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Biochemical and Biophysical Research Communications 299 (2002) 49–56
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Human brain acyl-CoA hydrolase isoforms encoded by a single gene☆,☆☆

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Received 24 September 2002

Abstract

Acyl-CoA hydrolases are a group of enzymes that catalyze the hydrolysis of acyl-CoA thioesters to free fatty acids and CoA-SH. The human brain acyl-CoA hydrolase (BACH) gene comprises 13 exons, generating several isoforms through the alternative use of exons. Four first exons (1a–1d) can be used, and three patterns of splicing occur at exon X located between exons 7 and 8 that contains an internal 3′-splice acceptor site and creates premature stop codons. When examined with green fluorescent protein-fusion constructs expressed in Neuro-2a cells, the nuclear localization signal encoded by exon 9 was functional by itself, whereas the whole structure was cytosolic, suggesting nuclear translocation of the enzyme. This was consistent with dual staining of the cytosol and nucleus in certain neurons by immunohistochemistry using anti-BACH antibody. The mitochondrial targeting signals encoded by exons 1b and 1c were also functional and directed mitochondrial localization of BACH isoforms with the signals. Although BACH mRNA containing the sequence derived from exon 1a, but not exon X, was exclusively expressed in human brain, these results suggest that the human BACH gene can express long-chain acyl-CoA hydrolase activity in multiple intracellular compartments by generating BACH isoforms with differential localization signals to affect various cellular functions that involve acyl-CoAs.

Keywords: Acyl-CoA thioesterase; Gene structure; Cytosol; Nucleus; Mitochondria; Neuron

Long-chain acyl-CoA hydrolases/thioesterases (EC 3.1.2.2) are a group of enzymes that catalyze the hydrolysis of acyl-CoA thioesters to free fatty acids and CoA-SH. The enzyme activity is ubiquitously found in organisms and cell types and in most cellular compartments, although in some fractions it is often accounted for by microsomal carboxylesterases with multi-substrate specificities [1]. The potency of acyl-CoA hydrolases/thioesterases may be a mechanism that regulates intracellular levels of acyl-CoAs, free fatty acids, and CoA-SH to affect various cellular mechanisms and functions, because acyl-CoAs participate in a large number of reactions that include lipid synthesis, energy metabolism, and regulation.

Acyl-CoAs are the source of signaling molecules like ceramide and diacylglycerol, and also serve as regulators of certain enzyme systems such as fatty acid synthesis, ion fluxes, vesicle trafficking, and protein phosphorylation [2]. Protein acylation anchors proteins to specific membranes and enables them to function correctly [3]. In addition, it has become evident that acyl-CoAs as well as free fatty acids can act as signaling molecules involved in regulating gene expression, as shown with the nuclear receptors, peroxisome proliferator-activated receptor α (PPARα), and hepatocyte nuclear factor 4α (HNF-4α) [4,5]. However, the physiological functions of acyl-CoA hydrolases/thioesterases are not fully established.

In previous studies, we and others have identified novel members of long-chain acyl-CoA hydrolases/thioesterases in rats, mice, and humans by means of protein purification and molecular cloning (reviewed in [1]). These enzymes, tentatively termed type-I and type-II

☆ *Abbreviations:* aa, amino acid(s); ACBP, acyl-CoA binding protein; hBACH, mBACH, and rBACH, human, mouse, and rat brain acyl-CoA hydrolase; GFP, green fluorescent protein; MTS, mitochondrial targeting signal; NLS, nuclear localization signal; RACE, rapid amplification of cDNA ends; RT, reverse transcription.

☆☆ Nucleotide sequences reported in this paper have been submitted to the DDBJ databank under Accession Nos. AB074415–AB074419 and Nos. AB088411 and AB088412.

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PII: S0006-291X(02)02587-1

acyl-CoA thioesterases according to the nomenclature proposed by Alexson's group [1], have been characterized as highly specific for hydrolyzing long-chain acyl-CoAs and implicated in lipid metabolism [6–9]. To date, it has been revealed that mouse type-I acyl-CoA thioesterases comprise four isoforms with cytosolic, mitochondrial, and peroxisomal localizations (CTE-I, MTE-I, PTE-Ia, and PTE-Ib) to form a highly homologous multi-gene family, each gene of which is less than 9 kb in size and organized by three exons divided by two introns, and that their human orthologs are located within a very narrow region (<166 kb) of chromosome 14 as a gene cluster [10]. On the other hand, type-II acyl-CoA thioesterases include cytosolic and mitochondrial isoforms (CTE-II and MTE-II), although the molecular identity of MTE-II is unclear as it has been neither purified nor cloned yet [8]. We have referred to CTE-II as brain acyl-CoA hydrolase (BACH) (also called ACT by Hajra's group [9]) because of its constitutive expression in the brain at a level much higher than in other tissues, and localized it to neurons in rodents [11]. The BACH gene is well conserved in humans and rodents, and the human gene spans about 130 kb, mapped to 1p36.31–p36.11, and comprises at least nine exons [12]. In studies with rats and mice [11,13], we cloned several isoforms of BACH, for example, rat LACH1, whose structures were highly related to each other, and speculated the alternative exon usage of a single gene as a mechanism to generate those isoforms.

In this study, we systematically searched for human BACH isoforms by molecular cloning and reinvestigated the genomic organization of the BACH gene, in order to figure out how these isoforms are generated. Moreover, the intracellular distribution of each isoform was examined to investigate the role of the alternative exons that encode signal motifs. We here report that the human BACH gene can express acyl-CoA hydrolase activity in multiple intracellular compartments by generating isoforms with differential localization signals.

Materials and methods

Materials. Total RNA from normal human adult brain was obtained from Gibco-BRL and BioChain Institute. Tissue slides of the human neural system were purchased from BioChain Institute. Slides of adult mouse brain, rabbit anti-rBACH antibody, and the mouse neuroblastoma cell line Neuro-2a were previously described [7,11]. Xenopus oocytes were obtained from a local fish farm (Tokyo, Japan).

cDNA cloning. cDNAs for hBACH isoforms were isolated by PCR after oligo$(dT)_{18}$-primed RT of human brain total RNA, cloned into the TA-cloning vector pGEM-T (Promega), and sequenced. The nucleotide sequences of the PCR primers used are indicated in Fig. 1A. The 5′-RACE was performed with 5′RACE system version 2.0 (Gibco-BRL). In the 3′-RACE, 5′-CAATGGATCCGTATGCCTAA$(T)_{18}$ VN-3′ (N is any base and V is G, A, or C) was used as a RT primer.

Expression of GFP-fusion protein. To construct the plasmids encoding the GFP-fusion proteins shown in Fig. 3, coding regions of cDNAs for hBACH isoforms were amplified by PCR and directionally inserted into the GFP expression vector pEGFP-N1 (Clontech) at *Nhe*I and *Eco*RI sites or pEGFP-C1 (Clontech) at *Eco*RI and *Bam*HI sites. The PCR primers (see Table 1 for the nucleotide sequences) and the template cDNAs (databank Accession No.) used were as follows: GFP-NLS (A), GFP-NLS* (B), and GFP-BACHa (C), primers S1, S2, or S3, and A1 with D88894, respectively; MTSb-GFP (E) and BACHb-GFP (F), S5 and A4 or A3 with AB074417, respectively; GFP-npNLS (G), S6 and A5 with Y00204; coMTS-GFP (H), S7 and A6 with NM_004074. The cDNA fragments encoding nucleoplasmin NLS (npNLS) and MTS of cytochrome *c* oxidase subunit VIII (coMTS) were prepared by RT-PCR using total RNA from frog oocytes and human brain, respectively. To construct the plasmid for GFP-BACHa-npNLS (D), PCR was performed using primers S4 and A2 with a cDNA clone for hBACHa (D88894) as a template. The PCR product was digested with *Hin*dIII and *Eco*RI, and ligated to the expression plasmid for GFP-npNLS described above that had been constructed in pEGFP-C1 using *Eco*RI and *Bam*HI sites and digested with *Hin*dIII and *Eco*RI.

Neuro-2a cells were plated onto glass coverslips (diameter 18 mm) precoated with 0.1% (v/v) polyethylenimine at 1.5×10^4 cells/cm^2 and cultured for 24 h as described previously [11]. These cells were transfected with the plasmids (0.7 μg) using Lipofectamine plus (Life Technologies) and, after 24 h, treated with 300 μM dibutyryl cAMP for induction of cell differentiation [14]. Cells were maintained for another 24 h. Before fixation with 4% (w/v) paraformaldehyde, some of these cells were incubated with 100 nM MitoTracker Orange (Molecular Probes) for 30 min. For microscopic examination, a μRadiance AG-2 confocal scanning system (Bio-Rad) was used.

Quantitative RT-PCR. To estimate mRNA levels of hBACHa–d, real-time quantitative PCR was performed using the ABI PRISM 7700 sequence detection system and SYBR Green PCR core reagents kit (PE Biosystems) as described previously [11]. After oligo$(dT)_{18}$-primed RT of human brain total RNA, PCR was performed using primers S8-11 specific to hBACH gene exons 1a–1d, respectively, and A7 specific to exon 2 (Table 1). For comparison of mRNA levels among hBACHa,

Table 1
List of PCR primers used

Sense primers	
S1 (E)	5′-ACGAATTCGAAGAAGCGCTTTGAGGAA-3′
S2 (E)	5′-ACGAATTCGGAGGAGGGCTTTGAGGAA-3′
S3 (E)	5′-ACGAATTCAGCCCCCAGCATGTC-3′
S4 (H)	5′-ACAAGCTTCAGCCCCCAGCATGTC-3′
S5 (N)	5′-CAGCTAGCCCACTTACCGCAGGATGA-3′
S6 (E)	5′-ACGAATTCACCCAAAGCTGTAAAGAGA-3′
S7 (N)	5′-CAGCTAGCGTACTCCGTGCCATCATGTC-3′
S8	5′-GCAGCCCCCAGCATGT-3′
S9	5′-GGTGCTGCGCTCCCGTCCA-3′
S10	5′-TTCGGAGAAGCTTGTCACTAAAC-3′
S11	5′-CCTTTCTCTGCATCTGATCCTG-3′
S12	5′-GCCAGACACCTGCGCCCTT-3′
S13	5′-CACCTGGTGGGGCCTTCAGA-3′
Antisense primers	
A1 (B)	5′-GAAGGATCCAGTCTAGGGCTGAGGCT-3′
A2 (E)	5′-CAGAATTCTTCTCGTCCTCGGTCTC-3′
A3 (B)	5′-CAGGATCCGGCTGAGGCTCCGCGTG-3′
A4 (E)	5′-GCGAATTCGGATGGTCCCCCCGTGGACAT-3′
A5 (B)	5′-CAGGATCCAAGTTTCTTCTTCTTTGC-3′
A6 (E)	5′-CTGAATTCGCTTCCCCTCCGGCGGCAACG-3′
A7	5′-TGCCGGCCACGTTGGCATCAT-3′
A8	5′-CCAGCCAAGCAGCAGCTTTA-3′
A9	5′-CAACAGGGTCGGCGTCCACCAA-3′

Restriction enzyme sites are underlined with the corresponding enzyme names shown in parentheses. N, *Nhe*I; E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI.

hBACHa/X, and hBACHa/Xi, PCR was performed with those RT products as the template using primers S12 and A8 specific to exons 1a and 9, respectively, after which the PCR products were, in turn, used as templates for nested PCR with primers S13 and A9 specific to exons 7 and 8, respectively. The reactions were run on an agarose gel and stained with SYBR Green (Molecular Probes), and the intensity of bands corresponding to each isoform was measured by LAS 1000plus luminoimage analyzer (Fuji Photo Film, Tokyo, Japan).

Other methods were performed as described previously [11,12].

Results and discussion

cDNA cloning and genomic organization of the hBACH gene

To seek out novel isoforms of hBACH, 5′- and 3′-RACE were performed with total RNA from human

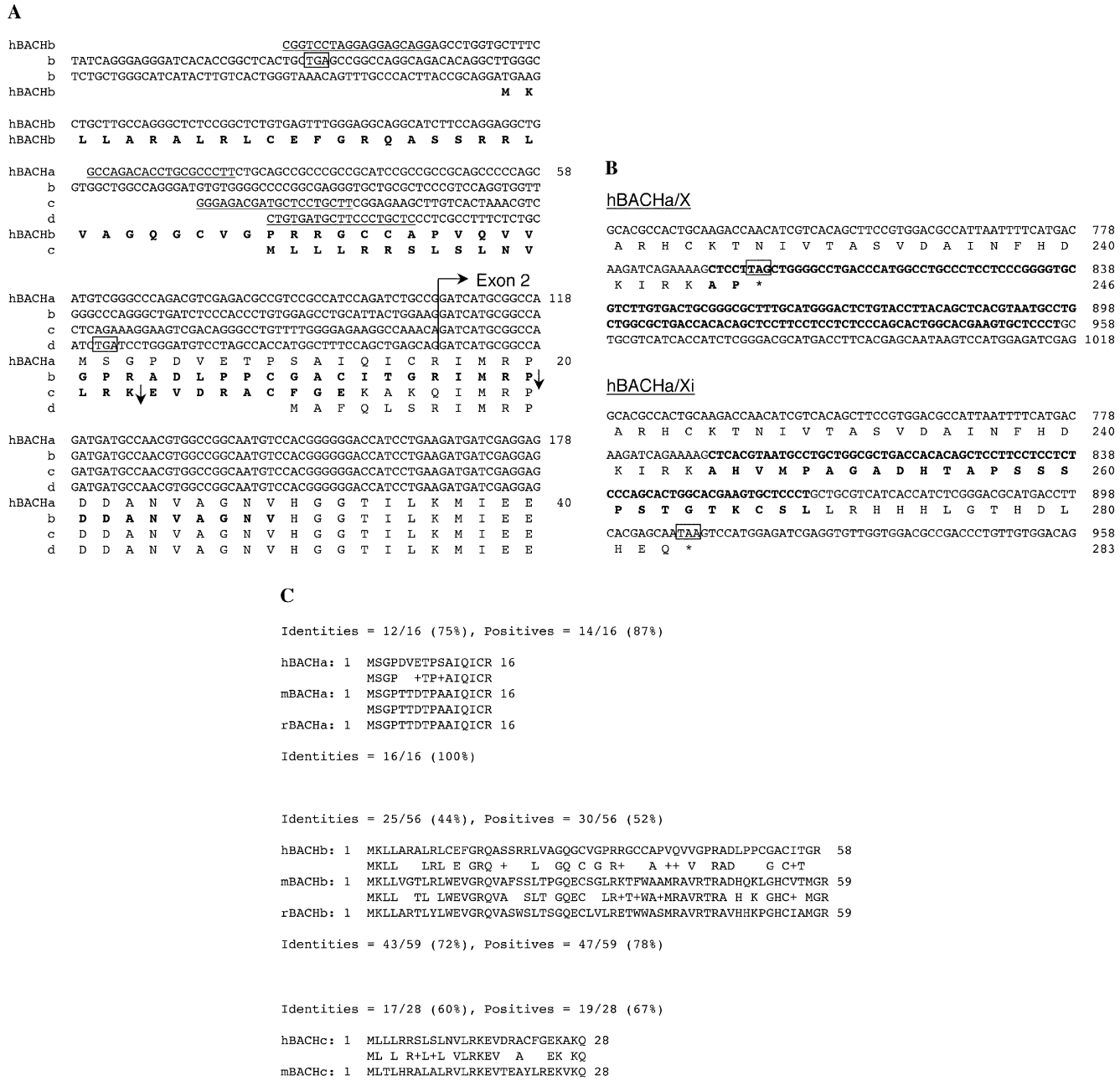

Fig. 1. (A) Sequence comparison of cDNA 5′-ends and N-termini of hBACH isoforms. In-frame stop codons are boxed. The MTSs (aa 1–71 and 1–24 for hBACHb and hBACHc, respectively) are shown in boldface with predicted cleavage sites for the presequences indicated by arrows. The nucleotide sequences of the PCR sense primers that were used to amplify the full-length of the respective cDNAs in combination with a common antisense primer A8 (see Table 1) are underlined. For hBACHa, the nucleotides and amino acids are numbered. (B) Partial sequences of hBACHa/X and hBACHa/Xi cDNAs. The 165-bp (X) (nt 792–956) and 73-bp nucleotide sequences (Xi) (nt 792–864) and their deduced amino acid sequences are shown in boldface. (C) Comparison of amino acid sequences encoded by BACH gene exon 1 between humans and rodents. For pairwise sequence comparison, the BLAST 2 sequences program (http://www.ncbi.nlm.nih.gov/BLAST/) was used. Conservative substitutions are indicated by +.

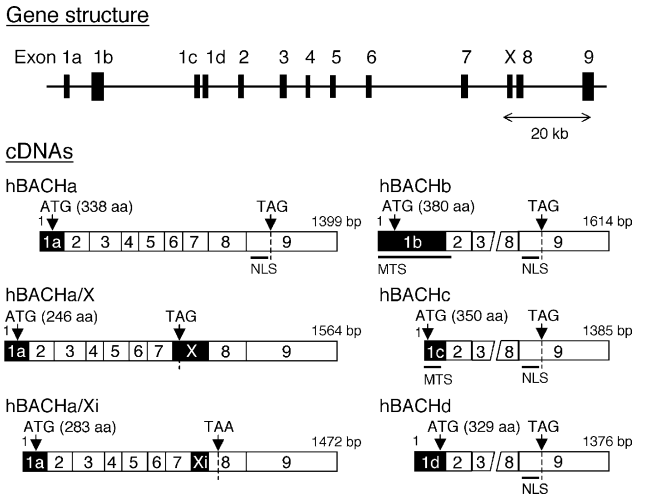

Fig. 2. Schematic representation of the structural organization of the hBACH gene and cDNAs for its transcripts. Exons are depicted as closed boxes and introns and flanking regions by solid lines. Open boxes represent cDNA sequences with the number of exons from which the sequences are derived, and highlighted are the sequences derived from alternative exons. Exon X had an internal 3′-splice acceptor site, giving rise to transcripts with a 165-bp (X) or 73-bp insert (Xi). Positions of the start and stop codons are indicated by arrows with total numbers of deduced amino acids shown in parentheses. MTS, mitochondrial targeting signal; NLS, nuclear localization signal.

brain. The expressed sequence tag (EST) database was also searched (Figs. 1 and 2). As shown in Fig. 1A, this screening resulted in four distinct sequences of cDNA 5′-ends related to the hBACH gene. For the 3′-end, however, there was only a single sequence in which a single polyadenylation site was found at 5′-TGCTTGGCT GG-poly(A), albeit with several variations with 3′-extensions or deletions of a few bases. Then, to search for an unknown exon(s) present within introns of the hBACH gene previously defined [12], RT-PCR was performed using various pairs of primers that were specific to adjacent exons. Size estimation followed by the sequencing of PCR products revealed 165-bp and 73-bp inserts between the sequences derived from exons 7 and 8, with the 73-bp insert being identical to nt 93–165 of the 165-bp sequence (Fig. 1B). An attempt to find another insert sequence using the EST database failed. When run against a DNA database, all of the cDNA sequences described above completely matched the corresponding genomic sequences (databank Accession No. NT_028054) within the range of about 130 kb that had previously been assigned to the hBACH gene (1p36.31–p36.11) [12]. Therefore, it was concluded that the hBACH gene comprises 13 exons, of which four first exons (1a–1d) can be used, and three patterns of splicing occur at exon X located between exons 7 and 8 that contains an internal 3′-splice acceptor site, giving rise theoretically to 12 isoforms through a mechanism of alternative use of exons. The structural organization of the hBACH gene is summarized in Table 2.

Table 2
Comparison of genomic organization between hBACH and mBACH genes

hBACH gene (129 kb)			mBACH gene (94 kb)		
Exon	Exon size (bp)	Intron size (kb)	Exon	Exon size (bp)	Intron size (kb)
1a	>105		1a	>207	
		<7.4			<7.9
1b	>320		1b	>180	
		<24.7			<13.5
1c	>91		1c	>148	
		<1.7			6.8
1d	>82		—	—	
		9.0			—
2	118		2	118	
		10.2			10.9
3	157		3	157	
		5.9			5.2
4	92		4	92	
		6.1			6.6
5	115		5	115	
		8.7			7.8
6	87		6	87	
		23.5			15.8
7	117		7	117	
		12.0			7.6
X	165		—	—	
		1.4			—
8	185		8	185	
		16.4			10.4
9	423		9	374	

For calculations, the NCBI reference sequences (databank Accession Nos. NT_028054 and NW_000222 for human and mouse genomes, respectively) were used. The size of exon 1 was not determined because the transcription start site was not known. No exons corresponding to exons 1d and X of the hBACH gene were found in the mBACH gene.

In practice, full-length cDNAs for six kinds of hBACH isoforms (hBACHa–d, hBACHa/X, and hBACHa/Xi, shown in Fig. 2) were cloned from human brain total RNA. These clones had unique sequences derived from the respective exon 1's and shared the same sequence corresponding to exons 2–9, with or without exon X-derived sequences. Of these isoforms, hBACHa was that which had previously been cloned as hBACH [12] with a subunit size of 43 kDa and >95% identity in amino acid sequence to the rodent orthologs, rBACH and mBACH [11,13]. Moreover, rat LACH1 [13] and a mouse EST clone (databank Accession No. AA103190) [11] corresponded to hBACHb, and mouse MBACH-k2 [11] to hBACHc. Therefore, these rodent BACH isoforms are tentatively termed BACHa–c, respectively, in this paper (Fig. 1C). The BACH gene is well conserved in humans and mice, but mouse exons 1d and X could not be found in this study (Table 2). When expressed in bacteria, the cDNAs for hBACHa–d shown in Fig. 2 produced enzyme proteins that had acyl-CoA hydrolase activity with similar chain-length specificities (data not shown). However, hBACHa/X and hBACHa/Xi cDNAs

did not, in which the insertion of 165- or 73-bp resulted in a frame-shift and created premature stop codons, producing C-terminally truncated proteins with no enzyme activity (data not shown). The cleavage sites for the MTS presequences in hBACHb and hBACHc were not determined.

Intracellular localization of hBACH isoforms

As shown in Fig. 2, PSORT II (http://psort.ims.u-tokyo.ac.jp/), a computer program for the prediction of protein sorting signals and localization sites in amino acid sequences, predicted a bipartite NLS in the sequence encoded by the hBACH gene exon 9 and MTS's in those encoded by exons 1b and 1c. Whether or not these signals are functional was examined by expressing their GFP-fusion constructs in Neuro-2a cells (Fig. 3). When the NLS was expressed as its GFP-fusion protein, it was exclusively localized to nuclei (Fig. 3A), whereas the fusion protein with mutations in the NLS diffused over the cell (Fig. 3B) like GFP expressed alone (Fig. 3I), indicating that the NLS is functional by itself. However, a GFP-fusion construct of the entire hBACHa protein showed cytosolic localization with no distribution in nuclei (Fig. 3C). So, the NLS in hBACHa was replaced by the NLS derived from Xenopus nucleoplasmin [15], a typical bipartite NLS (Fig. 3G), and examined. As shown in Fig. 3D, this chimeric hBACHa was exclusively localized to nuclei. Thus, it was unlikely that there is a structure in hBACHa to rigidly prohibit or mask the NLS from factors involved in the nuclear import. These results suggest that hBACHa could be in the nucleus under particular conditions with stimulated nuclear import. Some fractions of hBACHa might translocate from the cytosol to the nucleus in response to some stimulus. A GFP-fusion construct of the entire hBACHd was also localized to the cytosol as was the case in Fig. 3C (data not shown).

The MTS encoded by exon 1b was also functional and directed mitochondrial localization of hBACHb as shown in Figs. 3E and F, in which the cytoplasmic space of the cell was dotted with a large number of fluorescent signals as seen with the MTS of cytochrome *c* oxidase subunit VIII [16], a typical MTS (Fig. 3H). These dotted patterns of GFP signals completely overlapped those of staining by MitoTracker Orange used as a mitochondrial marker (data not shown). Despite the presence of NLS in hBACHb, the MTS delivered the isoform to mitochondria, indicating that the MTS is dominant over the NLS as an intracellular localization signal. Similar results were obtained with hBACHc and its MTS (data not shown). These results suggest that hBACHb and hBACHc are mitochondrial enzymes and that the isoforms represent a type-II acyl-CoA thioesterase, MTE-II, that had previously been partially purified from liver mitochondria of rats treated with peroxisome proliferators [8].

Expression levels and histology of hBACH isoforms in the brain

Among the hBACH isoforms described above, the mRNA level for hBACHa was significant, but not for the remainder whose levels were not higher than 2% of

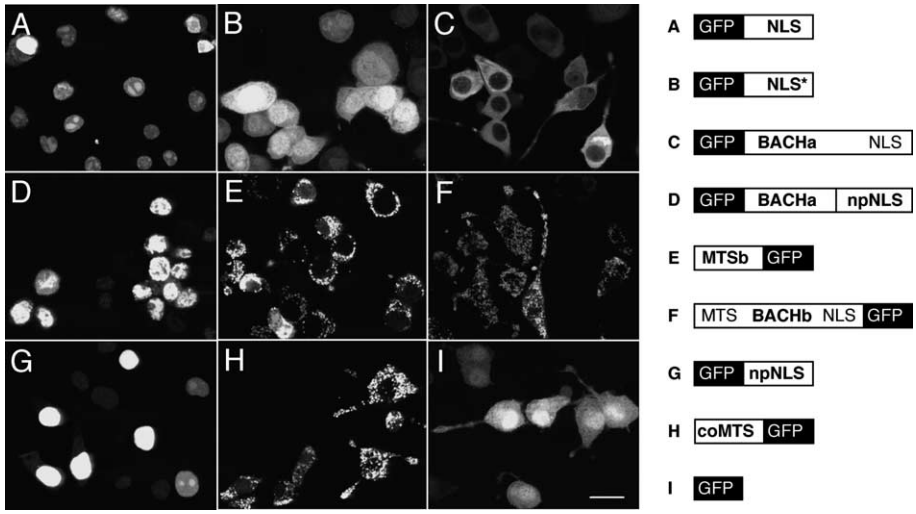

Fig. 3. Expression of hBACH isoforms as GFP-fusion proteins in Neuro-2a cells. Fused to the C-terminus of GFP were a polypeptide containing the NLS encoded by hBACH gene exon 9, KKRFEEGKGRYLQMKAKRQGHAEPQP-COOH (NLS underlined) (A) or its mutant, EEGFEEGKGRYLQMKAKRQGHAEPQP-COOH (B), a full-length hBACHa (C), the one whose NLS was replaced by that of nucleoplasmin, KAVKRPAATKKAGQAKKKKL-COOH (D), and the latter NLS alone (G). To the N-terminus of GFP, a polypeptide containing the MTS encoded by exon 1b (aa 1–76 of hBACHb) (E), a full-length hBACHb (F), and an MTS from cytochrome *c* oxidase subunit VIII, NH_2-MSVLTPLLLRGLTGSARRLPVPRAKIHSL (H) were fused. GFP alone was also expressed (I). The structural organization of each fusion protein is schematically represented on the right. Scale bar, 25 μm.

hBACHa mRNA, as estimated by quantitative RT-PCR with human brain total RNAs (Fig. 4, upper panel). It was thus indicated that among hBACH isoforms hBACHa is exclusively expressed in the brain, where it was localized by immunohistochemistry using anti-rBACH antibody to the cytosol of neurons, such as the pyramidal cells in the cerebral cortex (Fig. 4A) and Purkinje cells in the cerebellum (Fig. 4B). These findings are consistent with the case of mBACHa [11]. Interestingly, the immunoreactivity was found in the nucleus as well as the cytosol of certain large neurons in the cerebellum, medulla oblongata, and spinal cord in mice (Figs. 4C–F). Although the human samples could not be examined, this would be the case for hBACHa in human,

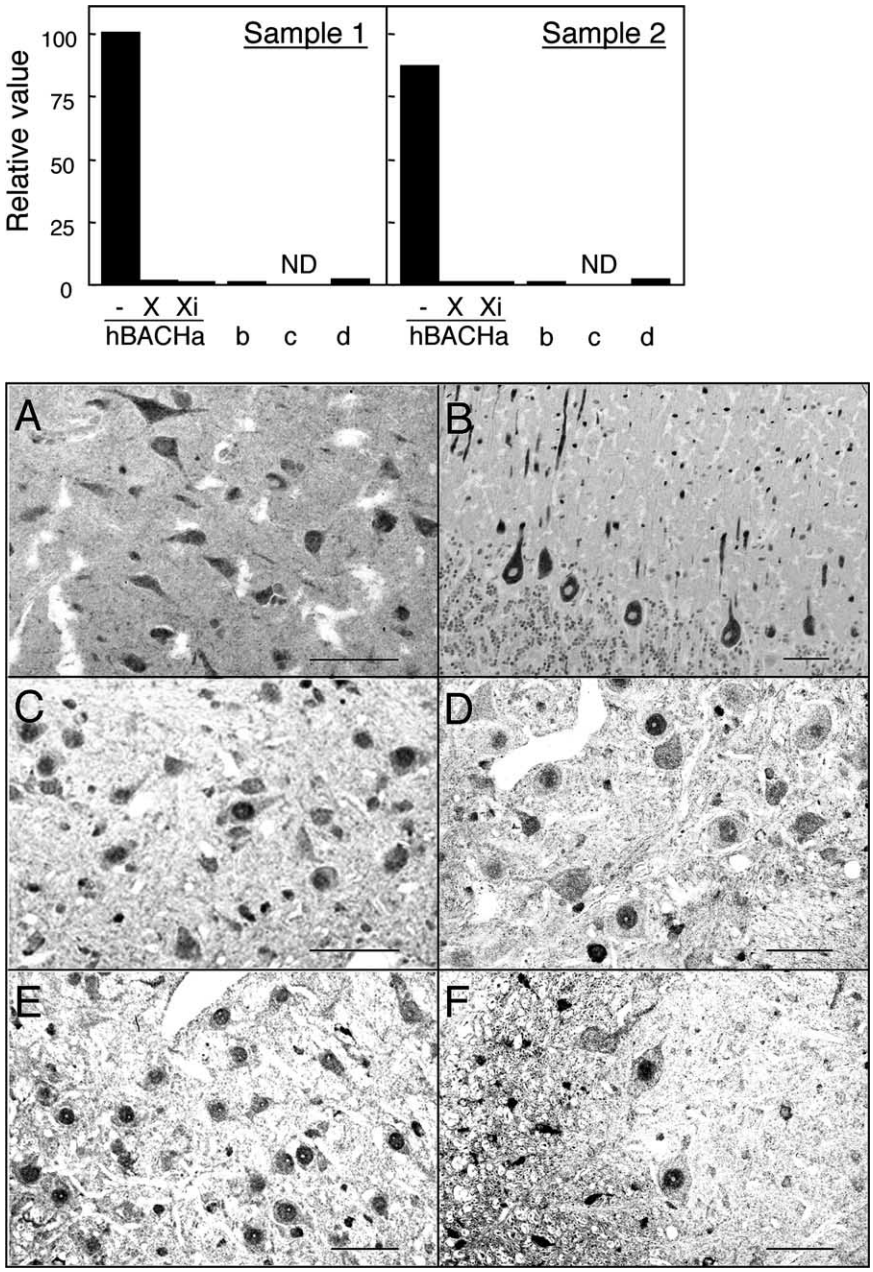

Fig. 4. Upper panel, mRNA levels of hBACH isoforms in human brain. Two samples of human brain total RNA were subjected to quantitative RT-PCR. The assay was carried out twice in duplicate for each sample, and the results are expressed as relative values of the average (variation <10%), taking the value for hBACHa in sample 1 as 100 after normalization with mRNA levels of glyceraldehyde-3-phosphate dehydrogenase as an internal standard. ND, not detectable (less than 0.5). The values for hBACHb–d represent the sum of values for the respective isoforms and those with an insert X or Xi if there are any. Lower panel, Immunohistochemistry of human (A and B) and mouse brain (C–F) with anti-rBACH antibody. The immunoreactivity was visualized by a peroxidase reaction with 3,3′-diaminobenzidine as the substrate, following which the sections were counterstained by methylgreen. For a negative control, the primary antibody was replaced by nonimmunized rabbit IgG, with which no staining was observed (data not shown). (A) Cerebral cortex; (B)–(D) cerebellum; (E) medulla oblongata; (F) spinal cord. Scale bar, 50 μm.

because of the high similarity of hBACHa and mBACHa with the completely conserved NLS [11,12], giving support to the dual localization of BACHa in the cytosol and nucleus, suggested above.

Functional significance of the isoform formation

In this study, we suggested that the hBACH gene can express long-chain acyl-CoA hydrolase activity in the cytosol, nucleus, and mitochondria by generating isoforms with differential localization signals via a mechanism of alternative use of exons. The physiological functions of these isoforms are unclear. However, a possible role for the mitochondrial isoform may be to act as an auxiliary enzyme in fatty acid β-oxidation, and that of the nuclear one may be in the regulation of gene expression by modulating the supply of acyl-CoA and/or free fatty acid as ligands for nuclear receptors, as described in [1].

In the cytosol, BACH may serve as a potent scavenger of free long-chain acyl-CoAs that can be a regulatory molecule, as exemplified by protein kinase C activation [17], Ca^{2+} release [18], and K(ATP) channel activation [19], and a molecule with cytotoxicity at high concentrations due to their own property as a detergent [20], as well as substrates for lipid metabolism. Cytosolic long-chain acyl-CoAs are specifically bound to acyl-CoA binding protein (ACBP) with high affinity to be protected from hydrolysis by acyl-CoA hydrolases [2]. They are also converted to the acyl-carnitine by carnitine palmitoyltransferase (CPT-I), resulting in a very low concentration of free long-chain acyl-CoAs in the cytosol [2,21]. In addition to these enzymes, the potent hydrolytic activity of BACH would provide another mechanism to maintain the acyl-CoA level, ensuring the turning-off of the regulatory acyl-CoA signal and prevention of cytotoxicity and uncontrolled substrate supply caused by increased levels of free long-chain acyl-CoAs. These functions of BACH may also be effective in the nucleus. Recently, an immunohistochemical study in mice showed a lack of ACBP in neurons [22]. This suggests an emphasized contribution of BACHa as an acyl-CoA scavenger in the neuron, and so may explain the selective expression of the BACH gene, especially BACHa, in this cell type (Fig. 4, [11]). Long-chain acyl-CoAs were reported to play a role in synaptic vesicle formation [23], long-term potentiation in the hippocampus [24], and inhibition of dopamine sulfotransferase [25], on which BACHa may possibly have influence.

In the brain, hBACHa is virtually the sole isoform expressed and the expression levels of others are insignificant (Fig. 4, upper panel). This appears to be reflected by the frequency of hits in the BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) against a human EST database: 50, 19, 1, 3, and 1 hit for ESTs that contained the sequences corresponding to exons 1a–1d and X(i), respectively. While BACHa and BACHb (probably MTE-II [8]) can significantly be expressed to function, the mRNA expression of other isoforms might represent a deviation from the usual regulation of transcriptional initiation and/or splicing at a negligible level. So, increased expression of, for instance, the isoforms containing exon X-derived sequences would possibly reflect some specific or rather pathological conditions. Functional differences between hBACHa and hBACHd, both with the NLS, and between hBACHb and hBACHc, both with the MTS, are also unknown. Several questions, thus, remain to be answered. However, in this study, we defined the hBACH isoforms and suggested the functional significance of the isoform formation.

Acknowledgments

This work was supported in part by grants from The Ministry of Education, Culture, Sports, Science and Technology, and The Promotion and Mutual Aid Corporation for Private Schools of Japan.

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