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ORIGINAL ARTICLE

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Identification of a novel candidate gene in the iron-sulfur pathway implicated in ataxia-susceptibility: human gene encoding HscB, a J-type co-chaperone

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Abstract Iron-sulfur proteins participate in a wide range of biochemical processes, including many that are central to mitochondrial electron transfer and energy metabolism. Mutations in two such proteins, frataxin and ABCB7, cause Friedreich ataxia and X-linked sideroblastic anemia with ataxia, respectively, rendering other participants in this pathway functional candidates for hereditary ataxia syndromes. Recently frataxin was shown to have an identical phylogenetic distribution with two genes and was most likely specifically involved in the same sub-process in iron-sulfur cluster assembly as one gene, designated *hscB*, in bacteria. To set the stage for an analysis of the potential role of this candidate gene in human disease, we defined the human *HscB* cDNA, its genomic locus, and its pattern of expression in normal human tissues. The isolated human *HscB* cDNA spans 785 bp and encodes a conserved 235-amino-acid protein, including a putative mitochondrial import leader. The *HscB* gene is found at chromosome 22q11-12 and is composed of six exons and five introns. Northern blot analyses of RNA from adult and fetal tissues defined a pattern of expression in mitochondria-rich tissues similar to that of frataxin, an expression pattern compatible with its implied role in mitochondrial energetics and related disease phenotypes.

Keywords Human gene · Hsc20 · Expression · Iron-sulfur pathway · Ataxia

The GenBank accession number for *HscB* is AY191719

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Introduction

Iron-sulfur proteins participate in a wide range of biochemical processes including many that are central to mitochondrial electron transfer and energy metabolism. Several studies have provided the evidence that this class of proteins play an important role in the development of human neurological disease, particularly ataxia. Loss of function alleles of frataxin produce functional mitochondrial defects in patients with Friedreich ataxia (MIM 229300) (Patel et al. 2001), and nulls of the yeast orthologue, *YFH1*, display a respiratory-deficient phenotype and a defect in Fe-S cluster formation (Muhlenhoff et al. 2002). Other hereditary ataxia syndromes, including specifically X-linked sideroblastic anemia with ataxia (MIM 300135), caused by mutations in the ABCB7 ATP-binding cassette protein (Bekri et al. 2000), seem to share these features (Kaplan 2002). Several genes encoding proteins involved in the biogenesis of iron-sulfur proteins have been identified in bacteria and yeast, and structural and functional studies of their encoded proteins are underway. Thus, their human homologues become richly annotated functional candidate genes in heritable ataxia syndromes.

A recent study found that frataxin showed an identical phylogenetic distribution with only two genes and most likely was specifically involved in the same subprocess in iron-sulfur cluster assembly as one of these genes, designated *hscB* in bacteria and *JAC1* in yeast (Huynen et al. 2001). The *hscB* gene in bacteria encodes a 20-kDa J-type co-chaperone, designated Hsc20, that functions with the hsp70-class molecular chaperone HscA/Hsc66 (Seaton and Vickery 1994; Kawula and Lelivelt 1994; Vickery et al. 1997). In vitro studies have shown that HscB/Hsc20 regulates interactions between the Fe-S cluster assembly protein IscU and the HscA/Hsc66 chaperone (Hoff et al. 2000; Silberg et al. 2001; Hoff et al. 2002). Homologues of the bacterial *hscB* gene are found in eukaryotes, and the homologue in *Saccharomyces cerevisiae*, *JAC1*, is a nuclear gene whose

protein product is targeted to mitochondria (Strain et al. 1998; Kim et al. 2001; Lutz et al. 2001; Voisine et al. 2001). The *JAC1* gene is essential, and mutations in the gene have been found to cause defects in iron-sulfur protein activities, mitochondrial iron accumulation, and reduced mitochondrial respiratory activity (Strain et al. 1998; Kim et al. 2001; Lutz et al. 2001; Voisine et al. 2001). Because the phylogenetic distribution of *hscB*/*JAC1* is identical to that of frataxin, both being repeatedly lost or transferred in concert, it is likely that Hsc20/Jac1p and frataxin are involved in similar highly conserved cellular processes (Huynen et al. 2001). Furthermore, the finding that mutations in both genes in yeast produce similar defects in Fe-S cluster formation and in mitochondrial respiration and that these processes are implicated in the pathogenic mechanism of ataxia syndromes suggests that mutations in HscB may likewise produce disease susceptibility. To set the stage for an analysis of the potential role of the *hscB*/*JAC1* gene in human disease, we defined the human *HscB* cDNA, the map position of its locus, the intron-exon boundaries within the locus, its pattern of expression in normal human tissues, and place this in the context of structural and functional studies of its model system homologues.

Materials and methods

The *HscB* cDNA

A similarity search for the human *HscB* cDNA was carried out using the *Escherichia coli* Hsc20 protein sequence with the tBLASTN program against the human EST database (http://www.ncbi.nlm.nih.gov/BLAST). The search revealed several significant alignments with several EST clones (AI739537, AI796137, AW250733, BE279981, BE782421). The IMAGE clone number 2391082 (AI739537) was obtained and the complete sequence of its insert obtained sequencing both strands using T7 and T3 primers that bind sites flanking the insert. Potential reading frames were identified using Translate Tool (available at http://us.expasy.org/tools/dna.html).

Defining the genomic locus of *HscB*

The BLASTN program was used with the defined *HscB* cDNA sequence against the non-redundant human genomic database (http://www.ncbi.nlm.nih.gov/BLAST) yielding two positive entries (AL117330 and AL023494). The identification of exons and exon-intron junctions was carried out by merging the cDNA sequence with these genomic sequences.

Defining the *HscB* gene expression pattern using Northern blot analysis

Human multiple tissues Northern (MTN) and human fetal multiple tissues Northern blots were obtained from CLONTECH. Each MTN blot lane contains 2 μg of purified poly(A^+) RNA. A 231-bp fragment of *HscB* cDNA (complementary to messenger sequence 472–703 bp downstream from the initiation codon) was obtained by PCR and purified. This fragment was labeled with α-^{32}P-UTP (3,000 Ci/mmol) by in vitro transcription labeling using a MAXIscript kit (Ambion, Austin, Texas, USA). Blots were hybridized with the labeled probe, washed and exposed to X-ray film (Fuji) at −70 °C with two intensifying screens. Blots were stripped with 0.5% SDS at 95 °C, and re-hybridized with labeled β-actin cDNA probe as a standard.

Results

The *Escherichia coli* Hsc20 protein sequence was used to carry out a similarity search in the human EST database, revealing several significant alignments. Sequencing the complete insert of one such clone, the IMAGE human cDNA clone number 2391082 (AI739537), revealed the full-length sequence of human *HscB* cDNA. This sequence has been deposited with GenBank and given accession number AY191719. It has a length of 785 bp and encodes a predicted protein of 235 amino acids, with 34% and 29% overall identity to the *E. coli* Hsc20 and yeast *JAC1* homologues, respectively (Fig. 1), with higher identity in core domains (see below). The conservation of this sequence through distant phylogeny suggests a conserved functional role in a core cellular process.

The two genomic entries containing portions of the human *HscB* sequence (AL117330 and AL023494) map to chromosome 22q11-12, and together include nearly the complete sequence of *HscB*. The *HscB* gene has six exons and five introns spanning 15.3 kb. The exon and intron sizes and the exon-intron junction sequences are shown in Table 1, and the organization of the *HscB* gene is shown in Fig. 2. Two monogenic familial ataxia syndromes have been mapped to chromosome 22q, spinocerebellar ataxia type 10 (SCA10; MIM 603516) and mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE; MIM 603041), but both are significantly telomeric to *HscB* and genes for both have already been assigned (Matsuura et al. 2000; Nishino et al. 1999).

The predicted protein encoded by the human *HscB* gene has features consistent with those determined for the *E. coli* Hsc20 homologue. The crystal structure of *E. coli* Hsc20 revealed that the protein consists of distinct N- and C-terminal domains that make contact through an extensive hydrophobic interface (Cupp-Vickery and Vickery 2000). The N-terminal domain (residues 1–75) is folded into a compact α-topology similar to the evolutionarily conserved J-protein co-chaperone motif required for chaperone activation (Kelley 1998). The J-domain is connected by a short loop to the C-terminal domain (residues 84–171), which is folded into a compact three-helix bundle (Cupp-Vickery and Vickery 2000). Residues 72-145 of human HscB are 43% identical to the J-domain region of *E. coli* Hsc20, and the His-Pro-Asp J-domain signature motif is found at positions 102–104. Residues 154–235 are 30% identical to the C-terminal domain of *E. coli* Hsc20. The predicted human protein has an N-terminal extension of 71 residues not present in the bacterial protein. This extension is enriched in positively charged, hydroxylated, and hydrophobic residues consistent with a role as

a mitochondrial-targeting signal (Pfanner 2000; Pfanner and Wiedemann 2002).

The exon structure of the human *HscB* gene appears to reflect the functional domains of the protein (Fig. 2). Exon 1 encodes the putative mitochondrial presequence (residues 1–71) as well as several amino acids presumed to occur at the N-terminus of the J-domain (residues 71–79). Exons 2 and 3 encode the core of the J-domain, encompassing residues 80–111 and 112–143, respectively. Exon 4 encodes residues 144–189 that form the short loop connecting the J- and C-domains and the initial portion of the C-domain. Exons 5 and 6 encode residues 190–235 and comprise the majority of the C-domain.

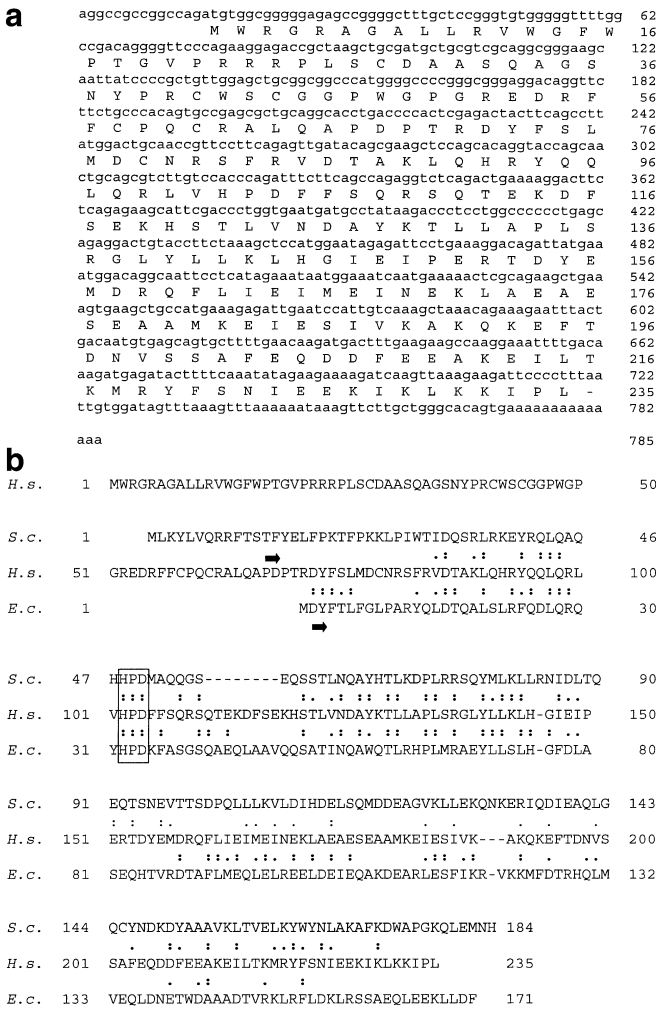

Fig. 1a, b cDNA and deduced amino acid sequence of human HscB. **a** Sequence of IMAGE clone no. 2391082 and deduced amino acid sequence. **b** Alignment of the deduced amino acid sequences of HscB proteins from *Homo sapiens* (*H.s.*), *S. cerevisiae* (*S.c.*) and *E. coli* (*E.c.*). Sequence identities (:) and similarities (.) are indicated. The J-protein signature sequence, His-Pro-Asp, is *boxed*, and the N-termini of the mature forms of *S. cerevisiae* mitochondrial Jac1p and *E. coli* HscB are indicated by *arrows*

The structure and implied function of human HscB suggest a mitochondrial role for the protein. In order to determine if the pattern of expression of human *HscB* was compatible with this hypothesis, Northern blot analyses were carried out using labeled *HscB* to probe poly(A^+) RNA from multiple adult and fetal human tissues. Figure 3 shows that the highest levels of the mRNA for *HscB* are found in liver, muscle and heart, with very low levels elsewhere. This pattern of expression in mitochondria-rich tissues is similar to that observed for human frataxin, both sharing very low CNS expression levels (Campuzano et al. 1996). Unlike frataxin, however, the transcript size of human *HscB* varied depending on tissue type. The highest level of human *HscB* expression was found in adult and fetal liver in which a transcript of ~2.5 kb was observed. Adult heart and skeletal muscle also displayed significant expression, and two transcripts, ~1.4 kb and ~ 6 kb, were observed. The higher level of expression of *HscB* in these mitochondria-rich, metabolically active tissues is compatible with the implied role of human HscB in iron-sulfur cluster formation, interaction with frataxin and mitochondrial energetics, and is consistent with the observed N-terminal extension mitochondrial import sequence. The presence of different transcript sizes in different tissues may suggest alternatively spliced variants, possibly reflecting tissue-specific regulation.

Discussion

The predicted human HscB protein is similar in its general features to the homologous bacterial (Hsc20/HscB) and yeast (Jac1p) co-chaperones. Each is predicted to be approximately 20-kDa in the mature form, and each has a highly conserved N-terminal J-domain linked to a less well-conserved C-terminal region. The J-domains of Hsc20 and Jac1p have been implicated in interactions with their Hsp70 partners, and it is likely that this region of human HscB carries out the

Table 1 Sequences at the exon-intron boundaries and the sizes of exons and introns of human *HscB*. Intron sequence is shown in *lowercase* and exon sequence in *uppercase*; *tss* translation start site

Exon no.	Exon size(bp) (coding region)	Splicing acceptor	5′-Exon junction	3′-Exon junction	Splicing donor	Intron size (bp)
1	326		tss	-TTATGGACTG	gtacgagcga-	1,551
2	97	-ttccttccag	CAACCGTTCC-	-GAG GTCTCAG	gtagcttatt-	636
3	95	-ccactaacag	ACTGAAAAGG-	-ACCTTCTAAG	gtgatttccc-	1,160
4	139	-atagctaaag	CTCCATGGAA-	-ATTGTCAAAG	gtgaaagata-	> 5,332
5	49	-tcttt ttcag	CTAAACAGAA-	-TTTGAACAAG	gtactttctt-	5,788
6	92	-tctatttcag	ATGACTTTGA-	-poly(A)		

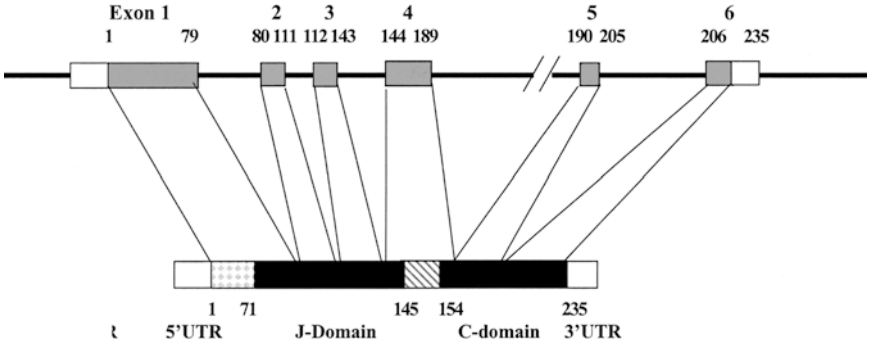

Fig. 2 Genomic organization of *HscB*. The gene structure is represented at the *top*, with introns indicated as a *thin line* and exons indicated as a *box*, which is labeled *above* with the exon number and the amino acid residue positions encoded. The structure of the transcript is presented below, with the translated sequences shown as *filled boxes* and untranslated sequences as *open boxes*. Regions encoding the predicted J- and C-domains of the protein are indicated by *black boxes*, with residue numbers shown *below*; the proposed mitochondrial signal sequence is indicated by a *stippled box*

orthologous function. The role of the C-terminal region of Hsc20/Jac1p has not been defined. C-terminal regions of Hsp40 class J-type co-chaperones have been implicated in peptide substrate binding, but the C-terminal domain of HscB lacks structural features found in the larger Hsp40 proteins. Huynen and co-workers have suggested the possibility that frataxin may fill the role of the substrate-binding regions missing from HscB and that the co-evolution of frataxin with HscB may reflect the interaction of a J-type co-chaperone and a substrate binding protein required for its function (Huynen et al. 2001). While there are no experimental results to support this hypothesis, HscB and human frataxin both have conserved regions on the protein surface that may play a role in protein-protein interactions (Cupp-Vickery and Vickery 2000; Dhe-Paganon et al. 2000); several of these residues are found to be altered in pathogenic point mutant alleles of human frataxin (Dhe-Paganon et al. 2000). In addition, studies with recombinant human frataxin have shown that frataxin is able to sequester iron in vitro (Cavadini et al. 2002), and a role in iron binding could provide a possible functional link to HscB in iron-sulfur cluster biogenesis.

HscB shares many functional aspects in common with frataxin suggesting a common role in cellular physiology and perhaps additionally in pathophysiology. The features of mitochondrial expression and relatively low CNS expression observed for HscB have previously been recognized for frataxin (Campuzano et al. 1996) as has the shared energy-deficient phenotype of mutants of both in model organisms and their implied shared role in Fe-S cluster biosynthesis (Strain et al. 1998; Muhlenhoff et al. 2002; Kim et al. 2001; Lutz et al. 2001; Voisine et al. 2001). The relatively low level of expression in the CNS the two share may render this tissue particularly vulnerable. Their remarkable evolutionary association, perhaps based upon a potential for

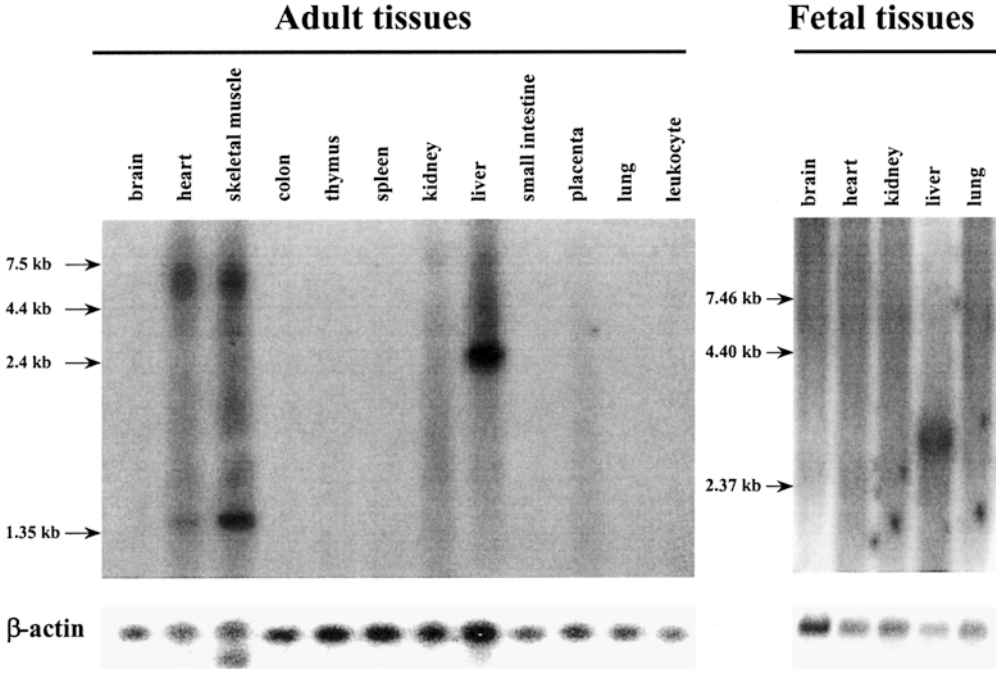

Fig. 3 Northern blot analysis of *HscB*. RNAs from the human tissues indicated on the top were loaded. High stringency hybridization with the *HscB* probe was followed by hybridization with a β-actin probe to demonstrate uniformity of RNA loading. Positions of the RNA standard markers are indicated

functional and/or physical interactions in Fe-S cluster formation, makes *HscB* a promising candidate gene for evaluation in hereditary ataxia syndromes. This is particularly intriguing from the perspective of ataxia pathogenesis, since in a screen for second-site suppressors of polyglutamine toxicity, an important but still incompletely explained pathogenic mechanism underlying several dominant ataxia syndromes (Ross 2002; Cummings and Zoghbi 2000), the two different suppressor genes recognized encoded J proteins (Kazemi-Esfarjani and Benzer 2000). This suggests that defects in J-protein mechanisms might predispose to pathogenic processes leading to an intermediate shared with polyglutamine toxicity, both thereby producing susceptibility to ataxia (Opal and Zoghbi 2002). Demonstration of a direct interaction of HscB with frataxin, a shared functional role in Fe-S cluster formation, or in suppressing polyglutamine toxicity would further strengthen the functional candidacy of HscB in the spectrum of mitochondria-related disease phenotypes observed in Friedreich ataxia and the polyglutamine-expansion diseases, including cardiomyopathy, neuropathy, diabetes mellitus and ataxia itself.

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