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Identification and molecular analysis of *BANP*

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Abstract

BTG3 belongs to a family of structurally related genes whose biochemical functions remain elusive. In order to investigate the mechanism underlying *BTG3*-mediated functions, we tried to identify BTG3 potential partners. The use of the yeast 'two-hybrid system', with BTG3 as bait, enabled us to isolate BANP (BTG3 Associated Nuclear Protein). Other commonly used protein-binding assays did not confirm this yeast interaction. However, *BANP* had never been described before, and this prompted us to further characterise this gene. In this paper, we present data on its molecular organization in mouse, then we speculate on the nature of this nuclear protein, and finally we localise *BANP* on the human chromosome 16q24 subregion; we discuss the fact that frequent loss of heterozygosity within this region has been observed in different tumours.

Keywords: ANA; BTG; BTG3; rBTG3; 16q24

1. Introduction

The mouse *BTG3* gene (Guéhenneux et al., 1997) whose orthologues are *ANA* in human (Yoshida et al., 1998) and *rBTG3* in rat (Seo et al., 1999), belongs to the *BTG* gene family: its N-terminal region exhibits the common signature of the BTG protein family, the two well-conserved boxes A and B. Several members of the BTG family (BTG1, BTG2/TIS21/PC3, TOB, TOB2) are supposed to play a role in the negative control of the cell cycle, since their RNA expression is associated with different cell-cycle arrest processes (e.g., differentiation commitment, genotoxic treatment, P53-regulated expression of *BTG2*) and their in vitro ectopic overexpression is antiproliferative (Fletcher et al., 1991; Rouault et al., 1992, 1996; Matsuda et al., 1996; Montagnoli et al., 1996; Iacopetti et al., 1999; Ikematsu et al., 1999). Moreover, *BTG2* mutated ES cells present an altered G2/M block as compared with naive cells when they are treated with genotoxic agents (Rouault et al., 1996). The underlying mechanisms of this inhibition remain to be clearly established, but it has been recently proposed that BTG2/TIS21/PC3 overexpression could keep the RB protein in its hypophosphorylated form and thus prevent cell division (Guardavaccaro et al., 2000).

As for BTG3/ANA, the situation is even less clear although its overexpression has been shown to impair serum-induced cell-cycle progression (Yoshida et al., 1998). Some clues regarding its presumptive functions can also be gathered from the analysis of *BTG3* RNA expression patterns. *BTG3* RNA expression is ubiquitous with variations between different tissues. Its RNA level peaks at the end of the G1 phase of the cell cycle (Guéhenneux et al., 1997). It is noteworthy that *BTG3* RNA expression is induced by redox changes (Seo et al., 1999). Furthermore, Yoshida et al. (1998) have shown that *BTG3/ANA* is expressed in the ventricular zone of the developing nervous system, and they speculate on a possible role of *BTG3* in the differentiation process of neural precursors. These observations, combined with the fact that its ectopic overexpression is antiprolifera-

Abbreviations: aa, amino acid; EST, expressed sequence tag; GST, glutathione *S*-transferase; L/W/H, leucine/tryptophan/histidine; nt, nucleotide; ORF, open reading frame; UTR, untranslated region.

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tive, suggest that *BTG3* plays a role in the negative control of the cell cycle.

As mentioned above, the biochemical role of BTG3 has not yet been elucidated. Like other BTG proteins, BTG3 physically interacts with CAF1 (CCR4-associated factor1), a component of a general transcription multisubunit complex highly conserved throughout evolution (Bogdan et al., 1998; Rouault et al., 1998; Ikematsu et al., 1999). The exact role of this complex (i.e. the genes finally controlled by this complex) has not yet been unravelled, in spite of intensive research (Bai et al., 1999 and references therein). It should be emphasized that BTG3, unlike BTG1 and BTG2, does not interact with PRMT1 (our unpublished data), an arginine *N*-methyltransferase that could play a pivotal role in the antiproliferative function of the BTG proteins (Lin et al., 1996; Abramovich et al., 1997).

To broaden our knowledge on BTG3 function(s), we decided to search for associated proteins which may provide some insight into the biochemical pathways controlled by BTG3. We thus used BTG3 as a bait in 'two-hybrid' experiments in yeast, and identified BANP. In this paper, we describe the isolation/characterization of BANP, discuss the nuclear localization of this protein, and present data on its human chromosomal assignment.

2. Materials and methods

2.1. Yeast 'two-hybrid' experiments

The BTG3 bait for the yeast 'two-hybrid' system was based on the pLex yeast expression vector. PLex9 was cut with *Eco*RI/*Sal*I and ligated with the mouse *BTG3* cDNA obtained by PCR (sequences of synthetic oligonucleotides used: cggaattcatgaagaacgaaattgcggct and cgtcgacctagtgaggtgctaacatgtga). Thus, we generated a fusion protein consisting of the Gal4 DNA-binding domain fused to BTG3.

To identify genes encoding proteins that interact with BTG3, we used the 'two-hybrid' system to detect such associations via the reconstitution of a functional transcription activator in yeast with, as recipient, the L40 strain which was transformed with pLexBTG3. This BTG3 expressing yeast cell was subsequently transformed with a Gal4-transactivation domain-tagged 11-day-old embryo Matchmaker cDNA library purchased from Clontech (Palo Alto, CA, USA). We followed the protocol provided by the manufacturer. After 4 days at 30°C on L/W/H amino acid depleted SC medium plates, the transformants were tested for β-galactosidase activity using a colony filter assay. Positive (blue) colonies were further analysed, the electromax DH10B bacterial strain being used to recover the expression plasmids from the selected transformed yeast.

2.2. BANP DNA analysis

2.2.1. Northern Blot analysis

Total cellular RNA (10 μg) obtained from different mouse tissues (Tri-reagent, Sigma) was size fractionated in formaldehyde 1.2%–agarose gel and treated as described previously (Guéhenneux et al., 1997). *BANP* cDNA labelling reactions (Redi-primeII, Amersham) and hybridization procedures were carried out as in Guéhenneux et al., 1997.

2.2.2. 5′RACE experiments

We used the 5′/3′RACE kit (Roche Molecular Biochemicals) according to the manufacturer's instructions. mRNA from mouse spleen were reverse transcribed with the primer SP1 (ACAGCGCATCGCACT) and dA-tailed. *BANP*-specific cDNA was amplified with the provided oligo-dT primer and a BANP nested primer SP2 (TCTAGGATGCAAGTGGCCTCGAGAG), cloned into Bluescript and sequenced.

2.2.3. Mouse DNA library screening

The mouse BANP genomic clone 2C was isolated by screening clones of a λEMBL3 mouse genomic library using *BANP* cDNA (nucleotides 5–369) as a probe, following the usual procedures (Rouault et al., 1996).

2.2.4. Sequencing procedures

BANP DNA and cDNA cloned into Bluescript SK(−) (Stratagene, La Jolla, CA, USA) were sequenced by the dideoxy chain termination procedure with Sequenase II (USB, Cleveland, OH, USA) as described by the manufacturer.

Sequence similarity searches were performed at the NCBI BLAST Web server (http://www.ncbi.nlm.nih.gov/BLAST/) using the BLASTP, BLASTN and BLASTX programs (Altschul et al., 1990). Homologous sequences were aligned with the CLUSTALW program (Thompson et al., 1994).

2.2.5. Luciferase assay

Promoter activity was estimated with the Dual-Luciferase Reporter Assay System (Promega). All the luciferase reporter vectors were purchased from Promega. The putative promoter region of BANP upstream of the cDNA of *BANP* (Accession No. AF247179) was subcloned from the λEMBL3 *BANP* mouse genomic clone 2C and inserted into the pGL3-basic vector (pGL3ES1.1). Human HeLa cells and mouse NIH3T3 fibroblasts were seeded in six-well plates at 2.5×10^5 cells/well. After 24 h, cells were transiently cotransfected using Lipofectin Reagent (GibcoBrl) (HeLa cells) or Exgen (Euromedex) (NIH3T3 cells), respectively, with 1 or 2 μg of pGL3ES1.1, pGL3basic vector, or pGL3 control vector for luciferase constitutive expression, and 50 ng of the

Renilla Luciferase Reporter Gene pRL-SV40 vector for internal control. On the following day, cells were washed once with phosphate-buffered saline (PBS), lysed with passive lysis buffer, then Firefly and Renilla luciferase luminescence assays were successively performed as described by the manufacturer. Firefly reporter gene values were normalized to the activity of the internal control.

2.3. Cellular localization of BANP by immunofluorescence microscopy

A pSG5flagBANP vector was constructed by cloning the *Bam*HI/*Bgl*II coding sequence of *BANP* obtained by polymerase chain reaction with primers *Bam*HIBANP-138 and *Bgl*IIBANP-1893, in frame with the sequence of the flag peptide (IBI Flag system, Kodak) into the *Bam*HI/*Bgl*II digested pSG5flag vector (Rouault et al., 1998). The cloned product was checked by DNA sequencing. *Bam*HIBANP-138: 5′-CGGATCCATGATGTCAGAGCAG-3′; *Bgl*IIBANP-1893: 5′-CAGATCTTGTCTGAATGCACTC-3′.

HeLa cells were seeded on microscope slides in six-well plates at 2.5×10^5 cells/well. After 24 h, the cells were transiently transfected by Lipofectin Reagent (GibcoBRL) with 1 μg of the pSG5flagBANP vector. Two days later, cells were washed once with PBS, fixed with cold ethanol/acetic acid 95/5 (v/v) for 5 min at room temperature, and washed three times with PBS.

Immunodetection was carried out using M2 monoclonal antibody (IBI Flag System, Kodak) at a 1/100 dilution in PBS/0.2% gelatin, followed by a fluoresceine isothiocyanate-conjugated goat anti-mouse secondary antibody. The coverslips were mounted on microscope slides and the immunofluorescence was visualized with a Zeiss Axioplan 2 microscope.

2.4. BANP human chromosomal assignment

Fluorescence in situ hybridization (FISH) of normal human metaphases was performed using two independent BANP recombinant phages as probes as described in (Cherif et al., 1990; Rouault et al., 1996). The human *BANP* genomic recombinant phages were isolated by screening clones of a λEMBL3 human genomic library with a mouse *BANP* probe. Previously, we had checked that the mouse *BANP* probe detected a locus on a human Southern blot.

3. Results and discussion

3.1. Isolation/characterization of BANP

In this study, we used the 'two-hybrid' interaction system developed by Fields and Song (1989). To screen for cDNA encoding proteins able to interact with BTG3, the L40 yeast strain was transformed with a mouse 11-day-old embryo cDNA library cloned in pGad10. Out of 2.10^7 transformants plated on L/W/H amino acid depleted SC medium, clone 20 produced β-galactosidase and was further analysed. It is worth noting that we also trapped CAF1 that is known to interact with all the BTG proteins (Rouault et al., 1998). Clone 20 contains a 1.9 kb cDNA in frame with the Gal4 activation domain of pGAD10. No expression of the reporter gene was observed when the L40 yeast strain was cotransfected with clone 20 and the wild-type pLex9 vector. Clone 20 was then named BANP for BTG3 associated nuclear protein. The in vitro association assays commonly used when performing such studies failed in the case of BTG3 and BANP (in vitro translation followed by co-immunoprecipitation, affinity chromatography with different GST-fusion proteins). Several explanations can account for this: posttranslational modifications could be required for BANP/BTG3 interaction. If this were the case, in vitro translation would not be the appropriate system to demonstrate interactions between these two proteins. It is also possible that a third partner present in yeast bridged BTG3 and BANP. Furthermore, the in vivo BANP/BTG3 association could be very labile and difficult to assess while using classical co-immunoprecipitation procedures. Finally, it is possible that standard extraction methods were unable to detect these proteins without impeding their interaction.

It is worth noting that we did not observe any interaction when we performed a mammalian 'two-hybrid system' with these two proteins. Furthermore, the transcription factor CAF1 does not react with BTG3 in this assay, although CAF1 is highly suspected to be associated with BTG3 (Rouault et al., 1998). It should be emphasized that these experiments are positive when BTG1 or BTG2 are used in combination with CAF1 (Rouault et al., 1998). On the other hand, immunocytochemical studies have described BTG3 as a cytoplasmic protein (Yoshida et al., 1998). We can therefore imagine that BTG3 has a strong cytoplasmic retention signal and cannot be used in such a system that requires a nuclear localization of both partners, the role of BTG3 being to trap nuclear proteins outside of the nucleus. Another explanation could be that this BANP/BTG3 association is indeed an artefact.

Comparison of *BANP* (1969 bp) with sequence databases revealed one highly similar uncharacterized human cDNA (AK001039, 2124 bp) that had been sequenced in the course of the NEDO human cDNA sequencing project (Isogai and Otsuki, 2000; unpublished) (NB: GenBank contains another human sequence, AK000545, that corresponds to a chimeric sequence of *U6 snRNA* and *BANP* cDNA). Moreover, numerous matching ESTs from human, bovine, mouse and rat, and one

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   1 CTCTGCGAGG ACGGACGCCA TTATCGCAGC TCCCCGACAA ACACCACGAG AATTCCGCAG
  61 CCCACACGGT GACAGAAACC CCATACCCTG TGACTTCTGG ACTCTTGCTG TCAGTGTGCC
 121 CCTTCCTCGT GCTCTGGATG ATGTCAGAGC AGGACCTGGC GGATGTGGTT CAGATTGCAG
 181 TGGAAGACCT GAGCCCTGAT CACCCAGTTG TTTTGGAGAA TCATGTCGTG ACAGATGATG
 241 ATGAACCTGC CTTGAAGCGC CAGCGACTAG AGATCAATTG CCAGGACCCC TCTATAAAGT
 301 CTTTCCTGTA CTCTATTAAC CAGACGATAT GTTTGCGGTT GGATAGCATT GAGGCCAAGC
 361 TGCAAGCTCT CGAGGCCACT TGCAAATCTC TGGAAGAGAA GCTAGACCTG GTCACCAATA
 421 AACAGCACAG TCCCATCCAG GTCCCCATGG TGGCAGGTTC CCCACTTGGC GCCACCCAGA
 481 CCTGCAACAA AGTGCGATGC GCTGTTCCTG GGCGTCGGCA GAACACCATC GTGGTGAAAG
 541 TGCCTGGTCA GGACGACAGC CACAACGAAG ATGGGGAGAG CGGGTCAGAG GCCAGTGACT
 601 CCGTGTCTAA CTGTGGCCAG CCAGGAAGCC AGAACATTGG AAGCAACGTC ACACTCATCA
 661 CCCTGAACTC CGAAGAGGAC TATCCCAATG GCACCTGGCT GGGCGATGAG AATAACCCTG
 721 AGATGCGGGT ACGCTGTGCC ATCATCCCTT CCGACATGTT GCACATCAGC ACCAACTGTC
 781 GCACGGCCGA GAAGATGGCG CTGACACTGC TGGACTACCT GTTCCACCGT GAGGTGCAGG
 841 CTGTGTCCAA CTTGTCCGGC CAGGGCAAGC ACGGGAAGAA GCAGCTGGAC CCCCTCACCA
 901 TCTACGGCAT CCGGTGTCAC CTCTTCTATA AATTTGGAAT CACGGAATCT GACTGGTATC
 961 GGATCAAGCA GAGCATTGAC TCCAAGTGCC GGACAGCCTG GCGGCGGAAG CAGCGAGGCC
1021 AGAGCCTGGC GGTCAAGAGC TTCTCTCGGA GGACGCCATC CTCATCCTCT TACAGTGCCT
1081 CAGAGACCAT GATGGGAACC CCTCCTCCCA CCAGTGAGCT ACAGCAGTCA CAGCCACAGG
1141 CCCTACACTA CGCCCTGGCC AACGCCCAGC AGGTCCAGAT CCACCAGATT GGGGAGGATG
1201 GACAGGTGCA AGTAATCCCA CAGGGCCACC TCCACATTGC CCAGGTGCCT CAAGGGGAGC
1261 AGGTGCAGAT CACACAGGAC AGCGAGGGCA ATCTGCAGAT CCATCATGTG GGTCAGGATG
1321 GCCAGTCGTG GGGCCTGTGC CAGAATCCCA TTCCTGTCAG CGGTGACTCA GTGGCCCAGG
1381 CTAATCCCTC CCAGCTTTGG CCTCTGGGAG GAGACACACT TGATCTGCCT GCTGGAAATG
1441 AAATGATCCA GGTACTGCAG GGTGCTCAGC TCATAGCCGT GGCCTCTTCA GACCCTGCTG
1501 CTACAGGAGT AGATGGGTCG CCTCTCCAGG GCAGTGACAT TCAGGTTCAG TATGTCCAGC
1561 TGGCGCCTGT GAGTGACCAC ACAGCCGCAG CGCAGACCGC AGAGGCCCTG CAGCCCACTC
1621 TGCAGCCCGA CATGCAGCTT GAACATGGGG CCATCCAGAT CCAGTGAGGC CAGGCACTGC
1681 AGGAGCACCG AGTCACAGCT GCTCGCTGAC CCTGCCCCAC TCGTGCCCTG CTCTCTTGCT
1741 TCAGCAAGCA ACTGCAGGTT CTGCTGGGCA TCTGAGAGCT GCTCCTCCCA GGGGAAGGTC
1801 CTGGCCACCC CTGCTGGAAG GCGCCTCAGG GTTGGAGTCT CACTACTGGT CGTCTCCAAA
1861 GGAGAAGCAT AGTGCAGAGT GTTGAGTGCA TTCAGACAGA CAAGAACTAC GATATTTTGT
1921 TTAAACAGCT TTTTTAATTT GCTATGGTGT TTATAACAAA AAAGAAAAT
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Fig. 1. Nucleotide sequence of the mouse *BANP* cDNA (GenBank Accession No. AF091234). The deduced BANP coding sequence is indicated in bold type.

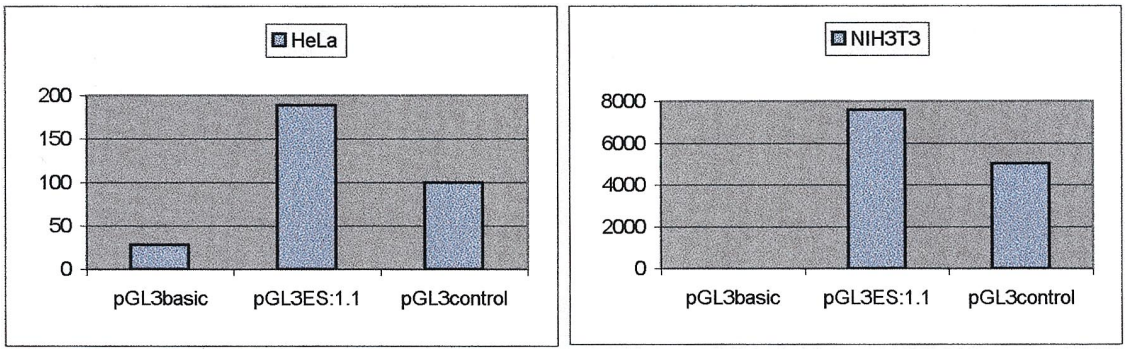

Fig. 2. *BANP* promoter analysis. The 5′ region of *BANP* (pGL3ES1.1) confers a transcriptional activity when cloned upstream of a luciferase coding sequence, as shown in these experiments. pGL3basic is an empty control vector. PGL3control is a SV4O promoter luciferase expression vector and was used as a positive control.

EST from chicken (AJ397040) and from zebrafish (AI882954) have been detected. Both mouse and human cDNAs contain one open reading frame (ORF) (positions 138–1664 in mouse, 153–1547 in human) beginning by two contiguous in frame AUG codons. The second one fulfils the criteria for a eukaryotic start codon with an A in −3, and could be the initiating codon (Cavener and Ray, 1991). The human cDNA contains a canonical polyadenylation signal (AATAAA) 20 bp upstream of its 3′ extremity. The 3′ UTR (including the polyadenylation signal) is well conserved in humans, mouse and rat, and the 3′ extremity (before the poly-A track) of ESTs from these three species all end at the same position. It is therefore likely that the human 3′ UTR is complete. Our mouse cDNA does not encompass the polyadenylation signal. As judged from mouse and rat ESTs and from the human cDNA, about 250 nt of the 3′ UTR were missing. Three mouse 3′ ESTs correspond-

A

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human       MMSEHDLADVVQIAVEDLSPDHPVVLENHVVTDEDEPALKRQRLEINCQDPSIKSFLYSI
mouse       MMSEQDLADVVQIAVEDLSPDHPVVLENHVVTDDDEPALKRQRLEINCQDPSIKSFLYSI
            ****:****************************:************************

human       NQTICLRLDSIEAKLQALEATCKSLEERLDLVTNKQHSPIQVPMVAGSPLGATQTCN---
mouse       NQTICLRLDSIEAKLQALEATCKSLEEKLDLVTNKQHSPIQVPMVAGSPLGATQTCNKVR
            *************************:****************************

human       -AVPGRRQNTIVVKVPGQEDSHHEDGESGSEASDSVSSCGQAGSQSIGSNVTLITLNSEE
mouse       CAVPGRRQNTIVVKVPGQDDSHNEDGESGSEASDSVSNCGQPGSQNIGSNVTLITLNSEE
             *****************:***:**************.***.***.**************

human       DYPNGTWLGDENNPEMRVRCAIIPSDMLHISTNCRTAEKMALTLLDYLFHREVQAVSNLS
mouse       DYPNGTWLGDENNPEMRVRCAIIPSDMLHISTNCRTAEKMALTLLDYLFHREVQAVSNLS
            ************************************************************

zebrafish                                   <DWYRIEQSIDSKCLTAWRXKQXXQILTVK
chicken                                  <DAWAESDWYRIKQSIDSKCRTAWRRKQRGQSLAVK
human       GQGKHGKKQLDPLTIYGIRCHLFYKFGITESDWYRIKQSIDSKCRTAWRRKQRGQSLAVK
mouse       GQGKHGKKQLDPLTIYGIRCHLFYKFGITESDWYRIKQSIDSKCRTAWRRKQRGQSLAVK
            ************************************:******* **** **  * *:**

zebrafish   SSSRRTPASQSSSDGVSAAET----SAIETSTQ--QALHYALAGATQQVQIHRIGEDGQV
chicken     SFSRRTPSSSSYSGSEGPQSSVSASNEIQQNQP--QALHYALAN-AQQVQIHQIGEDGQV
human       SFSRRTPNSSSYCPSEPMMSTPPPASELPQPQPQPQALHYALAN-AQQVQIHQIGEDGQV
mouse       SFSRRTPSSSSYSASETMMGTPPPTSELQQSQP--QALHYALAN-AQQVQIHQIGEDGQV
            * ***** *.* . .     :    . :       ********. :******:*******

zebrafish   QVIPQGHLHIAQVPQGEQVQITQDSEGNLQIHQVHVGQDGQ-------------------
chicken     QVIPQGHLHIAQVPQGEQVQITQDSEGNLQIHQVHVGQDGQ-------------------
human       QVIPQGHLHIAQVPQGEQVQITQDSEGNLQIH--HVGQDGQ-------------------
mouse       QVIPQGHLHIAQVPQGEQVQITQDSEGNLQIH--HVGQDGQSWGLCQNPIPVSGDSVAQA
            ********************************  *******

zebrafish   ----------------------VNVLRGPQLIAVASTD-GTGGVDASPLQANDIQVQYVQL
chicken     -----------------------VLQGAQLIAVASAEPASGGVDGSPLQGSDIQVQYVQL
human       -----------------------VLQGAQLIAVASSDPAAAGVDGSPLQGSDIQVQYVQL
mouse       NPSQLWPLGGDTLDLPAGNEMIQVLQGAQLIAVASSDPAATGVDGSPLQGSDIQVQYVQL
                                  **:*.*******:* .: ***.****..*********

zebrafish   GTVTDHTGAVQ-AEALTPALQAEMDVKE-AIQLQ
chicken     TPVTDHSATSQGTDTLQSA
human       APVSDHTAGAQTAEALQPTLQPEMQLEHGAIQIQ
mouse       APVSDHTAAAQTAEALQPTLQPDMQLEHGAIQIQ
             .*:**:.  * :::* .:**.:*:::. ***:*
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B

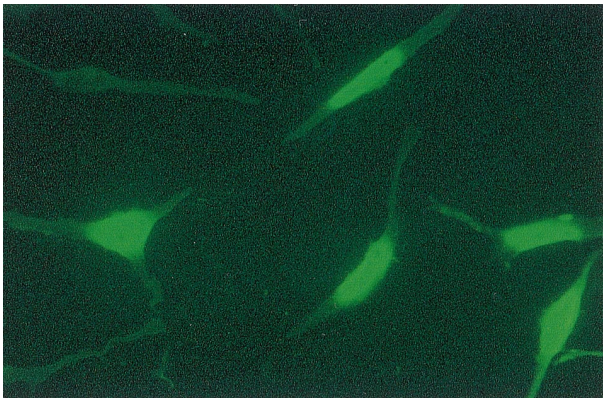

Fig. 3. (A) Alignment of vertebrate BANP proteins. Accession Nos. are: human cDNA, AK001039; mouse cDNA, AF091234; chicken (EST, partial cDNA), AJ397040; zebrafish (EST, partial cDNA), AI882954. (*) identical residues; (:) conservative substitution; (.) less conservative substitution. (B) Nuclear localization of BANP protein. Analysis of flag epitope-tagged BANP expression by transient transfection of Hela cells.

ing to this missing *BANP* 3′ UTR were found in the databases (AI597204, AI504266, AA561621). They complete the *BANP* cDNA since they exhibit a polyadenylation site followed by a polyA tail. One explanation could be that the internal poly-A track at the end of clone 20 prevents the total reverse transcription of *BANP*. This difference corresponds to the discrepancy between the size of our cDNA (about 2 kb) and the *BANP* RNA size on Northern blot (2.3 kb).

The human 5′ UTR contains one in-frame stop codon which suggests that this ORF corresponds to the complete protein. To ascertain whether the mouse 5′ UTR was complete, we performed a 5′RACE experiment to generate a full-length cDNA. Only a 4 bp extension was obtained (nt 1–4). It is therefore very likely that the 5′ UTR was complete. This 1969 nt sequence was submitted to GenBank database (Accession No.: AF091234) (Fig. 1).

To better characterize this *BANP* upstream region, we screened a mouse genomic library with a 5′ end BANP probe (nucleotides 5–369). From a recombinant phage, we subcloned a genomic region that encompassed part of the 5′ UTR (exon 1). Comparison of *BANP* cDNA and genomic DNA sequences revealed a typical splice donor site (CACACG**gt**aattg) beginning at nucleotide 68 (Fig. 1). Having established that this subclone was part of the *BANP* gene, we postulated that the sequence upstream of the *BANP* 5′ UTR (exon1) was the promoter. Furthermore, the PROMOTER SCAN II program (Prestridge, 1995) predicted this region as being a promoter (GenBank Accession No.: AF247179).

To test this assertion, we performed luciferase assays with this region cloned upstream of a reporter gene (luciferase). As illustrated in Fig. 2, this construction promoted a luciferase activity and thus can be considered as part of *BANP* promoter.

All these data indicate that the proposed mouse *BANP* cDNA (Fig. 1) is full length (except the extremity of the 3′ UTR that can be found in ESTs deposited in databases) and that the deduced ORF actually represents the BANP protein.

3.2. BANP protein analysis

The mouse sequence exhibits an ORF coding for a peptide of 509 amino acids of 55 384 predicted molecular weight.

The zebrafish and chicken ESTs correspond to partial cDNA sequences that encompass the 3′ part of the coding region. The alignment of the conceptual translation of these ESTs with human and mouse proteins shows that the *BANP* gene is highly conserved in vertebrates (Fig. 3A). The mouse BANP protein contains a 42-amino-acid-long insertion compared with the human, chicken and zebrafish sequences. The analysis of rat ESTs shows that this insertion is also present in the rat BANP protein. Except for this insertion, human and mouse proteins are 95% identical (Fig. 3A). The BANP protein does not show any significant sequence similarity to any other proteins and does not contain any of the functional signatures described in the PROSITE database (Hofmann et al., 1999). A glutamine-rich region (aa 329–509) of unknown significance was found and is highly conserved among the vertebrate BANP proteins (Fig. 3A). However, analysis of BANP protein with the PROPSEARCH program (Hobohm and Sander, 1995) indicated that its size and amino acid composition (content of bulky residues, content of small residues, average hydrophobicity, average charge and the content of selected dipeptide-groups) were similar to many different transcription factors. We therefore suggest that BANP might be a transcription factor.

In accordance with this assumption, immunofluorescence microscopy in Hela cells transfected with a pSG5flagBANP vector revealed an exclusive nuclear localization of the BANP protein (Fig. 3B).

3.3. BANP RNA expression

Expression of *BANP* in various organs was measured by Northern blot using *BANP* cDNA as a probe. It revealed a mRNA expression of 2.3 kb. As shown in Fig. 4, it is highly expressed in heart, spleen, thymus, and moderately expressed in muscle and bladder. No expression could be detected in liver even after prolonged film exposure. Furthermore, analysis of *BANP* EST showed that it is expressed during different stages of mouse embryo development (from 1 to 14.5 days post-conception). We conclude that *BANP* expression is rather ubiquitous.

3.4. Human BANP chromosome assignment

Since *BANP* is evolutionarily conserved, we used a mouse *BANP* probe (*BANP* cDNA, 86% identical to its human counterpart) to screen a human genomic DNA library. Two different recombinant phages were isolated and further amplified. As illustrated in Fig. 5, fluorescence in situ hybridization (FISH) of these probes on human metaphase chromosome mapped BANP to the 16q24 subregion. The human cDNA matches seven

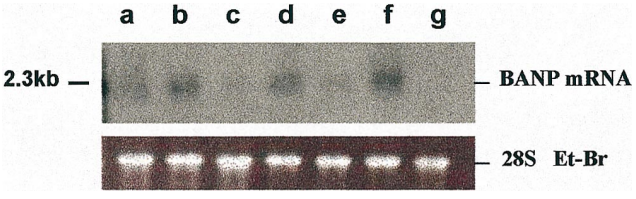

Fig. 4. *BANP* RNA expression was assessed in various organs. a, heart; b, brain; c, muscle; d, spleen; e, bladder; f, thymus; g, liver.

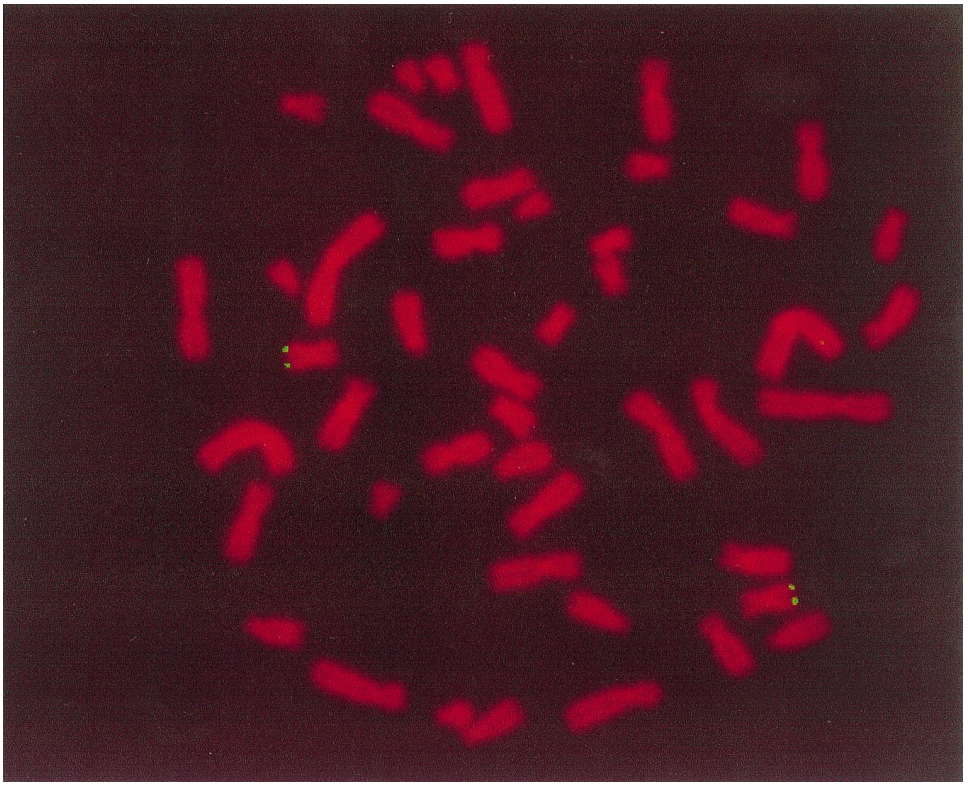

Fig. 5. Chromosomal localization of the human *BANP* gene by FISH in normal human chromosomes using *BANP* probes. With probe a, we observed a spot on the two sister chromatids at band 16q24 in the 20 metaphase cells examined. With probe b, we observed the same localization and, in addition, a colocalization at band 10q22–23. Thus, *BANP* is located at band 16q24.

unfinished genomic sequences from chromosome 16. These sequences span six exons, but the total number of exons cannot be determined since 5′ and 3′ parts of the gene and at least one internal exon have not yet been sequenced. The gene appears to be large since the six presently available exons span at least 200 kb. Several papers report data showing loss of heterozygosity of this region in different tumours (breast, prostate, stomach) (Tsuda et al., 1994; Radford et al., 1995; Mori et al., 1999; Wang et al., 1999). These data suggest the presence of one or several tumour suppressor genes on this chromosome arm. Although there is no evidence of BANP involvement in the genesis of these tumours, it could be interesting to establish whether *BANP* is rearranged in these cases. Nevertheless, *BANP* constitutes a marker and should be useful for linkage analysis in order to discover these tumour suppressor genes.

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