Structure and mRNA expression of a bovine trp homologue related to mammalian trp2 transcripts

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Abstract Mammalian homologues of the transient receptor potential (trp) gene product from *Drosophila melanogaster* function as Ca^{2+}-selective or non-selective cation channels. Complementary DNA was isolated from a bovine testis cDNA library which encodes bovine trp2 (btrp2), a protein of 432 amino acid residues comprising four predicted transmembrane segments. Btrp2 mRNA is expressed in bovine testis, spleen and liver but not in brain, heart, adrenal gland or retina. In bovine testis expression of btrp2 mRNA is restricted to spermatocytes but is not present in spermatogonia, Leydig or Sertoli cells suggesting that btrp2 may contribute to the formation of ion channels in sperm cells.

Key words: Trp homologue; Cation channel; Capacitative calcium entry; Store-operated channel; Testis

1. Introduction

The transient receptor potential (trp) and trp-like (trpl) gene products from *Drosophila* photoreceptor cells have been implicated to be cation channels involved in the invertebrate phototransduction process [1,2]. When expressed in vitro the trp channel appears to be activated by the depletion of intracellular Ca^{2+} stores and is primarily permeable to Ca^{2+} [3,4] whereas the trpl protein is a non-selective cation channel [5,6]. In vivo, both proteins may assemble to homo- or heteromultimeric complexes.

Recently, the cDNAs of several mammalian homologues of the dipteran trp/trpl proteins have been cloned. When expressed in common eukaryotic expression systems these cDNAs encode cation channels which are highly Ca^{2+}-selective like bCCE1 [7], or non-selective like htrp1 [8,9], htrp3 [9–11] and mtrp6 [12]. In addition to these full-length clones several cDNA fragments have been amplified from various mammalian tissues and species on the basis of sequence homology, which may represent parts of additional trp-related gene products.

Here we report the molecular cloning and characterization of bovine trp2 (btrp2), a trp homologue, and its cellular expression in testis. A part of its primary structure is most similar to the deduced amino acid sequence of a 315 bp mtrp2 cDNA fragment recently amplified from mouse liver [9] and the human expressed sequence tags (ESTs) T67673 and H61599. However, its open reading frame lacks the multiple stop codons found in the human ESTs which are thought to be encoded by the pseudogene htrp2 [13].

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2. Materials and methods

2.1. Isolation and sequencing of cDNA clones

The human expressed sequence tag T67673 was identified by searching databases for nucleotide sequences homologous to trp using the Fasta algorithm of the Heidelberg Unix sequence analysis resources (HUSAR). A 778 bp *Eco*RI fragment of the human EST clone T67673 was used to screen an oligo(dT)-primed bovine testis cDNA library and clone 781 was isolated and sequenced. A cDNA library was constructed by reverse transcription of testis poly(A)$^+$ RNA using the oligodeoxynucleotide 53 (Fig. 1A,B, complementary to nt 624–643 of clone 781) as specific primer. The oligodeoxynucleotide 52 (Fig. 1A,B, complementary to nt 577–606) was phosphorylated using T4 polynucleotide kinase and [γ-^{32}P]ATP and served as probe to screen this library. Four independent clones including clones E32 and E51 (Fig. 1A) were isolated and sequenced. To further elongate the cDNA in the 5′ direction two additional specifically primed cDNA libraries were constructed using primers 65 (complementary to nt −32 to −15) and 75 (complementary to nt 120–137) and screened with the oligodeoxynucleotides 66 (complementary to nt −78 to −49) and 86 (nt −68 to −49), respectively. No clones were obtained from these libraries which elongate the sequence given by clone E32. To confirm this finding, a random-primed λZAP cDNA library was constructed and 3.2×10^6 additional clones were screened with a 174 bp *Sty*I (−187)/*Bam*HI (−357) fragment of clone E32 (probe 1, Fig. 1A,B) and a 492 bp *Sst*I fragment (nt 928–1420) of clone 781 (probe 2, Fig. 1A,B). Several clones including clones 4, 12, 15, 16 and 29 were isolated and sequenced. The overlapping cDNA sequences were used to deduce the full-length btrp2 nucleotide sequence. The presence of the full-length transcripts within poly(A)$^+$ RNA from bovine testis was confirmed by PCR amplification using primers 100 (complementary to nt 1279–1299) and 200 (nt 1–22) corresponding to the 5′- and 3′-nucleotide sequences of the protein coding sequence of btrp2 (Fig. 1A,B) and reverse-transcribed bovine testis cDNA as template. The amplified cDNAs were subcloned and sequenced. Six out of eight independent clones including clone WW26 contained the 1296 nt sequence encoding the complete btrp2 protein. In addition two potential splice variants of btrp2 transcripts were identified containing an insertion of 74 nt or a deletion of 163 nt, respectively. All cDNA clones were sequenced on both strands using the dideoxy sequencing method [14] with radioactive [^{35}S]dATPαS or infrared dye-labeled primers and an automated sequencer (Licor).

2.2. Northern blot analysis

Total RNA was isolated from bovine and human tissues as described [15]. Northern blot analysis was performed as described [7]. Blots were hybridized with [α-^{32}P]dCTP-labeled probes for 20 h at 42°C. Complementary DNA probes were the 596 nt *Apa*I fragment of btrp2 and the 778 nt *Eco*RI fragment of T67673, labeled by random priming. Filters were exposed overnight to X-ray films at −80°C.

2.3. In situ hybridization

Tissue slices (8 μm) were mounted to siliconized glass slides, air dried and fixed in freshly prepared 4% (w/v) paraformaldehyde (PFA) in 1×PBS buffer (140 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4) at 4°C for 25 min. The slides were incubated in 3×PBS (5 min, 21°C), 1×PBS (10 min, 21°C), dehydrated in the presence of increasing concentrations of ethanol, air dried and stored at −80°C. Before hybridization, sections were rehydrated and incubated in proteinase K (1 μg/ml in Tris-HCl, 50 mM EDTA, pH 7.5) at 37°C for 30 min. Following a refixation step the sections were incubated in 100 mM triethanolamine, pH 8.0

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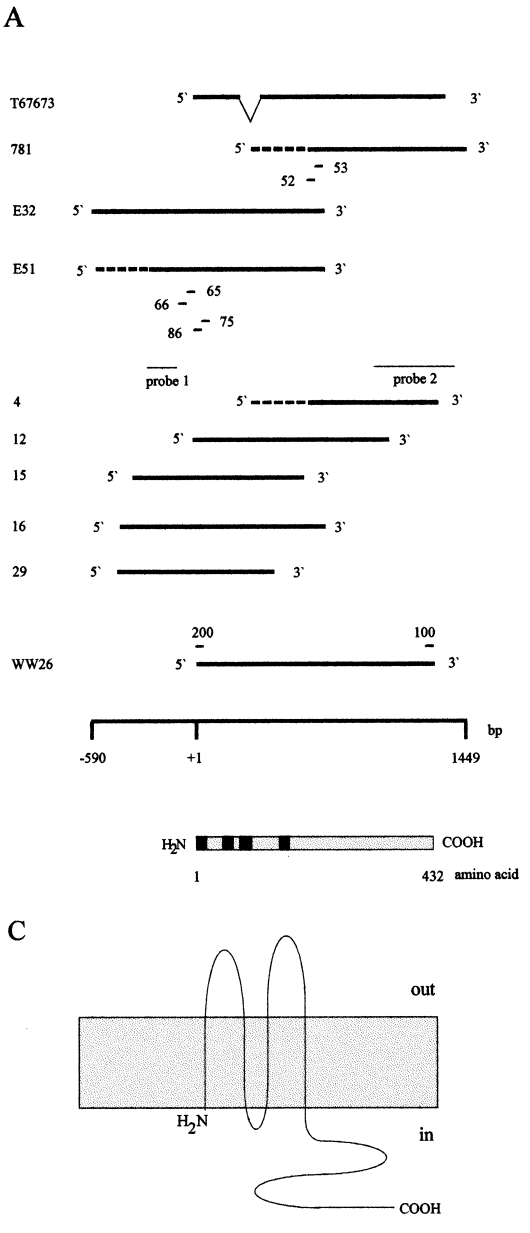

B

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                                                          TTCCATGCTGCC    -601
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TGTTCCCCTGCTCTCCTTCACCTCCTACAGCACTGACCACTCTGGGCTGTAACAACTTGG    -421
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TAGGATCCCACTGGCACATGATAGGAGCTTAATCAGTGTTTATTCAGTAGTGATTGCTGG    -301
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TGGTGACAGCATCACAGAGGTCTTAAGAGAGCTGTGAAACTCACTGTCCCCGTTAGAGAA    -121
GGCTTCGGAGAGGAGGTGAGATTTGTCCTGGGTCGCAAAGGCTTCCTGTGGTTTGAGTGT     -61
AAGGAAGTGTGGATCGAGGGCCTGCGCAGTTACCTCCTGGACTGGTGGAACTTCCTGGAT      -1
ATGGTCATCCTCTCCCTGTACCTGGCGGCCTTCACGCTGCGCCTCCTCCTGGCCGGGCTT      60
 M  V  I  L  S  L  Y  L  A  A  F  T  L  R  L  L  L  A  G  L      20
GCACACAAGCACTGCCGGGATGCCCCTGATGGGGCTGCCTGCCACTACTTCACCTCGGCT     120
 A  H  K  H  C  R  D  A  P  D  G  A  A  C  H  Y  F  T  S  A      40
GAGCGGAGTGAGTGGCGCACGGAGGACCCCCAGTTCCTGGCTGAGGTGCTCTTCGCTGTC     180
 E  R  S  E  W  R  T  E  D  P  Q  F  L  A  E  V  L  F  A  V      60
ACCAGCATGCTCAGCTTCACCCGCCTGGCCTCCATCCTGCCGGCCCACGAGTCGCTGGGC     240
 T  S  M  L  S  F  T  R  L  A  S  I  L  P  A  H  E  S  L  G      80
ACCCTGCAGATTTCCATGGGAAGGATGATTGACGACATGATCCGGTTCATGTTCATCCTC     300
 T  L  Q  I  S  M  G  R  M  I  D  D  M  I  R  F  M  F  I  L     100
ATGATCATCCTGACCGCCTTCCTCTGCGGCCTCAACAACATCTACGTGCCTTACCAGGAG     360
 M  I  I  L  T  A  F  L  C  G  L  N  N  I  Y  V  P  Y  Q  E     120
ACCGAGCGGCTGGGCAATTTCAATGAGACCTTCCAGTTTCTGTTCTGGACGATGTTTGGC     420
 T  E  R  L  G  N  F  N  E  T  F  Q  F  L  F  W  T  M  F  G     140
ATGGAGGAGCACAGTGTGGTGGACATGCCTCAGTTTCTGGTGCCGGAATTCGTTGGCCGG     480
 M  E  E  H  S  V  V  D  M  P  Q  F  L  V  P  E  F  V  G  R     160
GCCCTCTATGGCATCTTTACCATCGTCATGGTGATTGTGCTGCTCAACATGCTCATTGCC     540
 A  L  Y  G  I  F  T  I  V  M  V  I  V  L  L  N  M  L  I  A     180
ATGATCACCAACTCATTCCAGAAGATTGAGGACGCTGCTGACGTGGAGTGGAAGTTTGCT     600
 M  I  T  N  S  F  Q  K  I  E  D  A  A  D  V  E  W  K  F  A     200
CGCTCAAAACTCTACCTGTCCTACTTCCGAGAGGGCCTGACATTGCCCGTGCCCTTCAAT     660
 R  S  K  L  Y  L  S  Y  F  R  E  G  L  T  L  P  V  P  F  N     220
ATCCTGCCCTCCCCCAAGGCCATCTTCTACCTGCTCAGGAGAGTTTTCCGCTTCATTTGC     720
 I  L  P  S  P  K  A  I  F  Y  L  L  R  R  V  F  R  F  I  C     240
TGTTGCCACTTCTGCTGCAAAACCAAGAAGCCAGACTATCCCCCAATCCCCACCTTTGCC     780
 C  C  H  F  C  C  K  T  K  K  P  D  Y  P  P  I  P  T  F  A     260
AATCCTGGGGCAGGGGCAGGGCCAGGGGAGGGAGAGCGTGGATCCTACCGGCTTCGGGTC     840
 N  P  G  A  G  A  G  P  G  E  G  E  R  G  S  Y  R  L  R  V     280
ATCAAGGCCCTGGTGCAACGCTACATAGAGACTGCCCAGCGGGAGTTTGAGGAGACTCGT     900
 I  K  A  L  V  Q  R  Y  I  E  T  A  Q  R  E  F  E  E  T  R     300
CGGAAAGACCTGGGCAACAGACTGACAGAGCTCACCAAGACGGTGTCTCGACTGCAGAGT     960
 R  K  D  L  G  N  R  L  T  E  L  T  K  T  V  S  R  L  Q  S     320
GAGGTAGCAGGTGTGCAGCGGGCTGTGGTGGAGGCAGGGCCACGCCGGCCGCCCGGAGGA    1020
 E  V  A  G  V  Q  R  A  V  V  E  A  G  P  R  R  P  P  G  G     340
GCCAGCGTCCTCAGTCGCTACATCACCCGAGTGCGCAACAGCTTCCAGAACCTGGGGCCC    1080
 A  S  V  L  S  R  Y  I  T  R  V  R  N  S  F  Q  N  L  G  P     360
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 P  I  P  E  T  P  E  L  T  V  P  A  T  V  G  T  Q  E  S  S     380
GAAATAGGGCTTCCGGATGCTGGAGGGGCTCAGGCTCCAGCCTCTGGGGAATCTGGCCCC    1200
 E  I  G  L  P  D  A  G  G  A  Q  A  P  A  S  G  E  S  G  P     400
TCCTCCCCAGCTCACGTGTTAGTGCACAGGGAGCAAGAATCAGAGGGGGCTGGGGACCTG    1260
 S  S  P  A  H  V  L  V  H  R  E  Q  E  S  E  G  A  G  D  L     420
CCCCAGGAGGCAGACCTGGGAGCCAAGGAGGGGACCTGATGCTGGGGGAGGGGGTCATTT    1320
 P  Q  E  A  D  L  G  A  K  E  G  T  *                         432
CCCTTCTGTTGCTGACTAGAGCAGCCTAGATAGCTGAGGGCAGGGTCACATCGTGGGAGC    1380
CCCCGCTGCTTTCCTTGCCTCAATAAAGCCTCTGCTCTGGAGCTCAGATGTCTCCAGAGG    1440
ATGTGAAAA                                                       1449
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Fig. 1. Cloning strategy (A), nucleotide and deduced amino acid sequence (B) and model of transmembrane topology (C) of btrp2. A: Nucleotide sequences are indicated by black lines and the deduced amino acid sequence by gray bars. The predicted transmembrane domains are indicated by black boxes. In addition to the human EST T67673 the following bovine cDNA clones are shown: 781 (isolated from an oligo(dT) cDNA library using the *Eco*RI fragment of T67673 as probe); E32, E51 (isolated from a specifically primed cDNA library using oligodeoxyribonucleotides 53 and 52 as specific primer and probe, respectively); 4, 12, 15, 16, 29 (isolated from a random-primed λZAP cDNA library screened by probes 1 and 2); WW26 (RT-PCR-amplified using primers 100 and 200). Dashed lines in clones 781, E51 and 4 are sequences resulting from incomplete RNA processing. The first ATG triplet in frame downstream of a stop codon was selected as translation start site leading to a deduced primary structure of 432 amino acids. Specifically primed cDNA libraries using oligodeoxynucleotides 65 and 75 as primers and 66 and 86 as probes did not lead to further elongation of the cDNA indicating that the 5′ end of E32 represents the 5′ end of btrp2 transcripts in bovine testis. B: Nucleotide and amino acid residues are numbered on the right with the first residue of the ATG triplet encoding the initiating methionine and the initiating methionine itself, respectively. The stop codon in frame is indicated by an asterisk. The sequence of the btrp2 cDNA was submitted to GenBank. C: Predicted transmembrane topology of btrp2.

(TEA) for 10 min followed by two incubations in 0.25% acetic anhydride in TEA (10 min, 21°C), dehydrated and air dried for at least 1 h. RNA probes were synthesized with [α-^{33}P]UTP using the linearized recombinant plasmid pcDNA3 WW26 as template for transcription with SP6 polymerase (antisense) or T7 polymerase (sense). The plasmid pcDNA3 WW26 covers the complete protein coding region of btrp2. Labeled cRNAs (specific activity 1–2×10^9 dpm/μg) were purified by gel chromatography, the probe length was adjusted to ∼200 nucleotides by alkaline treatment [16], precipitated and resuspended in 0.1 M DTT. 7–10 μl hybridization solution (50% deionized formamide, 1×Denhardt's solution, 0.5 mg/ml yeast tRNA, 10% dextransulfate, 10 mM DTT, 30 mM NaCl, 5 mM EDTA, 10 mM Na_2HPO_4/NaH_2PO_4, 20 mM Tris-HCl, pH 6.8) containing the denatured cRNA probes (∼5×10^6 cpm/μl) were applied to each slice and incubated for

16 h at 55°C [16]. After hybridization slices were washed twice with buffer A (30 mM NaCl, 10 mM NaH_2PO_4/Na_2HPO_4, 5 mM EDTA, 10 mM DTT, 50% formamide, 20 mM Tris-HCl, pH 6.8) for 60 min each, followed by incubation in 500 mM sodium chloride, 5 mM EDTA, 10 mM Tris-HCl, pH 7.5 (NTE) at 37°C for 15 min. After treatment with RNase A (20 μg/ml in NTE) for 30 min at 37°C and an additional incubation in NTE at 37°C for 15 min the slides were washed in buffer A for 60 min at 65°C. The slides were washed twice in 2×SSC (15 min, 21°C), followed by two washes in the presence of 0.1×SSC for 15 min at 65°C and at 21°C, respectively. After dehydration the slices were exposed to X-ray films for 5 days. The slides were dipped in nuclear track emulsion (NTB-2, Kodak) and exposed for 2 weeks. The emulsion was developed for 2.5 min (Kodak developer D-19) at 18°C and fixed (Kodak fixer) for 5 min at 18°C. Slides were washed, stained with hematoxylin and eosin, transferred into xylene and mounted with Eukitt. Photographs were made with a Contax 167 MT camera adapted to an Axioskop microscope (Zeiss). Kodak TMAX 100 pro- films were used for prints which were scanned and processed using Corel Photo Paint.

3. Results

3.1. Primary structure of btrp2

The human EST T67673, which was isolated from a human cDNA library, comprises 1336 nucleotides and a poly(A)$^+$ tail of 170 residues (Fig. 1A). It includes the sequence present in the EST database (nucleotides 1–412) as well as the nucleotide sequence recently suggested to represent the human trp2 homologue (nucleotides 1–1196, GenBank accession number X89067). Its primary structure contains three stop codons in frame which led to the assumption that htrp2 might be a pseudogene product [13]. In many cases the pseudogene and its closely related functional gene are present in one organism and, therefore, we looked for the expression pattern of htrp2-related transcripts using Northern blot analysis. The 778 nt *Eco*RI fragment of the T67673 cDNA used as probe hybridized to mRNA from bovine testis, whereas no hybridization signals could be detected in adrenal gland or human placenta mRNA (Fig. 3A). An oligo(dT)-primed cDNA library was constructed using poly(A)$^+$ RNA isolated from bovine testis and thereafter screened with the same probe used for Northern analysis. Several cDNA clones of different length were obtained including clone 781 (Fig. 1A). Sequence analysis revealed that this clone comprises 1123 bp encoding a protein sequence similar to the htrp2 sequence and was therefore termed bovine trp2 (btrp2). Btrp2 differs from htrp2 in that its open reading frame lacks the stop codons found in the human cDNA. At these positions arginine residues are encoded by the bovine sequence (Fig. 2). Interestingly, a similar substitution has been indicated to be present in the partial mtrp2 cDNA [9] suggesting a closer relationship of btrp2 to mtrp2 than to htrp2. To get the full-length btrp2 cDNA, additional cDNA clones were isolated from specifically and randomly primed cDNA libraries, including clones E32, E51, 4, 12, 15, 16 and 29 (Fig. 1A). These clones partially overlap and, therefore, make it possible to deduce the full-length sequence, which comprises 2061 bp encoding a putative protein of 432 amino acid residues with a calculated molecular mass of 48 038 Da (Fig. 1B). The ATG triplet that appears downstream of the stop codon TGA (nucleotides −179 to −177) was selected as the translation initiation site but it cannot be excluded that ATG triplets downstream of the selected one may encode the initiation methionine (for example nt 187–189 or 256–258). The presence of transcripts corresponding to the protein coding region was confirmed by RT-PCR using poly-(A)$^+$ RNA from testis as template (Fig. 1A). Amplified cDNAs were subcloned and sequenced including clone WW26 (Fig. 1A). Comparison of the deduced amino acid sequence of btrp2 to the available sequences of mtrp2 and htrp2 (Fig. 2) reveals 96% (btrp2/mtrp2) and 82% (btrp2/htrp2) identity. The close relationship between btrp2 and mtrp2 is further supported by the finding that the bovine and murine cDNAs but not the human cDNA contain an insertion of 93 bp encoding 31 amino acids (Fig. 2). Analysis of the btrp2 amino acid sequence for local hydropathicity [17] suggests the presence of four transmembrane domains (segments of at least 19 residues with an average hydropathicity index of greater than 1.6, Fig. 1C). This predicted topology differs from the topology of other trp-related gene products including trp, trp1, htrp, htrp3, mtrp6 and bCCE1, which comprise at least six transmembrane domains with the putative pore region between domains five and six [7,9,12,18–21]. Sequence comparison reveals that the predicted transmembrane domains of btrp2 correspond to domains three, four, five and six of trp and related gene products.

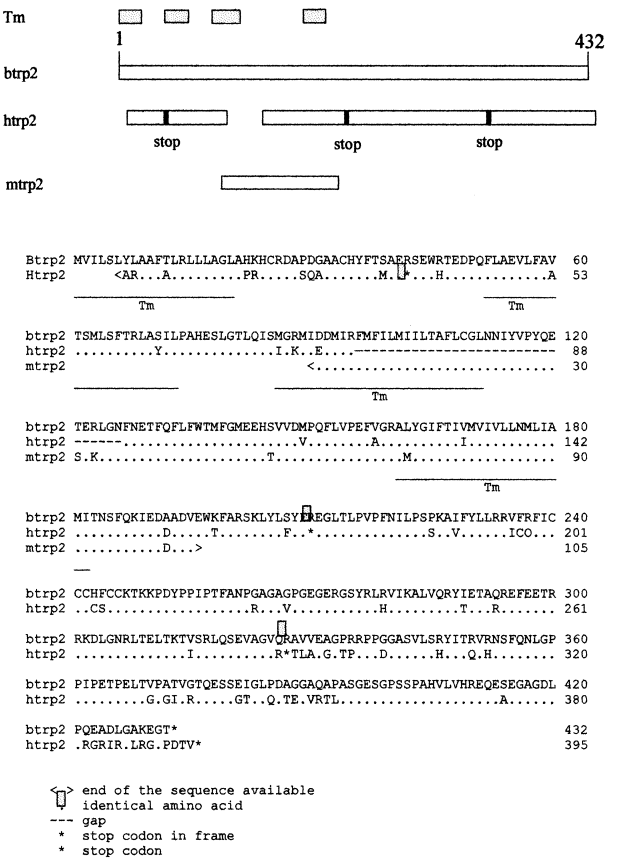

Fig. 2. Amino acid sequence alignment of btrp2 with the available sequences of trp2 homologues from mouse (mtrp2, [9]) and man (htrp2). The deduced amino acid sequences are indicated by white bars; gray bars represent predicted transmembrane domains (Tm). Htrp2 is derived from the human EST T67673 clone. The positions of the stop codons interrupting the open reading frame are indicated by black lines. Htrp2 lacks 31 amino acid residues compared to btrp2 and mtrp2. The deduced amino acid sequences are numbered on the right, the predicted transmembrane segments are underlined. Sequence alignments were carried out using the Clustal W algorithm.

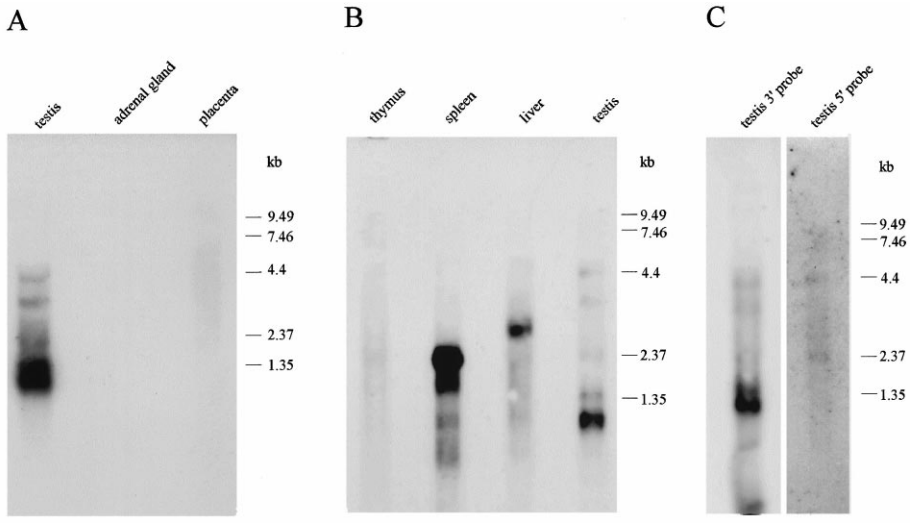

Fig. 3. Expression of trp2 transcripts. Northern blot analysis of 5 μg polyadenylated RNAs isolated from bovine tissues and human placenta probed with the 778 nt *Eco*RI fragment (nt 1–779) of htrp2 (A), the 596 nt *Apa*I fragment (nt 484–1079) of btrp2 (B) and a cDNA fragment derived from the 3′ region (3′ probe, nt 1230–1444) and the 5′ region of the btrp2 cDNA (5′ probe, nt −180–254) (C).

3.2. Tissue-specific expression of btrp2 mRNA

Northern blot analysis using the 778 bp *Eco*RI fragment of T67673 as probe revealed hybridization to 1.1, 1.3, 2.3, 3.3 and 4.4 kb transcripts present in poly(A)$^+$ mRNA from bovine testis but not from bovine adrenal gland or human placenta (Fig. 3A) as well as from bovine brain, retina and heart (data not shown). Transcripts of similar size were detected in testis using the 596 bp *Apa*I fragment (nt 484–1079) of btrp2 as probe (Fig. 3B). Using a 3′ probe of btrp2 (nt 1230–1444) similar mRNA species were detected as with the *Apa*I cDNA fragment of btrp2 (Fig. 3C). The size of the 2.3 kb transcripts is consistent with the size of the cloned btrp2 cDNA (2061 nucleotides) indicating that this mRNA species may correspond to the btrp2 cDNA. In fact a 2.3 kb transcript was also detectable in Northern analysis (Fig. 3C) using a cDNA covering the 5′ region of the btrp2 cDNA (nt −180–254). The identity of the other transcripts recognized in mRNA from testis is not known but they may represent alternative splicing products or RNA species resulting from incomplete RNA processing. In fact several cDNAs representing such splicing products and heterogeneous nuclear RNA derived cDNAs were isolated from various cDNA libraries employed (data not shown). However these c,lones did not lead to further elongation of the amino acid sequence encoded by btrp2. The identity of the transcripts recognized in mRNA from liver or spleen is not known.

3.3. Spatial distribution of btrp2 transcripts in testis

Recently it has been shown that thapsigargin elevates cytosolic Ca^{2+} in sperm in part by stimulating Ca^{2+} influx by a voltage-insensitive pathway [22]. This influx could be accomplished by trp-related cation channels and therefore we looked for the localization of btrp2 transcripts in testis by in situ hybridization histochemistry. To detect all btrp2-like transcripts in bovine testis, a full-length probe of btrp2 (nt 1–1299) was used. As shown in Fig. 4A, transcripts are restricted to some seminiferous tubules. Spermatogenesis does not occur simultaneously in all seminiferous tubules and btrp2 mRNA

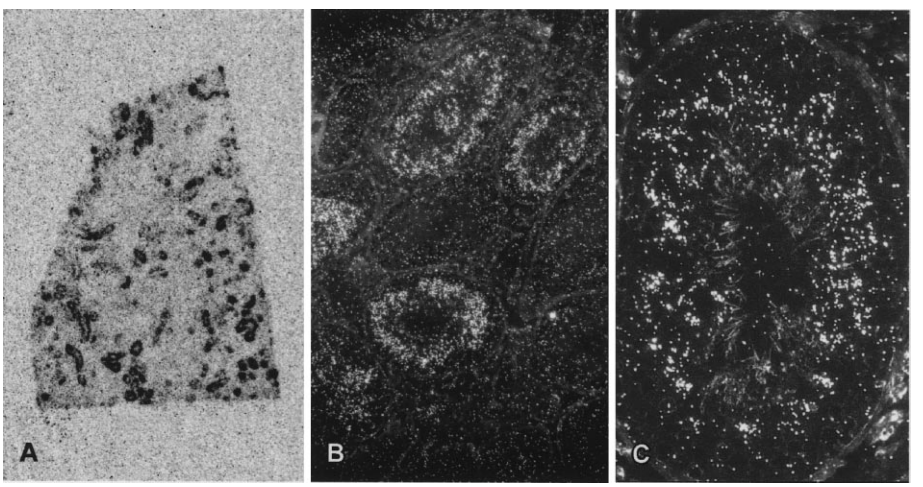

Fig. 4. Expression of btrp2 transcripts in testis. Overview (A), expression in seminiferous tubules (B, magnification 100×) and localization of transcripts in spermatocytes (C, magnification 400×).

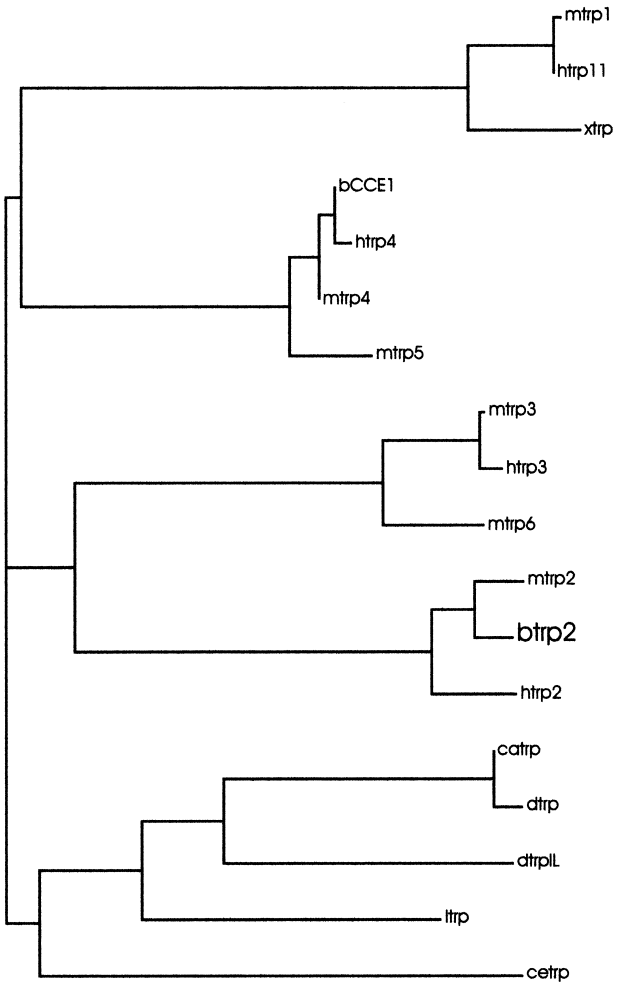

Fig. 5. Evolutionary relationship of trp-related proteins. The phylogenetic relationship was obtained by the Clustal/Clustree program of the available amino acid sequences that span the putative pore region and the predicted transmembrane domains which flank this region (~180 amino acids), bCCE1 [7]; btrp2, (this study); catrp [27]; cetrp (unpublished, GenBank accession number L16685); dtrp [19]; dtrp1 [20]; htrp1 [8,13,18]; htrp2 (this study, [13]); htrp3, htrp4, mtrpl, mtrp2, mtrp3, mtrp5, mtrp6 [9]; mtrp4 [4,24]; ltrp [28]; xtrp [4]; b, bovine; ca, *Calliphora*; ce, *Caenorhabditis*; d, *Drosophila*; h, human; l, *Loligo*; m, murine; x, *Xenopus*.

expression might be restricted to cells just differentiating during spermatogenesis. In fact btrp2 transcripts appear to be specifically expressed in spermatocytes (Fig. 4B,C), whereas no signals are present in spermatogonia, Leydig and Sertoli cells (Fig. 4B,C). Using a 5′ cRNA probe of the btrp2 cDNA (nt −550–189) hybridization signals were hardly detected in bovine testis. These results are consistent with the low expression level of the 2.3 kb mRNA species (Fig. 3B) supposed to encode the full-length btrp2 cDNA and support the conclusion that the 1.1/1.35 kb transcripts (Fig. 3B) are predominantly expressed in bovine testis.

4. Discussion

In summary, this study reports the primary structure of bovine btrp2, its tissue-specific expression and the cellular localization of its transcripts in testis. The deduced amino acid sequence of btrp2 is related to the sequences of other trp/trpl homologues and most closely resembles mtrp2. As shown by the phylogenetic analysis of trp/trpl proteins (Fig. 5), btrp2, mtrp2 and htrp2 are closely related to each other and to mammalian trp3 and trp6, whereas they are more distinct from trp1, trp4 and trp5 gene products, including bCCE1 as well as trp-related products from invertebrates. Htrp2 has been suggested to be a pseudogene product [13]. Both the pseudogene and the corresponding functional gene may be present in the same organism, but so far we could detect neither the htrp2 orthologue in bovine tissues nor the btrp2 orthologue in human tissues, indicating that the trp2 gene is unique and resembles a pseudogene in man but not in cattle. A similar finding was described in the case of the C4BP single copy gene, a subunit of the human plasma glycoprotein C4b binding protein, which resembles a pseudogene in mouse but not in man [23]. The btrp2 protein comprises four predicted transmembrane domains and lacks most of the amino-terminal sequences present in other trp/trpl-related proteins. In this respect it resembles truncated bCCE1 gene products which are abundantly expressed in adrenal gland [24]. The btrp2 and truncated bCCE1 gene products contain the putative pore forming region, suggesting that the ion conducting properties might still be preserved. Btrp2 transcripts are expressed in spermatocytes, liver and spleen and might contribute to cation channel formation in these tissues and cells. For example, there is extensive evidence that non-voltage-dependent Ca^{2+} influx is necessary for various functions of mammalian sperms including capacitation and the acrosome reaction [25,26]. The specific expression pattern of btrp2 transcripts in testis might indicate the contribution of btrp2 to this non-voltage-dependent calcium influx pathway.

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