

Research article

## Identification of the gene encoding Brain Cell Membrane Protein I (BCMPI), a putative four-transmembrane protein distantly related to the Peripheral Myelin Protein 22 / Epithelial Membrane Proteins and the Claudins

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### Abstract

**Background:** A partial cDNA clone from dog thyroid presenting a very significant similarity with an uncharacterized mouse EST sequence was isolated fortuitously. We report here the identification of the complete mRNA and of the gene, the product of which was termed "brain cell membrane protein I" (BCMPI).

**Results:** The 4 kb-long mRNA sequence exhibited an open-reading frame of only 543 b followed by a 3.2 kb-long 3' untranslated region containing several AUUUA instability motifs. Analysis of the encoded protein sequence identified the presence of four putative transmembrane domains. Similarity searches in protein domain databases identified partial sequence conservations with peripheral myelin protein 22 (PMP22)/ epithelial membrane proteins (EMPs) and Claudins, defining the encoded protein as representative of the existence of a novel subclass in this protein family.

Northern-blot analysis of the expression of the corresponding mRNA in adult dog tissues revealed the presence of a huge amount of the 4 kb transcript in the brain. An EGFP-BCMPI fusion protein expressed in transfected COS-7 cells exhibited a membranous localization as expected. The sequences encoding BCMPI were assigned to chromosome X in dog, man and rat using radiation hybrid panels and were partly localized in the currently available human genome sequence.

**Conclusions:** We have identified the existence in several mammalian species of a gene encoding a putative four-transmembrane protein, BCMPI, which defines a novel subclass in this family of proteins. In dog at least, the corresponding mRNA is highly present in brain cells. The chromosomal localization of the gene in man makes of it a likely candidate gene for X-linked mental retardation.

## Background

We recently developed a screening procedure for the selection of sequences encoding proteins targeted to the cell nucleus. Our method relies on the expression in transfected cells of enhanced green fluorescent protein (EGFP) fusion proteins from cDNA library constructs [1]. The selected clones encode EGFP fusion proteins that accumulate in the cell nucleus. Many of them were shown to harbor cDNA sequences corresponding to nuclear proteins that were translated in frame with the EGFP coding sequence. However, in nearly half of the selected clones the production of a fusion protein able to accumulate in the nucleus was shown to result from out of frame translation of the cDNA sequence fused to the EGFP coding region. On the average indeed, only one out of three cDNAs was positioned in frame with the EGFP coding sequence in the starting library. It was not expected that functional nuclear localization sequences would be generated at random (i.e. by out of frame translation of cDNA sequences) as often as was observed.

One clone, called "C60", that was isolated in this approach exhibited a significant DNA sequence similarity with a mouse EST sequence present in the EMBL/GenBank database (clone MNCb-0941, accession #: AU035837) [1]. No open reading frame (ORF) had been identified in this sequence yet, but the comparison of our dog sequence with the one from mouse identified a putative ORF on the basis that in the 385 bp-long region of similarity most of the differences occurred at the third position of base triplets in frame with a starting ATG codon. However, both sequences diverged before the stop codon was reached. Assuming that this was the correct reading frame, the cDNA portion in our EGFP fusion construct was translated out of frame (frame +2). This out of frame translation generated a 201aa-long sequence presenting several neighbouring clusters of arginine residues, which somehow resembled basic type nuclear localization signals. Although it could explain why this cDNA was isolated in the screening, it did not allow us to conclude whether the protein normally encoded by the cDNA is a nuclear protein or not. To further characterize the protein encoded by the cloned sequences we decided to isolate a complete copy of the corresponding mRNA.

## Results and Discussion

### *Identification of the complete dog BCMP1 mRNA*

The random primed cDNA insert harbored by clone C60 [1] was used as probe to screen a dog thyroid oligo-dT primed cDNA library in  $\lambda$  ZAPII phage vector [2]. Sixty positive clones were obtained out of the 500,000 cDNA clones screened. The longest insert (from clone C60-1) had a size of 4 kb and was entirely sequenced. Compared to the sequence of the insert of clone C60, this cDNA ex-

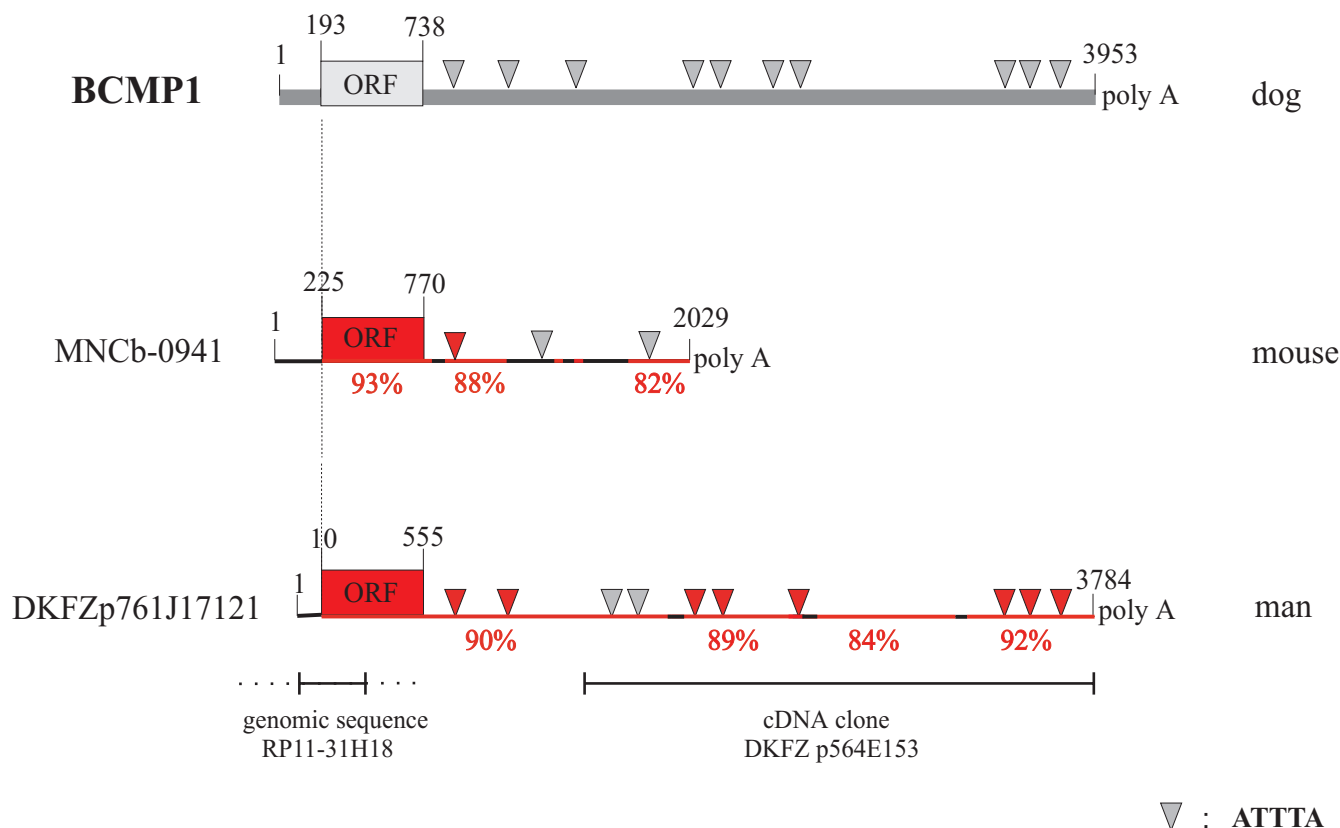
hibited a 2 bp extension in 5' and a 2,944 bp extension in 3'. The 3' poly-A tail was preceded by a correctly placed AATAAA motif (fig. 1). The longest ORF corresponded to the putative ORF identified previously by comparing the sequence from clone C60 with that of the mouse EST present in the database (see background section). It extended over 543 bp (181 aa), from position 193 to 735 in the cDNA sequence. The translation initiator codon was located in a suitable sequence context according to Kozak's rule [3]. As in the interval an updated homologous mouse sequence had been deposited in the database (clone MNCb-0941, EMBL/GenBank acc. #: ABO41540), the comparison of both sequences revealed that the coding region was entirely conserved in dog and mouse (fig. 2).

The 3.2 kb-long sequence located in 3' of the TAG codon (3' UTR) in the dog cDNA was distinctly AT-rich and contained 9 ATTTA motifs. These characteristics have been implicated in the rapid decay and restricted translation of mRNA molecules [4,5,6]. This 3' UTR was shorter in the mouse (1.3 kb) but several portions of it exhibited a remarkably high sequence conservation when compared with the dog sequence (fig. 2). Especially, the AT-rich character and the occurrence of multiple ATTTA motifs were preserved. A search in the database also identified a human sequence (DKFZp564E153, EMBL/GenBank acc. #: ALO49257) presenting a very high degree of sequence conservation over 2.5 kb with the 3' part of our dog cDNA (fig. 2). The coding region of the mRNA was not contained in this human sequence and the observation of such an extended conservation of DNA sequence between UTRs from different species was unexpected. During the preparation of this manuscript, a completed human sequence appeared in the database (DKFZp761J17121, EMBL/GenBank acc. #: AL136550). The coding region was entirely conserved between dog, mouse and man, and most of the ATTTA motifs present in the dog sequence were also preserved in man (fig. 2). It may suggest that BCMP1 mRNA is indeed subjected to tight post-transcriptional controls. However, whether the presence of these sequences really confers instability to the mRNA and restricts its translation remains to be determined experimentally.

A number of EST sequences from various species which were clearly homologous to dog BCMP1 could be retrieved from the database by BLAST searches. They indicated that the BCMP1 gene must also exist in the rat (e.g. acc. # BG381247), beef (e.g. acc. # AW352911), pig (e.g. acc. # BF704530) and in the fish *Gillichthys mirabilis* (acc. # AF266205), in addition to the already cited dog, mouse and man.

1	CTG GCG GCG AGC GTC GCG GGC GGG GTC GCG GTC GTC GCG GTC GTC GTC CAA	88
89	GAC GCG GCA GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG	98
97	GCG GTC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG	108
102	GCG GCG GTC GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG GCG	112
1	Met Ala Ser Ala Gly Ser Gly Met Glu Glu Val Asp Val Ser Val Leu	10
193	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	249
17	Thr Pro Leu Lys Leu Val Gly Leu Val Cys Ile Phe Leu Ala Leu Cys	32
242	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	288
33	Leu Asp Leu Gly Ala Val Leu Ser Pro Ala Trp Val Thr Ala Asp His	88
289	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	356
49	Gln Tyr Tyr Leu Ser Leu Trp Gln Ser Cys Arg Lys Pro Ala Ser Leu	64
337	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	364
60	Asp Ile Trp His Cys Glu Ser Thr Leu Ser Ser Asp Trp Glu Ile Ala	80
385	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	432
81	Thr Leu Ala Leu Leu Leu Gly Gly Ala Ala Ile Ile Leu Ile Ala Phe	96
433	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	480
97	Leu Val Gly Leu Ile Ser Ile Cys Val Gly Ser Asp Arg Arg Phe Tyr	112
481	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	528
213	Arg Pro Val Ala Val Met Leu Phe Ala Ala Val Val Leu Glu Val Cys	128
529	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	576
129	Ser Leu Val Leu Tyr Phe Ile Lys Phe Ile Ile Thr Val Ser Leu Lys	144
573	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	624
195	Ile Tyr Ala Glu Phe Asn Trp Gly Tyr Gly Leu Ala Trp Gly Ala Thr	160
628	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	672
162	Ile Phe Ser Phe Gly Gly Ala Ile Leu Tyr Cys Leu Asn Pro Lys Asn	176
673	Met Ser Thr Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser	720
373	Tyr Glu Asp Tyr Tyr ***	
723	TAC GAA GAC TAC TAC TAG AAC CAA GAG TGT CAG ATT TTA AAA ACA ACC	768
769	AAC CAT CCA ACA AAA GAA TTA CTT CTT TAT CTT TTT TAA CTC ACT GGT	816
817	TTC GAA AAC ACT GGT GGA GTA TCA AGC AGT TTT CTC ACT TGA TTT AAC	864
865	ATC TTT TCC GTC TCG GGT GCA ACC AGC AGC ATA ACC ACC TTT TAC ACC GAT	912
913	TTC ACC GAC CCG GAC AAA TTA AGA GAG CTG ATT AGA CAT AGG GAA AGC	960
961	ATG CAA ACT TCC AAT AAG TTA ACC GGA TTT TTT TTT ACA AGT GAA AGG	1008
2009	TTC TGT ATT ACA GCA ACC ATT GGG AGA TAC TAG TTT GAA TGA GAA AGT	1056
2057	CTC GAA TGT CTT ATT GCG TGC AGC GGA GCA GCT GGC ACA AGA AAC ATT	1104
1105	TAG AAG TTC CTT TGC TTT GAC AAG GAT TCC ACT GCT ACA GAG TTC TTA	1152
1153	TGA CTT GCT TAC GGC ACC GAA TGA CTA AAT TGG CCA TTT GTC ATT TGC	1200
1201	TGG AGT GAG CTT TGG AAA GGT GAA CTG CCG TTC ACC ACC TAA TGG GAG	1248
1249	TTC TGA GTC TAA TGG GTT AAA GGT GAA TAG ATT CTA CTT TCA AGA ATA	1296
1297	GTC TTT TTT AAT GGG AGC GAA TGC TTT GCG AGT CAG CAT CTC CCG GGG	1344
1345	AAA GGA GTC AAG ACT TGG AGA CAT GTC CTT TTT ATT ATG TGG TTA GAA	1392
1393	ATT GTT CCT GAG GCG TAT CTA GAA TGA GGA GAA TTT AGA GTC TTT ACT	1440
1441	TTC TCT GGG GCA ACT AGA AAG AAC TAT GCA TGA GTC GCT TTT GTC CTA	1488
1489	ACT ACC CTT TAA ACC ATT TAC CAA ACA TTA GCG CAA GAA ATA ATT GTC CCG	1536
1537	ATT TAA CAA GCG TAA ACC ATT TTA TAG AAG GAT ACC TAT TAG TAA GAA	1584
1585	TGG TAG TAA ACC ACT CCG TAG CCG TAT ACA TGC ACC TGC CTA TTA CAC	1632
1633	AGA AAA GTA ACA TTT GCT CTA GTC AAG TGA TTT GAA CAC TAA CCG TGT	1680
1681	ACA CTT TAA GAG GCT TAG ATT GGT GGT CAC ACC TAC TTA TGG TAC AAA	1728
1729	AGT ACA TAT ATT TTA AAG CTT TGG CCG TCG GTC GTC TAC TGT TTC ACC	1776
1777	GAA AAG TTT TAA TAG AAT TGT AAA GAA GAA AAA TTT CAA TGG TGT TAA	1824
1825	GTT GAA AAT TGA TGA TTC TTC TGT AAG CAC TAT AGT TCA AGC AAG AGT	1872
2873	AGC AAT TAA AAT TAT CAT TTT GGT AAA AAT TCA GTC AAA TAG AAG GCT	1920
2874	GCT TTT TTT TGC AAG TAC CTT AAA GGT CTT TAT TAA AAA ATA AAA GAA	1968
1969	TAG TGA TAT ATT TAT AAG AAT GTC TTA ATG TCT CAG TAG AGT AGT ACA	2016
2017	ATT CAT TGC TGA TCA CTT GGT CCG GAG TGA ACC AAT GGC CTA ACT	2064
2065	GAT TGG TGC ACA CTA TGA TGG ATT TAT GTC GTC ACA GGA ATC ATT TGT	2112
2113	TGG CTG TTC AAA TGG AAG GAA ATG TCA ATG ATA GGG AAT AAG TTT GGC	2160
2161	ACT AAT CTC AGA CTG CAA ACC TGT GGT AAT CCG GGT TAC TAT ACA AAT	2208
2209	TGG GAT AGT TGA AAT GTA AAT ATG TTT CTA TAT CAA GCA TAC AGT TCT	2256
2257	AAT ATA AAA GTC ATA AAT AAT TAC TTT TGT TAA CAA AGG CAC TAA AAC	2304
2305	AGT TTT CCG GGT TTT GGC CTT TTT GCA GAA AGA AGC ATT GGA AAA ATC	2352
2353	ATT TAA AAC ATG CTT ATG TTC TAG TGT ATT GCA AAA TCC GTT AAG AGC	2400
2401	AGC ACT ACA TGA GGA TTA TTT AAT AAT TTT TTT TAT CTC CCA GAA CAG	2448
2449	AGT TCT GCT TGC ACC AGC TCC TAC GAA CCG TTT GCG AAA ACA ACA GAA	2496
2497	AAA AAA ATC TAT TTT AAT ATT CAA ATG CTT AGA AAA AAA AAA CCG	2544
2545	TCC TAT CTT GGC ACT TTT CCG TAG GAT CAC AAA GCA AAT ATG AAG AAC	2592
2593	GAG GGT GAA TCT TAT TTT AGC AAG GAC AGA CAA AAG TTA TTA AAT GCA	2640
2641	TCC TAG AGA GAA ACC TTT AAG AAA GTA TAT GAT TCT CTG TAG AAT GAT	2688
2689	AAG AAA CTA TGG GTC AAG TTT TCC AGA AAA ATA TGA GGA TTA TAT AAT	2736
2737	CCT TTA AAG CAA TTT TCT TGA GAA GTA GTA AAA TCT GGG CCA GAC TGT	2784
2785	GTT GCA GTT AAT TGC GTA TTT TGA GAG CAG CAT GAG AAA TAC GAT AAT	2832
2833	TGT TGG AAG GGG AAT TGT GGT ACT AAC CAC TCT CTA TGG TGA AAG AAT	2880
2881	CTC TTT AAT CTT TAA TAA TGG AAG AAG AGT ACC TTT GTC TTT AGA TCA	2928
2929	ATG AGG CAC TTT TCT TAG TTT TAG TGG AGG ATG CAG TGT GCT GTC AGA	2976
2977	CCA GTA CCA CAC CTC GAG TCT TTT GAA GTC TTT ATT ATT CTG TGT CAA CAT	3024
3025	GAC AAG CTA GAA AAT GAC AAT AAT TTA TAG GGT AAG GCA CAC ATG TTT	3072
3073	GCA GTA CAC TGA AAA GTA GAT TTT TTT TAT AAC TTT TTT CCG CTC CCG	3120
3121	AGT GAA GAG CCG TAT GTT ATA TTT CTA CGA TCA CTA ATA TTT GAA AAT	3168
3169	ATT TGA TGA CCA ACC AAC TCA GCA CAA CAT GAA AGC TGT TGC TCA TCC	3216
3217	GAA ATA TGG TAT GGT TTA TGA AAA CAA ACA CTT GAA TTT TCA CTT ACA	3264
3265	TGA TGT CTT TGC GCG TTT AAT TTT TTT TTA TTT TTT CTT AGA AAG AAA	3312
3313	ACT GTC AAC TAC AAA TCC ATT GGT TAT CTT AGA TAT GCA CAT GTA CAC	3360
3361	ACA TAG AAT ACA AAA TAC TCA GCA GGG CTA GTT ATT TGG ATT TCT TGC	3408
3409	ACA ACT ATT TAC CTT TTT GTA AAT TCA ACA TGT AAT TTT TAA AGA CAA	3456
3457	AGA TAG AGA GAC CTA TGT GTC TGA AAT ATA AAT GAT ATA TAT GGA TTA	3504
3505	GCA TGT ACC TGT ATA TTA TTA AAC ATG CAA TGA AAT GAC TGG TAA GTG	3552
3553	AGT TGT AAT TGT AGT GCT AGC AAT GGA ATT TAT TCA SAC TGT ATT TTT	3600
3601	GTA CAG AAT AAT GCA CAC TAA CCG ATG CTT CTA TGT CTT CTT TAA TGC	3648
3649	CYA AAA CTA TGC CTA AAT ATT TGA TCT GTC TTA AAG AAT AAA ATT ACA	3696
3697	CTT GAT GAT GTC TAT GAT AAT GTC TTA CTA CTA CCG TGC TAT ATT TAT	3744
3745	TAT TTT TAA AAT TAT GAC ATT TTT ACT ACT TAA ATA TGA ATC CAT GGT	3792
3793	ATT CTG GTT ATT TTT TTT AAC AAT CTT TGG GGG GGA ACC TGT TTC TCA	3840
3841	CTC CAC TGC TTT TGA GTT TGC AAT TTT ACA ATC AAT TCT TCA TTT CAT	3888
3889	GAT TTT TGT AGC TGA CAT ATG AAG TTA TCT ATG TGG ATA AAA TAA AAA	3936
3937	TAA AAT TGC TTT CAC TGA AAA AAA AAA AAA AAA AAA AAA AAA AA	

**Figure 1**  
**Nucleotide sequence of dog BCMP1 cDNA.** The aminoacid sequence encoded by the ORF appears above the corresponding DNA sequence. The underlined sequence corresponds to the insert of the original clone C60 (see text). ATTTA motifs appear in bold and the polyadenylation signal is highlighted in blue.



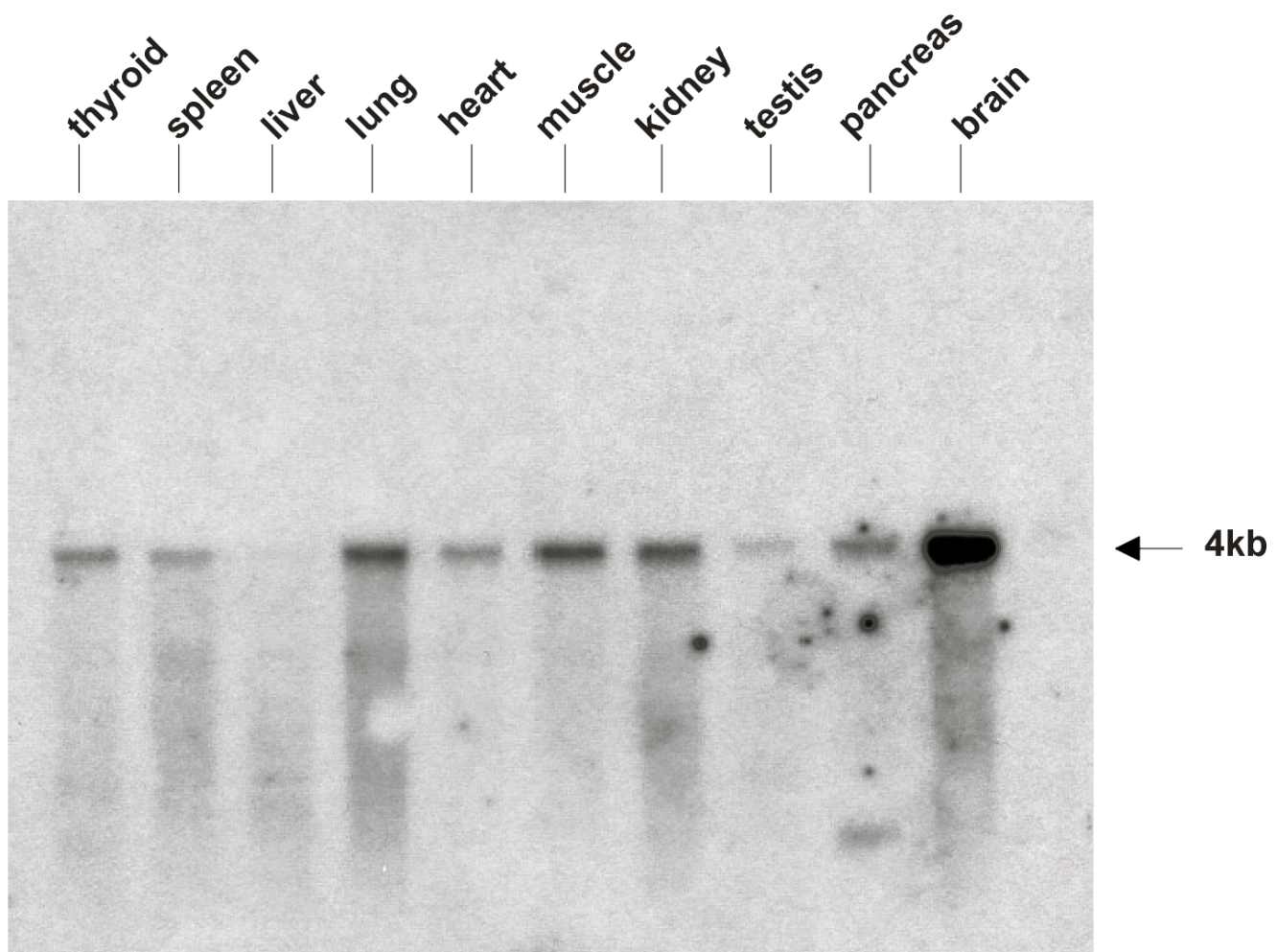
**Figure 2**  
**Structure of BCMP1 mRNA.** Schematic representation of known sequences from mouse and man that are homologous to dog BCMP1 sequence (short EST sequences were not considered). Coordinates are given for each sequence individually. Highly conserved regions are highlighted in red with indication of the percentage of identity relative to the dog sequence. Inverted triangles symbolize ATTTA motifs. They appear in red when the motif is conserved in the dog sequence.

**Analysis of BCMP1 mRNA expression in the dog**  
 Originally, the cDNA had been isolated from a dog thyroid cDNA library. In order to investigate whether the corresponding mRNA was also present in other cell types, a northern blot experiment was performed using poly-A+ RNA preparations from various dog tissues (fig. 3). Huge amounts of the 4 kb transcript were detected in brain cells. The presence of the mRNA was also detectable in most of the other RNA preparations but to lesser extents as compared to that found in brain RNA. The encoded protein is thus expected to be particularly abundant in the brain, unless the peculiar 3' UTR of the mRNA mediates a deep control on its translation (see above).

**Prediction of BCMP1 protein structure and subcellular localization of an EGFP-BCMP1 fusion protein**  
 The 181 aa-long protein sequence encoded by the mRNA did not present any significant resemblance with sequences present in protein databases. A search for the presence of protein family signatures (PfamHMM on Ex-pasy server) revealed the occurrence in the novel protein

of sequence motifs resembling significantly to one of the two motifs specific to the peripheral myelin protein 22 (PMP22) family of proteins and to the motif specific to the claudins (fig. 4). The two identified signatures overlapped partially in the novel protein sequence. PMP22 and the related epithelial membrane proteins (EMPs) [7], as well as the claudins [8], all belong to the super-family of four-transmembrane domain (4TM) proteins. As could be expected, the search for the existence of putative transmembrane domains in the novel protein (HMMTOP on Ex-pasy server) identified the presence of four of such domains (fig. 5). The protein thus appeared to be a novel member of this large family of proteins, somehow related to PMP22/EMPs and the claudins. As these are integral membrane proteins, and as the mRNA encoding the novel protein was predominantly found in brain, the newly identified protein was termed "brain cell membrane protein 1" (BCMP1).

According to the putative BCMP1 structure, the extracellular loop between TM1 and TM2 would be larger than the intracellular loop between TM2 and TM3 and the ex-



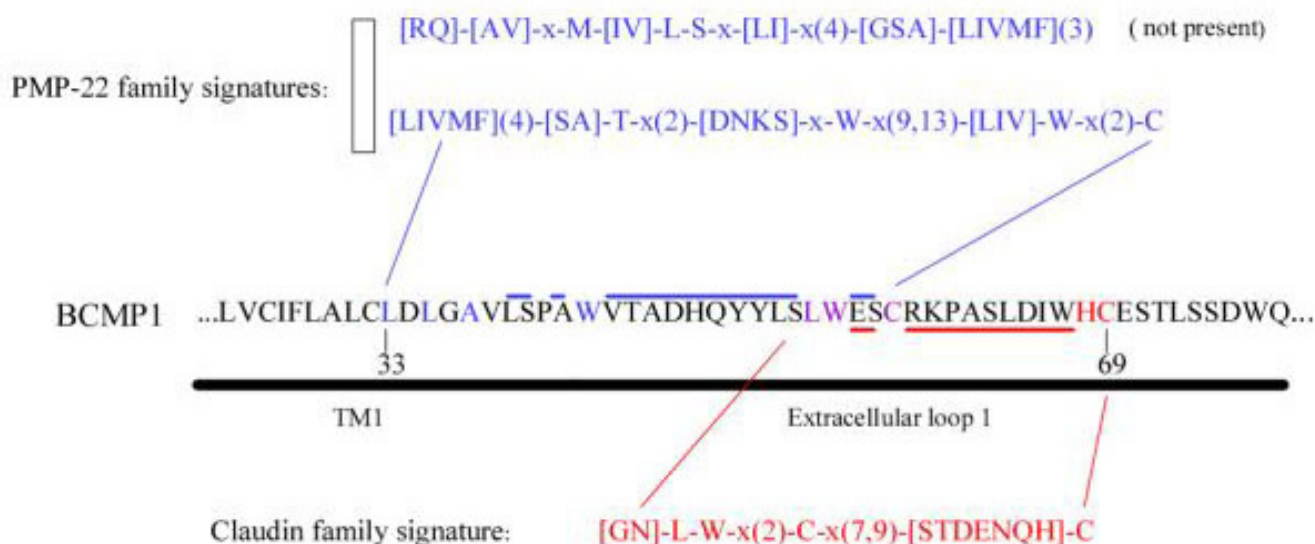
**Figure 3**  
**Northern-blot analysis of BCMP1 mRNA expression.** PolyA<sup>+</sup> mRNA preparations from various dog tissues were probed with the coding region of BCMP1 cDNA. The arrow points to the signal corresponding to the expected 4 kb-long transcript.

tracellular loop between TM3 and TM4, as it was supposed to be also the case in PMP22/EMPs and claudins. However, the intracellular amino-terminal arm proceeding the first transmembrane domain appeared to be much longer in BCMP1 than in its relatives.

In order to refine the classification of BCMP1 within the four-transmembrane domain protein family, a phylogenetic tree was constructed on the basis of the alignment of the available protein sequences related to the PMP22/EMPs and claudins (fig. 6). Dog BCMP1 and its mouse and human orthologs segregated as a distinct subgroup in the tree. Their closest relatives were the recently identified mouse PERP gene product [9] and the protein encoded by the CG6982 gene in drosophila (EMBL/GenBank acc. #: AAF56054). This group of proteins thus

shared primary structure determinants which defined a distinct subclass in the protein family.

In order to assess experimentally the postulated membranous localization of BCMP1, an EGFP-BCMP1 fusion protein was expressed in transiently transfected COS-7 cells and the subcellular localization of the hybrid protein was observed by fluorescence microscopy (fig. 7A and 7B). A fine granular fluorescence was observed all over the surface of cells expressing the EGFP-BCMP1 fusion protein, consistent with a plasma membrane localization of the tagged protein. A stronger fluorescence surrounding the cell nucleus was also observed. It indicated that a significant part of the expressed fusion protein accumulated in the endoplasmic reticulum. The pattern of EGFP fluorescence remained almost un-

**Figure 4**

**Identification of PMP22 and Claudin family signatures in BCMP1 primary structure.** Conserved residues are coloured (blue: PMP22 signature, red: claudin signature, violet: overlapping PMP22 and claudin signatures) and conserved spaces between specific residues are over - or underlined. The part of the predicted BCMP1 structure (see fig. 5) to which the primary sequence corresponds is shown (TM1 = first transmembrane domain).

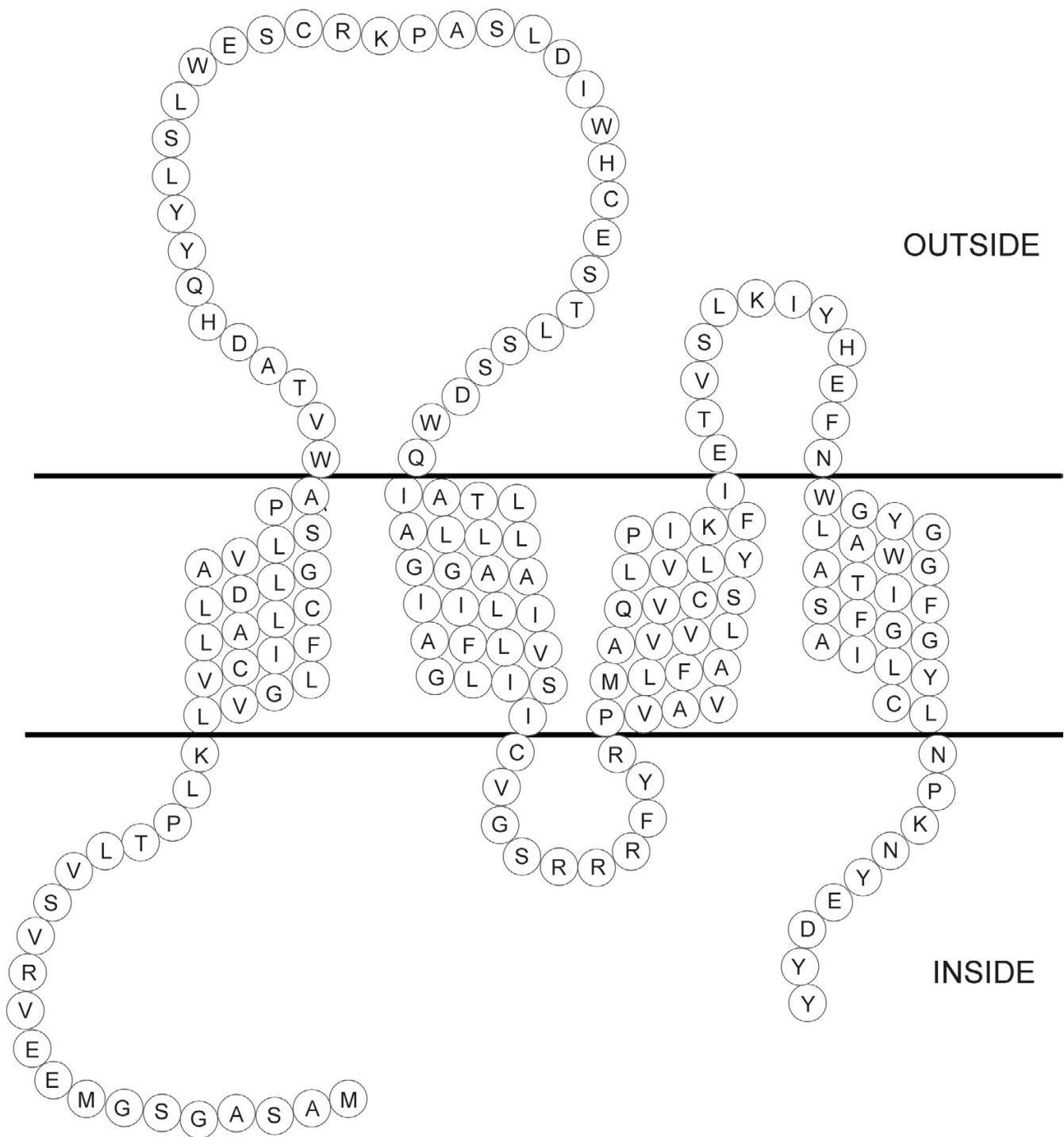
changed when the cells were permeabilized with saponin in order to stain the nuclear DNA (fig. 7C and 7D). This indicated that the fusion protein was embedded in the membranes and that it was not able to readily diffuse out of the cell.

#### Localization of the BCMP1 gene in dog, man and rat

In dog, the BCMP1 coding sequence was typed in duplicate on the 118 cell lines of the RHDF5000-2 radiation hybrid panel [10] on the latest version of the RH map [11]. The BCMP1 gene was linked to chromosome X close to FH2548 with a Lod score of 11.88. Marker FH2548 is located close to the DMD locus in dog (distance: 4.4 cR<sub>5000</sub>, approx. 500 kb). More informations about dog RH maps can be found at <http://www-recomgen.univ-lyon1.fr/doggy.html>.

The human EST sequence DKFZp564E153 (EMBL/GenBank acc. #: AL049257) that corresponds to the 3' UTR of dog BCMP1 mRNA had been localized on chromosome X. The corresponding human genomic sequence could not be found by BLAST searches against sequences available in the database. However, by using the coding region of dog BCMP1 a significant match was identified with genomic sequences assigned to human chromosome 8 (clone RP11-31H18, EMBL/GenBank acc. #: ACO41003). The similarity extended from position 1 to 418 in the human cDNA sequence (fig. 2), which corresponded to the amino-terminal part of the protein up to

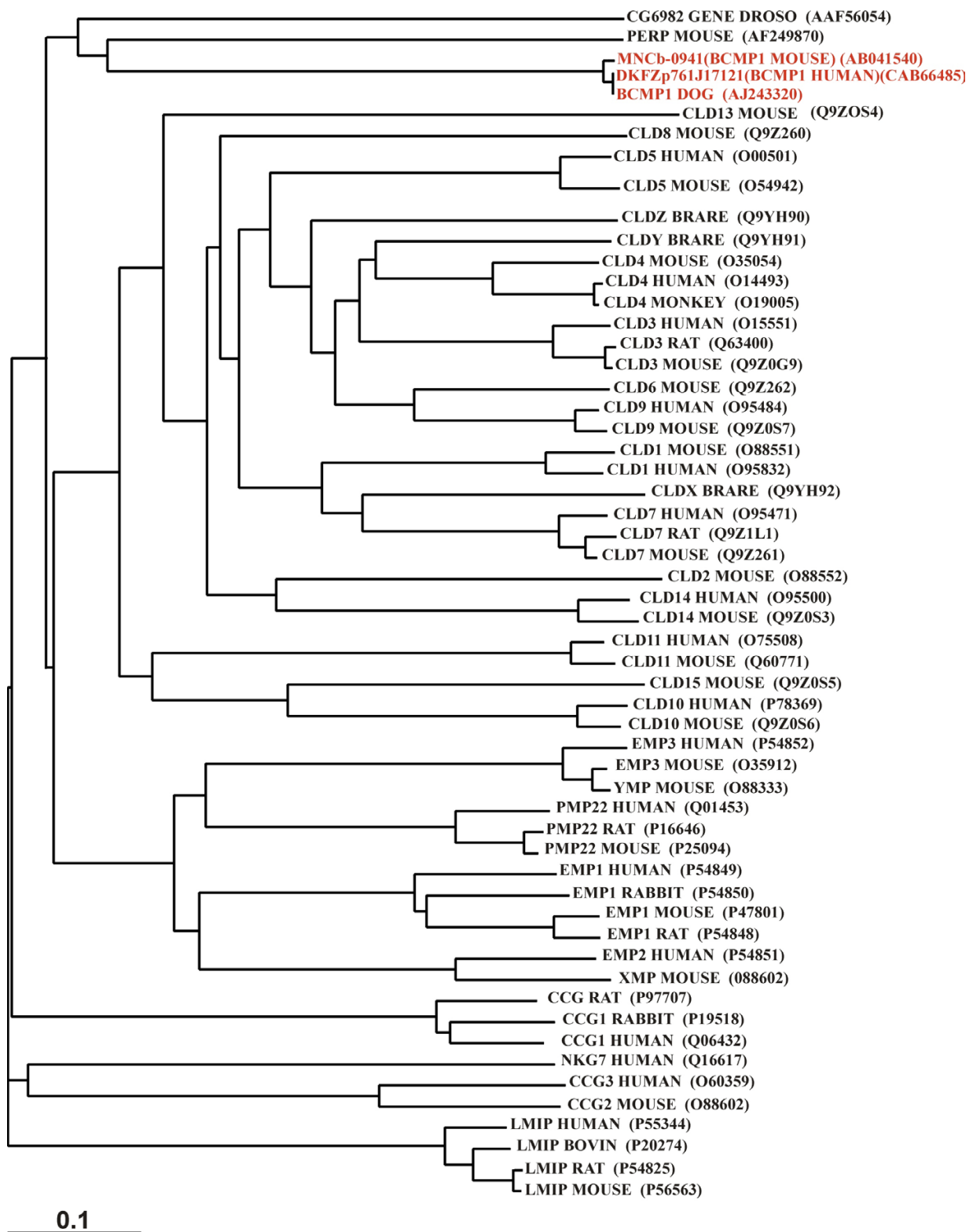
the first extracellular loop. As an intron was found at this same position in PMP22, EMP-1 and EMP-3 genes, it appeared likely that we had identified the first coding exon of the human BCMP1 gene. PMP22, EMP-1 and EMP-3 genes all contain an additional intron separating the sequences encoding the first transmembrane domain and the first extracellular loop into two exons [7]. This intron is clearly not present in the human BCMP1 gene. In order to clarify the location of the gene in the human genome (chromosome X or chromosome 8?), the GeneBridge-4 WGRH panel was used to map the sequences encoding human BCMP1 using a pair of primers directing the amplification of a 666 bp-long fragment encompassing the entire first coding exon and the exon-intron junction. It revealed that the amplified segment was located on chromosome X, 0.20 cR<sub>3000</sub> from marker W1-7096 and 6.51 cR<sub>3000</sub> from marker DXS1214. This location agreed with the previous assignment of the EST sequence DKFZp564E153. It also corresponds to the cytogenetic location Xp11.4. As the DMD gene maps at Xp21.2 in man, it is thus also close to the BCMP1 gene in this species. The chromosomal localization result revealed unambiguously the existence of a single BCMP1 locus in the human genome. As a consequence, it indicated that the sequences of the genomic clone RP11-31H18 had been inappropriately assigned to chromosome 8 instead of chromosome X in the database.



**Figure 5**  
**Predicted structure of BCMP1 in the plasma membrane.** The structure was drawn on the basis of the predictions obtained from HMMTOP on the ExPASy server.

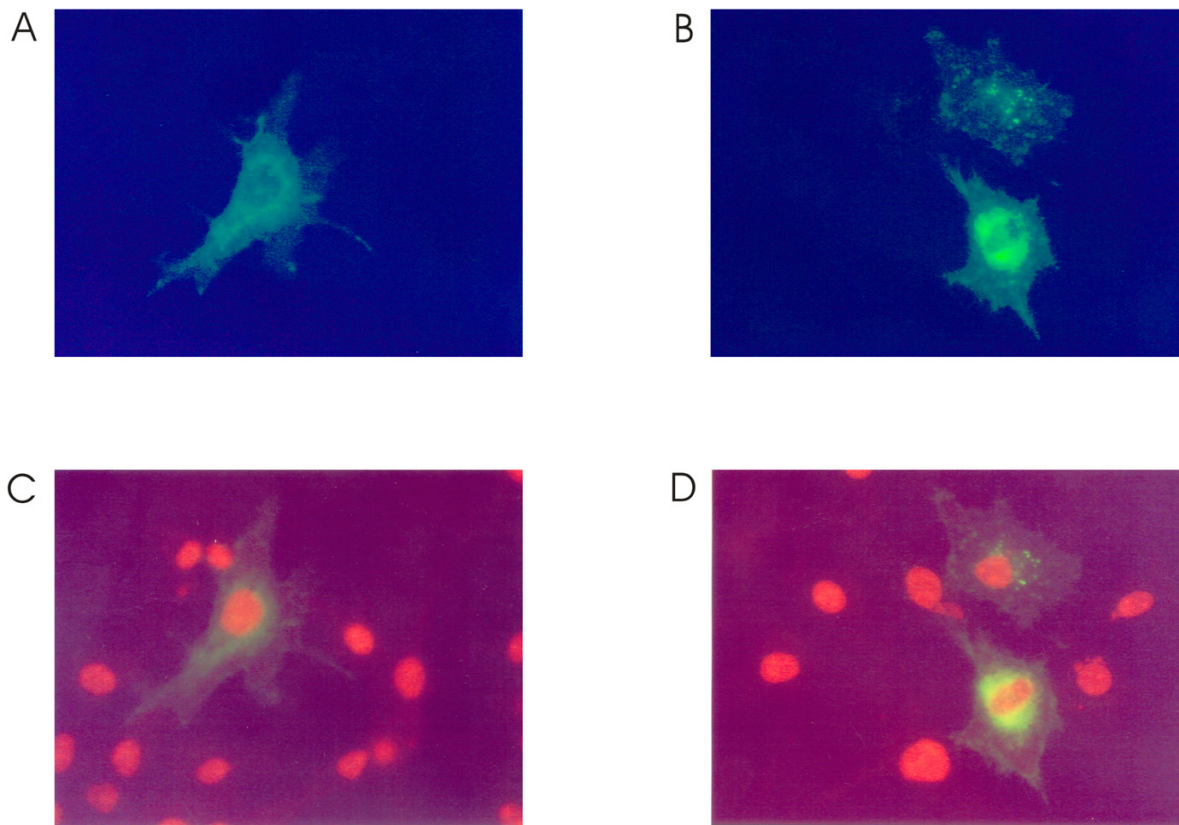
In the annotated human genome sequence available on the Ensembl server, the first coding exon of the human BCMP1 gene (gene ID:ENSG00000101959) is present in the chromosome X sequence (the sequences of clone

RP11-31H18 have now been properly reassigned to chromosome X; see ContigView on Ensembl server). Part of the coding region of human BCMP1 and the whole 3'



**Figure 6**  
**Phylogenetic tree of four-transmembrane proteins related to PMP22/EMPs and the claudins.** See materials and methods section for the description of the tools used for the construction of the tree. Database accession numbers of individual sequences are given in parentheses. Dog BCMPI and its mouse and human orthologs appear in red.





**Figure 7**  
**Subcellular localization of the EGFP-BCMP1 fusion protein.** The fusion protein was expressed in COS-7 cells. Parts A and B: observation of EGFP fluorescence in the perinuclear region and at the cell membrane. Parts C and D: cells were permeabilized and the nuclear DNA was stained with ethidium bromide in order to visualize the cell nucleus.

UTR region corresponding to DKFZp564E153, are still missing in the currently available human genome sequence.

Using primers derived from the rat EST236642 (EMBL/GenBank acc. # AI408352), which is 91% identical to the segment 2028-1434 of the mouse brain cDNA MNCb-0941, itself similar to dog BCMP1 cDNA, the rat gene (symbol: *Bcmp1*) was assigned to the chromosome X, between DXRat67 and DXRat28, at 497.9 cR along the MCW map (LOD score: 9.0; the local map is: DXRat67 - 29.9 CR - *Bcmp1* - 0.4 cR - DXRat28). The marker DXRat67 co-localizes with the gene *Dmd* [12], itself cytogenetically assigned to Xq22 [13]. The rat genes *Bcmp1* and *Dmd* are thus closely linked, as was already observed in dog and man.

## Conclusions

We have described here the identification of the gene encoding a novel protein, called Brain Cell Membrane Protein 1 (BCMP1), which belongs to the large family of four-transmembrane proteins and appears to be highly expressed in the brain. The gene seems to be conserved on chromosome X within mammals, in close association with the DMD locus in man, rat and dog at least. The encoded BCMP1 protein shares significant resemblances with both PMP22/EMPs [7] and the claudins [8], but exhibits distinct features, notably a predicted intracellular amino-terminal extension, which distinguishes it from the other known members of the family.

PMP22/EMPs are integral membrane proteins that seems to be implicated in various cellular processes, such as cellular differentiation, control of proliferation, and apoptosis [7]. PMP22 has been shown to play a critical role in peripheral nerves, where it is involved in the as-

sembly of peripheral nerve myelin and in the regulation of proliferation and differentiation of Schwann cells. The claudins also constitute integral membrane proteins which are localized exclusively at tight junctions [8]. Claudin-1, -2 and -3 have been shown to present calcium-independent cell-adhesion activity [14].

Alterations in the PMP22 gene are responsible for hereditary motor and sensory neuropathies in human and rodents, known as Charcot-Marie-Tooth type 1A (CMT1A) disease and *Trembler (Tr)* mouse respectively [7]. Individuals presenting nonsyndromic recessive deafness (autosomal recessive deafness DFNB29) were recently shown to harbor mutations in the gene encoding claudin-14 [15]. The Xp11.4 region of the human genome which comprises the BCMP1 gene has been linked to several forms of syndromic X-linked mental retardation, such as MRXS-2, -4, -6 and -10, and to a number of non-syndromic MRX cases [16]. The TM4SF2 gene which apparently encodes another member of the superfamily of four transmembrane proteins, a tetraspanin [17] more distantly related to BCMP1 than are PMP22/EMPs and the claudins, is located very close to the BCMP1 gene in man. Mutations in the TM4SF2 gene and gene inactivation resulting from chromosomal translocation have been shown to be involved in several cases of X-linked mental retardation [18]. Whether the BCMP1 gene is also involved in such genetic disorders and what is the function of the encoded protein thus constitute the obvious questions which will support our future investigations.

## Materials and methods

### DNA constructions

Standard DNA manipulations were conducted according to published procedures [19]. The full length BCMP1 cDNA clone was obtained by screening a dog thyroid cDNA library in  $\lambda$  ZAPII phage vector [2] using the original clone C60 [1] as probe. The DNA sequences corresponding to the cDNA insert in clone C60 were amplified by PCR using primers complementary to the sequences flanking the insert in the construct, 5'CAGATCTCGACCCACGCG<sup>3'</sup> and 5'TACCTGCGGCCGCGATAT<sup>3'</sup> respectively, and were labeled with digoxigenin (DIG labeling and detection kit, Boehringer Mannheim). Hybridization, washing and signal detection were performed as recommended by the supplier of the labeling system. The cloned DNA was sequenced on both strands using the Big Dye Terminator methodology and a model 377 DNA sequencer (Applied Biosystems). The construct encoding the EGFP-BCMP1 fusion protein was obtained by inserting a PCR fragment corresponding to the BCMP1 ORF between the EcoRI and BamHI sites in the pEGFP-C1 vector (Clontech). The following primers were used to amplify these sequences from the DNA of clone

C60: 5'TTCGAATTCGGCGGGCAGCGGC<sup>3'</sup> and 5'TGTGGATCCTAGTAGTAGTCTTC<sup>3'</sup>.

### RNA analysis

Northern blot analysis was performed on 4  $\mu$ g of polyA+ mRNA from various dog tissues. Acridine orange staining of the gel confirmed that each lane contained identical amounts of RNA. A <sup>32</sup>P-labeled PCR fragment corresponding to the BCMP1 ORF was used as probe (see above for preparation of the DNA fragment). Hybridization and washes were conducted in standard conditions in the presence of 50 % formamide [19].

### Cell transfection

Transfection of COS-7 cells was performed using the DEAE-dextran method [20]. About 200 ng of a crude plasmid DNA preparation was engaged per dish (diameter: 3 cm). The subcellular localization of EGFP fluorescence was observed 48 hours after transfection using an Eclipse TE300 inverted microscope (Nikon) equipped with NB-2A and NG-2A filter blocks. The transfected cells were permeabilized using saponin (0.075% final concentration) and nuclear DNA was stained with ethidium bromide (1  $\mu$ g/ml final concentration) in order to visualize the cell nucleus.

### Chromosomal localization

Dog BCMP-1 could be readily typed on the dog x hamster radiation hybrid panel RHDF5000-2 composed of 118 cell lines from panel RHDF5000 [10]. The following pair of primers, 5'TCTGGAGTGAACAAATGGGCTAA<sup>3'</sup> and 5'GCAGTCTGAGATTAGTGGCAAA<sup>3'</sup> generated a PCR product of 137 bp on dog genomic DNA. PCR results were scored in terms of present, absent or ambiguous in the 118 hybrid cell lines. The typing data were incorporated into the latest radiation hybrid map [11], using the Multimap package [21]. The GeneBridge 4 human x hamster radiation hybrid panel DNA (Research Genetics Inc.) was screened by PCR using the following primers: 5'GGCAGCGGCATCCAGGAA<sup>3'</sup> and 5'TGGGGAAGACCAACAGAGAACC<sup>3'</sup>. The PCR results were analyzed according to the prescription of the supplier of the panel DNA.

The panel of rat x hamster radiation cell hybrids [12] was typed by PCR with the following primers: 5'-AACTGTGAATACCAATCTAAGT-3' and 5'-GTTTTTCATTATGCAGTTACAG-3'. The mapping results were obtained from the rat radiation hybrid map server at the Medical College Wisconsin [(<http://rgd.mcw.edu/RH-MAPSERVER/>)] .

### Bioinformatics

Sequences comparisons were performed using the BLAST tool (<http://www.nc->

bi.nlm.nih.gov/BLAST]). Protein sequences alignments and phylogenetic tree were constructed using Clustal X (Ver. 1.8) and TreeView (Ver. 1.6.1) respectively. Structural predictions based on protein sequences were obtained using programs available on the ExPasy server ([http://www.expasy.ch]). Localization of the BCMP1 gene in the human draft genome sequence was achieved using data and tools available on the Ensembl server ([http://www.ensembl.org]).

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