T
366  AAG CCC TAT GAG GGG GAG AGC TCG TAC ATC ATC TTC CAU CAG TTT TCT GGA TCG TCG TCT ATG CTC GGT GAT CAA CAC GTC GGA CAG AAT
142  I S I G E G C D Y K A I I E H R I L N A L G P F E S O S R T
456  ATT TCC ATT GGT GAG GGC TGC GAC TAT AAG GCC ATC ATT GAA CAT GAG ATC CTG CAT GCT CTG GGA TTC TTC CAT GAG CAG TCA AGG ACT
172  D R D D Y Y H I W H A E I T D Y E H N V H T Y D D K T I T
546  GAC CCG GAT GAT TAT CTG AAC ATC TCG TGG AAT GAA ATT ATG ACA GAT TAT GAA CAT AAT TTC AAC ACG TAT GAT GAC AAG ACC ATC ACA
202  D L N T P Y D Y E S L H H Y Q P F K F H K N' E T I P T I T T
636  GAC CTC AAC ACA CCC TAT GAT TAT GAG TCC TTG ATG CAC TAT GGG CCA TTT TCA TTT AAC AAG AAT GAA ACC ATC CCG ACC ATC ACT ACC
232  K I P E F H A G E C I I G A C C C L D CAT TTC AGT GCG AIT GAT TTA ACG AGA CTG AAT CCG ATG TAC AAC TGC ACC
726  AAG CTC CCT ATT GAG GGC ATC ATC GCA CAG CCG CTG CAT TTC AGT GCG AIT GAT TTA ACG AGA CTG AAT CCG ATG TAC AAC TGC ACC
262  R T H T L L D H C A F E K T N I C G H I O G T R D D A D W V
816  AGA ACA CAT ACC CTG CTG GAC CAC TGT GGU TTT GAG AAC ACC AAC ATC TGT GJA ATG ATC CAG GGG ACC AGA GAT GAT GCT GAC TGG GTT
292  H E D S E O P Q O V D H T D Y G C C G T X E A A G G T G C T G C T A C T T T C A T T A T T A A T A C C A G C
906  CAT GAG GAC AGC AGT GAC CCT GGA CAA CTG CAT CAC ACC TTG GTG GCA CCG T X E A A G G T G C T G C T A C T T T C A T T A T T A A T A C C A G C
322  E G V T C E V A L L E T C R I L Y P K R N O Q C L Q P P Y A H M
996  TCG GGC GTC ACC GGA GAG GTA GCC CTT CTG GAG TCT CCG ATC CTT TAC CCC AAG AGG AAG CAG CAG TGT TTG CAG TTT TTT TAT AAT ATG
352  T C G S P E D R L L I W V R R C D N T G L V R O L A K T F
1086  ACA GAG AGC CCT TCA GAG AGA CTC CTC ATC TGG GTC AGA AGA GAC GAC AAC AAG GGC AAC GTC GGC CAG CTC GGC AAC ATC CAG ACT TTT
382  Q G D S D H N W K I A H V T L N E K K K P R Y V F O G T X K G
1176  CAA GGA GAT TCT GAT CAC AAC TGG AAA ATT GCT CAC GTC ACA CTC AAC GAA GAG AAG AAA TTT CCG TAT GTT TTC CAG GGT ACC AAA GGT
412  D P G H E D G I V L D T L T L T P C P T G V W T I R N'
1266  GAC CCT GGT AAC TCA GAT GGC GGT ATT TAC CTT GAT GAC ATC I ACT CTG ACA GAA ACC GCG TCG CCT ACA GGG GTT TGG ACC ATC CCG AAT
442  I S O V L E N T V K C D R L V E P K P V N S E G V G F G V T
1356  ATC TCC CAG CTC CTT GAG AAC ACA GTG AAA GGG GAC AAG CTC GTG AGC CCG AGG TTC TAC AAT TCC GAG GGT TAT GGT TTT GCG GTC ACC
472  L Y P F N G R A T C A E H E G Y L G G L A F H L Y S G D N D V I L K
1446  TTG TAC CCC AAT GGT AGA ATC ACC AET H E T C G TAC CTG GGA CTC CCT TTC CAU TTU TAC ACT GGG GAT AAC GAT GTG ATT CTU GAG
502  W P V K H E Q A I M T I L D C O E P U A K N H M S L S L A H F T
1536  TGG CCA GTG GAG AAC GAG CAG GCC ATA ATG ACC ATC CTG GAC CAG GAA CCG CAT GCC AGG AAC AGG ATG TCC TTG AGT TTG ATG TTC ACT
532  T S K Y O T S T C A A I A A T G G C T C T A T C T W D A C A C C C A A C C A A G G T T G G A G T C T A T G A T A A G G A C T G T G A T
1626  ACT TCC AAC TAC CAA ACA TCT TCA GCT ATA AAT GGC TCT GTC ATC TGG GAC ACC CCA ACC AAG GTT GGA GTC TAT GAT AAG GAC TGT GAT
562  C P R E I D W G W G O A I E H O M I L H R H N V P L X D D T L I
1716  TGT TTT AGA AGC ATT GAC TGG GGT TGG GGT CAG GCC ATT TCC CAC CAG ATG CTG ATG AGA AGA AAT TTC CTT AAA GAT GAC ACC CTC ATC
592  I F V D F K D C L T H L R O T E V L T P I S E R S V I S E R G L L
1806  ATT TTC GTG GAT TTT AAA GAC CTT ACC CAC TTG AGG CAG ACT GAA GTC CCC ATT TTC ACC AGA AGC GTC ATA CCC CGA GGA CTC CTT CTG
622  Q G Q E P L A L G D S R I A H H E L S L P R H L D O R O P S
1896  CAA GGC CAG GAG CCT CTT GCC TTG GGA GAC TCC AGA ATA GCC ATG ATG GAG GAA TCC CTG CCA AGA AGG CTG GAC CAG AGA CAG CCC AGC
662  R P K R C S V E H T G T G P H F G A C C A C A A C T O U C C A C A G T A C T T C A G A C C C A T G A C C C A A C C C T T G C
1986  AGA CCA AAG CCG TCA GTG GAA AAT ACT GGT CCC ATG GAG GAC CAC AAC TGG CCA CAG TAC TTC AGA GAC CCA TGT GAC CCA AAC CCT TGC
682  Q H E G T C V H V K G H A E C R C V S G H A F P Y T G G E H C
2076  CAG AAT GAA GGC ACC TGT CTG AAC GTA AAG GGA ATG GCC AGC TCG AGC CTC TCT GGG CAT GCC TTT TTC TAC ACA GGG GAG CCG TGT
712  Q A H H V H G S L L G L L I G C I T A L I F I T F S N
2146  CAG GCT ATG CAT GTG CAC GGA AGC TTG CTG GGC CTG CTC ATT GCG TCG ATC ACT GCT TTG ATC TTC TTG ACC TTC ATC ACA TTC TCC AAC
742  T Y O K L R O
2256  ACT TAC CAA AAG CTG AGG CAG TGA GCT GAT CTC CAC TAG ACT GCA GAA GGG CCT CCT TCA CCT AGA CTT TCC TTC CAG TAG TCA GTA TAA
2346  TTT GGG GAT ATT CTT CAT CAA ACA TGT TTG AAA GAG CCT CCA AAA CCA AAC CCC CAA ACC TCA TGT GGG GCT CTT GCC ACT CCA TTT CTG
2436  TAC AGT TCT GCT ATC CTT CTA TGA GTT TCT GGT GAA TCC ATT ATG TCC TAA GAT AAC AGT CAT TGC ATT TTA CTA TAA ACA CTT TGC TTG
2526  CTT GAC CTT CTC CCC TTA AAT CAC CAA GTC TAG TTG GGT AGG AGC ATG TTA T X C TCT GCT ACC ACA TTG ACC CCA GTG CTA GAA GGA TAC
2616  CTT ACT GGT GTA TTG TGG GTA CTC AGT AAG CAT TAA ATG AAT GAA CCA ATA AGC AAG TGA ATA GCT GTG GTA TAA TAA GTC TTT TTT TCT
2706  TCC TCA TAT TGT AAA ACA TAT GAT TAA GTC TAT TTG CAG GTG TAT CAG AAC CJA ATA CCC AGT TTA CAC TAG ATG GGA AAA CTA CTG TCT
2796  TAT TGC TCA TGT AGT TTC TTG TGA AAT GAT TTC ACA GAT TCC TCT GGT CAG CTG AGT TTC CAT GGC ATG TAC TTT TTT CTT GTT CTG ATT
2886  TTA AGG AAA AGC CTT AAT AAA ACC ATT AAA TAT GAA AAA AAA A

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Fig. 2. Combined nucleotide sequence of clones 6.2, 4 and the PCR product, with the deduced amino acid sequence of the  $\alpha$ -subunit of rat E-24.18. The hydrophobic domains are underlined. The arrow indicates the possible cleavage site of a signal peptide. The solid triangle indicates the N-terminus of the papain-released form of the protein. Asterisks mark the potential N-glycosylation sites. The putative extended zinc-binding site appears in bold. In the 3' untranslated region the putative polyadenylation signal is indicated by a double line.

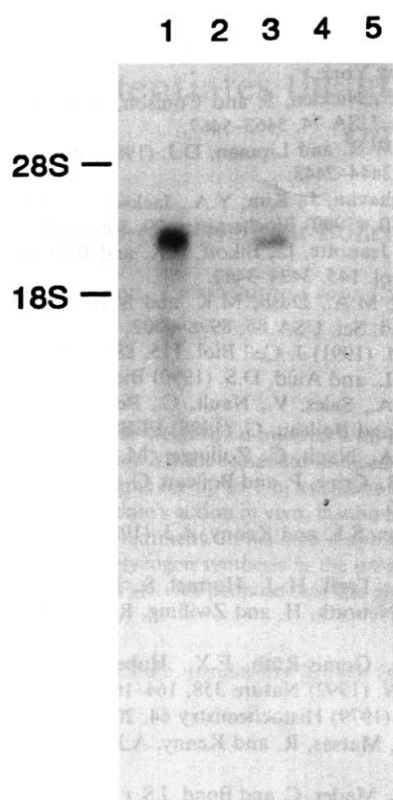


Fig. 3. Northern blot analysis of rat RNA. RNA samples were electrophoresed on a 1% agarose-formaldehyde gel, transferred to nitrocellulose membranes and hybridized with  $^{32}$ P-labelled cDNA (clone 6.2), as described in section 2. 10  $\mu$ g of total RNA isolated from kidney (lane 1), 1  $\mu$ g of poly(A) $^{+}$  RNA from skeletal muscle (lane 2), 1  $\mu$ g of poly(A) $^{+}$  RNA from kidney (lane 3), 1 and 2  $\mu$ g of poly(A) $^{+}$  RNA from brain (lanes 4 and 5, respectively). The blot was exposed for 10 days. Positions of 28 S and 18 S rRNA are indicated on the left margin.

nephrons in some other species [31,32]. No labelling was observed with E-24.18 sense RNA (Fig. 4c), nor with E-24.11 sense RNA (not shown). Consistent with the Northern and Western blots [3], no E-24.18-specific staining was observed in the rat brain by in situ hybridization after 3 weeks of exposure (not shown). The distribution of E-24.18 in rat kidney closely resembles that of meprin A in mouse kidney [3,33]. The co-localization of mRNAs for both E-24.18 and E-24.11 in the proximal tubules of the juxtamedullary nephrons suggest that these enzymes may have similar or complementary roles in the degradation of peptides and proteins filtered at the glomerulus. The preference of E-24.18 is for longer peptides and even some proteins [2,15], while E-24.11 is more efficient with a wide range of small biologically-active peptides [13,34].

Understanding the primary structure of E-24.18, especially details of its active site, should facilitate the design of highly potent and specific inhibitors. Such compounds would be valuable in elucidating the physiological role of this metallo-endoropeptidase.

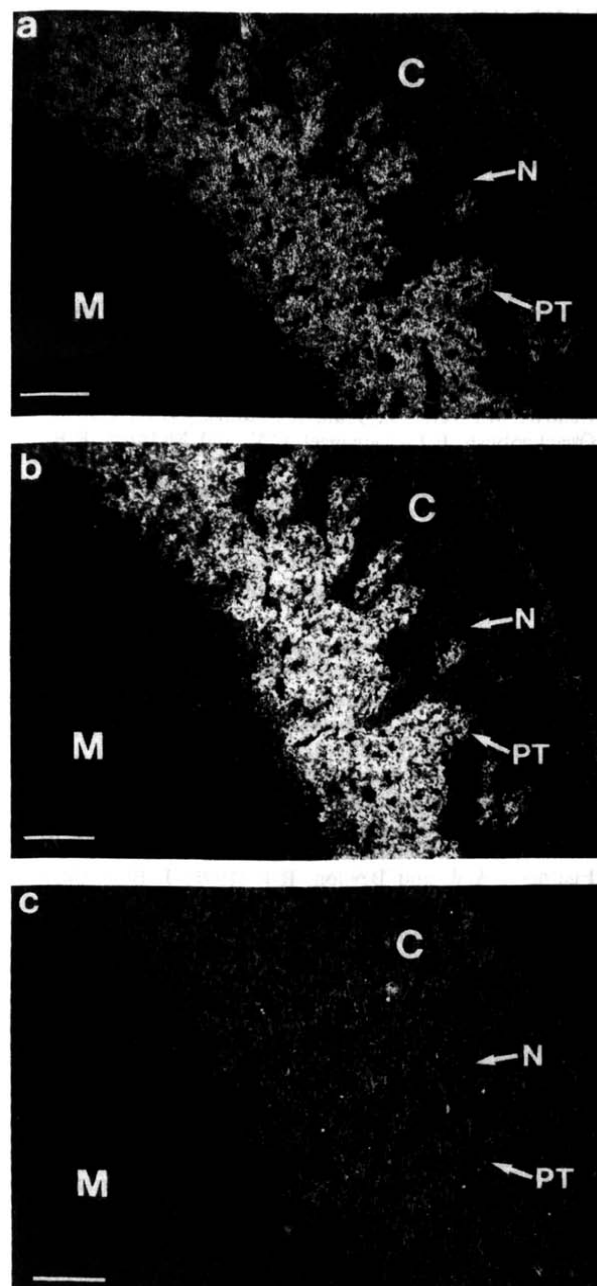


Fig. 4. In situ hybridization of adjacent longitudinal sections of rat kidney. Sections (5  $\mu$ m thick) were hybridized with  $^{35}$ S-labelled anti-sense cRNA for E-24.18 (a), E-24.11 (b) and sense cRNA for E-24.18 (c). C, cortex; M, medulla; PT, proximal tubules; N, nephron. Bars = 500  $\mu$ m.

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