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Human homologues of the bacterial heat-shock protein DnaJ are preferentially expressed in neurons

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The bacterial heat-shock protein DnaJ has been implicated in protein folding and protein complex dissociation. The DnaJ protein interacts with the prokaryotic analogue of Hsp70, DnaK, and accelerates the rate of ATP hydrolysis by DnaK. Several yeast homologues of DnaJ, with different proposed subcellular localizations and functions, have recently been isolated and are the only eukaryotic forms of DnaJ so far described. We have isolated cDNAs corresponding to two alternatively spliced transcripts of a novel human gene, *HSJ1*, which show sequence similarity to the bacterial DnaJ protein and the yeast homologues. The cDNA clones were isolated from a human brain-frontal-cortex expression library screened with a polyclonal antiserum raised to paired-helical-filament (PHF) proteins isolated from extracts of the brains of patients suffering from Alzheimer's disease. The similarity between the predicted human protein sequences and the bacterial and yeast proteins is highest at the *N*-termini, this region also shows a limited similarity to viral T-antigens and is a possible common motif involved in the interaction with DnaK/Hsp70. Northern-blot analysis has shown that human brain contains higher levels of mRNA for the DnaJ homologue than other tissues examined, and hybridization studies with riboprobes *in situ* show a restricted pattern of expression of the mRNA within the brain, with neuronal layers giving the strongest signal. These findings suggest that the DnaJ–DnaK (Hsp70) interaction is general to eukaryotes and, indeed, to higher organisms.

INTRODUCTION

Heat shock proteins have been found in a wide range of organisms and are phylogenetically highly conserved (Lindquist & Craig, 1988). They have been implicated as having roles in correct protein folding (Rothman, 1989), protein complex assembly (Ellis & Hemmingsen, 1989) and disassembly (Liberek *et al.*, 1990) and the uptake of proteins into organelles (Chirico *et al.*, 1988; Deshaies *et al.*, 1988). The *DnaK* gene, which encodes the prokaryotic analogue of the Hsp70 cognate protein of eukaryotes, and the *DnaJ* gene, constitute an operon in *Escherichia coli* (Saito & Uchida, 1978; Bardwell *et al.*, 1986; Ohki *et al.*, 1986). The protein products of the *DnaJ* and *DnaK* loci interact to disassemble a protein complex required for bacteriophage-λ replication (Liberek *et al.*, 1990), act together to dissociate RepA dimers in plasmid P1 replication (Wickner *et al.*, 1991) and are required for the correct renaturation of denatured λ repressor (Gaitanaris *et al.*, 1990).

In the yeast *Saccharomyces cerevisiae*, there are at least nine Hsp70 proteins (see Lindquist & Craig, 1988, for a review), which function in several cellular compartments, including the cytoplasm, mitochondria and endoplasmic reticulum (ER). Several yeast proteins, Sec63, SCJ1, YDJ1 and SIS1, which show sequence similarity to DnaJ, have recently been isolated. The first described, Sec63, contains a 70-amino-acid region similar in sequence to the *N*-terminal region of DnaJ, which is delimited by two membrane-spanning domains (Sadler *et al.*, 1989). Sec63 is assembled into a multisubunit membrane-associated complex (Deshaies *et al.*, 1991) and is required for the import of proteins into the ER (Rothblatt *et al.*, 1989; Sadler *et al.*, 1989). The SCJ1 protein appears to be localized to mitochondria and was identified as a high-copy-number suppressor in a screen to isolate genes whose products alter protein sorting (Blumberg & Silver, 1991). The YDJ1 protein was isolated as a component of the 'matrix lamina pore complex' (Caplan & Douglas, 1991). Although it is possible that SCJ1 and YDJ1 function in protein folding and/or protein import into various organelles, their functions are not known. The *SIS1* gene is a high-copy-number suppressor of the slow-growth phenotype of strains containing mutations in the *SIT4* gene, which encodes a predicted serine/threonine phosphatase (Luke *et al.*, 1991).

In human cells there are at least five distinct protein members of the Hsp70 gene family that exhibit constitutive and inducible regulation and share biochemical and antigenic properties (see Morimoto & Milarski, 1990, for a review). There is a high degree of evolutionary conservation among members of the Hsp70 family within a single species and between species; for example, *E. coli* DnaK and human Hsp70 are 50% identical at the amino-acid-sequence level (Bardwell & Craig, 1984; Hunt & Morimoto, 1985). However, to date no eukaryotic homologue of DnaJ in organisms higher than yeast has been described.

We have isolated human cDNA homologues of DnaJ from a human brain-frontal-cortex expression cDNA library screened with a polyclonal antiserum raised to isolated paired helical filament (PHF) proteins, the structural component of the neurofibrillary tangle, one of the characteristic histopathological features of Alzheimer's disease. These cDNAs represent two alternatively spliced transcripts which we have designated HSJ1a and HSJ1b (*Homo sapiens* DnaJ 1) of a previously undescribed human gene, *HSJ1*.

Abbreviations used: Hsp70, heat-shock-protein 70; PHF, paired helical fragment; ER, endoplasmic reticulum; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

The nucleotide sequences of HSJ1a and HSJ1b will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X63368 HSHSJ1MR.

MATERIALS AND METHODS

RNA extraction

Human brain, liver, kidney, and muscle samples obtained *post mortem* and kept at −80 °C were used to prepare RNA by homogenization in 5 M-guanidinium isothiocyanate buffer, followed by centrifugation for 18 h on a CsCl cushion (Chirgwin *et al.*, 1979). The RNA pellet was dried and resuspended in water. After phenol/chloroform extraction, the RNA was ethanol-precipitated, resuspended in RNasin-treated water and kept at −80 °C. RNA for RNase protection analysis was extracted by a modification of this method designed for frozen tissue samples (Ausubel *et al.*, 1989).

cDNA library construction and screening

RNA was isolated by the method of Chirgwin *et al.* (1979) from the frontal cortex of an 87-year-old male who had died with histologically confirmed diagnosis of Alzheimer's disease; tissue was obtained after a 5 h delay post mortem. Polyadenylated RNA was selected using oligo(dT)–cellulose affinity chromatography. First-strand synthesis was performed using 10 μg of polyadenylated RNA as a template in the presence of actinomycin D, using oligo(dT) as primer and avian-myeloblastosis-virus reverse transcriptase. Double-stranded DNA was generated by DNA polymerase I in the presence of RNAase H and *E. coli* DNA ligase. Double-stranded DNA was made blunt-ended by using T4 DNA polymerase and methylated by *Eco*RI methylase. *Eco*RI linkers (three sizes: 8-mer, 10-mer and 12-mer) were ligated separately, then recombined, digested with *Eco*RI; cDNA was size-selected and ligated into the *Eco*RI site of λgt11. Packaging was effected by using Gigapack gold (Stratagene). *E. coli* strain Y1090r− was used as the host bacteria for the screening. Fusion-protein production was induced by overlaying with nitrocellulose filters which had been soaked in 10 mM isopropyl β-D-thiogalactopyranoside. Screening was performed using a polyclonal antiserum raised to an enriched preparation of PHF (Brion *et al.*, 1985), which had been stripped of any anti-*E. coli* activity; Protein A coupled to alkaline phosphatase (Sigma) was used as the detection system for positive plaques.

A human frontal-cortex cDNA library in the Lambda ZAP II vector was purchased from Stratagene; the host strain was XL1-Blue. The library was plated at high density on bioassay plates (Gibco–BRL) and duplicate plaque lifts on to Hybond-N (Amersham) filters taken. The filters were hybridized with HSJ1′ DNA which had been random-primer-labelled (Amersham kit) with [α-^{32}P]dCTP (Amersham). Hybridizations were performed in the presence of 50 % (w/v) formamide, 5 × Denhardt's, 5 × SSC (1 × SSC is 0.15 M-NaCl/0.015 M-sodium citrate), salmon sperm DNA (100 μg/ml), poly(U) (25 μg/ml) and 20 mM-sodium phosphate, pH 6.5, for 18 h at 42 °C. The filters were washed at high stringency [0.1 × SSC/0.2% (w/v) SDS; 65 °C]. Positive plaques were plaque-purified and subjected to excision *in vivo* according to the manufacturer's (Stratagene) instructions.

Sequencing

The sequencing of HSJ1′ was performed by using the dideoxy-chain-termination method (Sanger *et al.*, 1977) and the 'Klenow' fragment of DNA polymerase (Amersham), by the subcloning of overlapping restriction (*Eco*RI, *Pst*I and *Taq*I) fragments into the single-stranded phage M13 and confirmed in both directions by the use of *Alu*I restriction fragments. The sequencing of clones isolated from the lambda ZAP library was performed by the double-stranded sequencing in the pBS vector using T3 and T7 primers and the Pharmacia T^7 polymerase sequencing kit. Plasmids purified by poly(ethylene glycol) precipitation (Sambrook *et al.*, 1989) were used to create unidirectional deletion mutants using *Bal*31 (Ausubel *et al.*, 1989), after linearization with *Bam*HI. The plasmids were then treated with mung-bean (*Phaseolus aureus*) nuclease, *Xho*I/*Hin*dIII and ligated into *Hin*dIII/*Sma*I-treated pBS. Competent SURE bacteria were used for transformation. Deletion mutants were sequenced directly from plasmid 'mini-preps' (Pharmacia). Sequence was confirmed in the reverse strand by the use of synthetic oligonucleotides as primers.

Computer-program analysis

A personal-computer-based software package, PCGENE (Intelligenetics), was useful for easy access and rapid-sequence analysis; however, the most versatile and powerful software was accessed via the S.E.R.C. VAX Seqnet facility at Daresbury, Warrington, Cheshire, U.K. Within this large resource the programs of the University of Wisconsin Genetics Computing Group (UWGCG) were found to be the most useful and, in particular, the database searching program FASTA (Devereux *et al.*, 1984).

Northern-blot analysis

RNA for Northern-blot analysis was extracted as above from post-mortem tissue and oligo(dT)-selected. Polyadenylated RNA (5 μg) was denatured at 65 °C for 15 min in the presence of 2.2 M-formaldehyde and 12.2 M-formamide and samples were loaded on to a 0.8%-(w/v)-agarose gel containing 7% (w/v) formaldehyde; the buffer used was 40 mM-Mops/10 mM-sodium acetate/1 mM-EDTA. Electrophoresis was carried out at 2 V/cm for 18 h. The gel was rinsed in water to remove formamide and then blotted on to Biodyne A (Pall) nylon membrane in accordance with the manufacturer's recommendations. Hybridizations were performed in the presence of 50 % (w/v) formamide, 5 × Denhardt's, 5 × SSC, salmon sperm DNA (100 μg/ml), poly(U) (25 μg/ml) and 20 mM-sodium phosphate, pH 6.5, for 18 h at 42 °C. Probes were labelled with [α-^{32}P]dATP using a random-primer-labelling kit (Boehringer). Filters were washed at high stringency [0.1 × SSC/0.2% (w/v) SDS] at 65 °C.

RNAase-protection analysis

RNAase-protection analysis was performed on 40 μg (Fig. 2*b* below) and 20 μg (Fig. 2*c* below) total RNA, essentially as described by Ausubel *et al.* (1989). A riboprobe labelled with [α-^{32}P]CTP was transcribed by T3 RNA polymerase (Stratagene) from a poly(ethylene glycol)-purified deletion-mutant plasmid, 15–27, which had been linearized with *Nco*I (Fig. 2*b* below) or a linearized plasmid containing the 5′ *Eco*RI fragment (Fig. 2*c* below). The template was digested with DNAase I (Promega), 10 μg of tRNA was added, and riboprobes purified by 'push-column' chromatography (Stratagene). The probes were ethanol-precipitated, and 2 × 10^5 c.p.m. of the probes was hybridized overnight at 45 °C with the target RNA in hybridization buffer: 40 mM-Pipes (pH 6.4)/1 mM-EDTA (pH 8.0)/0.4 M-NaCl/80 % (v/v) formamide. The RNA–RNA hybrids were digested with RNAase A and RNAase T1 for 1 h at 37 °C and phenol/chloroform-extracted; 10 μg of carrier tRNA was added and the RNA was ethanol-precipitated. The products were separated on a denaturing 8 %-polyacrylamide gel.

Hybridization *in situ*

Blocks of human hippocampus were dissected from a normal subject (81 years old; 17 h delay *post mortem*). Tissue samples were snap-frozen in isopentane at −80 °C and 10 μm-thick cryostat sections taken. The tissue sections were fixed for 5 min in 4% (w/v) paraformaldehyde in 0.1 M-sodium phosphate/

M-NaCl, pH 7.4, and dehydrated in graded ethanol solutions containing 300 mM-ammonium acetate. Sense and anti-sense riboprobes labelled with [α-^{32}P]CTP were transcribed from a full-length HSJ1a clone in pBS by T3 and T7 RNA polymerases (Stratagene) after linearization of the plasmid with either *Bam*HI or *Xho*I (Promega). The template was digested with DNAase I (Promega), 10 μg of tRNA was added and the riboprobes were purified by push-column chromatography (Stratagene).

Hybridization *in situ* was performed as previously described (Brion *et al.*, 1990). The anti-sense or sense [^{32}P]HSJ1a riboprobe (10^6 c.p.m./section) was added to the following hybridization solution: 50% (v/v) formamide, salmon sperm DNA (1 mg/ml), tRNA (0.6 mg/ml) dithiothreitol (10 mM), 10% (w/v) dextran sulphate and 2 × SSC. The hybridization was performed for 5 h at 50 °C. Sections were washed in 50% (v/v) formamide/2 × SSC at 52 °C, followed by a wash in 2 × SSC at 52 °C. Sections were finally treated with RNAase A (100 μg/ml) for 30 min at 37 °C, washed in 50% (v/v) formamide/2 × SSC at 52 °C and dehydrated in graded ethanol solutions. Slides were exposed for 2 weeks on autoradiographic Hyperfilm.

Southern blotting

DNA was extracted from transformed human lymphocytes. Cells were lysed with SDS (1% w/v) and proteinase K (100 μg/ml) for 16 h at 37 °C in lysis buffer (100 mM-NaCl/25 mM-EDTA); protein contaminants were removed by phenol/chloroform extraction and the DNA was ethanol-precipitated. The DNA was resuspended in TE (10 mM-Tris/HCl/1 mM-EDTA, pH 8.0) and 10 μg was digested overnight with either *Apa*I, *Hin*dIII, *Bam*HI or *Eco*RI (Promega) in accordance with the manufacturers' recommendations. The DNA was separated by 0.8%-(w/v)-agarose-gel electrophoresis. The gel was depurinated, denatured, neutralized and transferred to Hybond-N, essentially as described by Ausubel *et al.* (1989).

A 'Zoo blot' was purchased from Clontech (catalogue no. 7753-1), which contained *Eco*RI-digested genomic DNA from a variety of animals. Both blots were hybridized with probe HSJ1′ in the presence of 50% (w/v) formamide/5 × Denhardt's/5 × SSC/salmon sperm DNA (100 μg/ml)/20 mM-sodium phosphate, pH 6.5, for 18 h at 42 °C. The probe was labelled with [α-^{32}P]dCTP using a random-primer-labelling kit (Amersham). Filters were washed at high stringency [0.1 × SSC/0.2% (w/v) SDS] at 65 °C.

Western blotting

Total human brain-frontal-cortex homogenate was separated by SDS/10%-(w/v)-PAGE and transferred to nitrocellulose and probed with antisera. The nitrocellulose was blocked with 5% (w/v) dry skimmed milk (Marvel). Protein–antibody complexes were revealed with biotinylated anti-rabbit second antibody (1:200) and streptavidin-conjugated alkaline phosphatase (1:1500) (Amersham). Nitro Blue Tetrazolium and bromochloroindolyl phosphate (Sigma) were used as chromogens.

Antisera were produced by immunizing rabbits with peptide 15 (see Fig. 1*a* below) conjugated to keyhole-limpet haemocyanin (Calbiochem) and purified protein derivative of tuberculin (Central Veterinary Laboratory, Weybridge, Surrey, U.K.) by an *N*-terminal cysteine residue (Ausubel *et al.*, 1989) or by immunizing with HSJ1′–β-galactosidase fusion protein partially purified as inclusion bodies (Nagai *et al.*, 1985) and further purified by electroelution (Schleicher and Schuell, Biotrap) from an SDS/PAGE gel before immunization. Antisera were affinity-purified on purified HSJ′–β-galactosidase fusion protein as previously described (Robinson *et al.*, 1987) and used as dilutions equivalent to 1:500 (*a*) and 1:1000 (*b*) of native antisera.

Transcription *in vitro* using an 'mCAP mRNA capping' kit and an '*in vitro* Express' translation kit were used according to the manufacturer's (Stratagene) recommendations.

RESULTS

Isolation and sequence analysis of HSJ1a and HSJ1b

A λgt11 cDNA expression library (6.5×10^5 recombinant clones) made from polyadenylated RNA extracted from the frontal cortex of one brain of an Alzheimer's-disease patient was screened (5×10^5 plaque-forming units) with a polyclonal antiserum raised to a preparation enriched in PHF (Brion *et al.*, 1985). One strongly positive cDNA clone (HSJ1′) that was isolated was found to encode a previously undescribed sequence.

This cDNA clone was used to isolate longer cDNA clones by screening another human brain-frontal-cortex cDNA library, since the 5′ end of HSJ1′ was terminated by an internal *Eco*RI site and did not contain an initiation codon. A commercially available human frontal-cortex library in the vector lambda ZAP II (Stratagene) was purchased and 4×10^5 plaque-forming units were screened at high stringency [0.1 × SSC/0.1% (w/v) SDS; 65 °C] using HSJ1′ DNA; 14 putative positive clones were isolated, and of these 11 were successfully subjected to excision *in vivo* and nine of the 11 were cross-hybridized with HSJ1′ DNA by Southern blotting. The size of the positive cDNA clones, as judged by agarose-gel electrophoresis, ranged from 0.9 to 3.0 kb; several of the clones possessed internal *Eco*RI sites. Two clones encoded two putative full-length open reading frames which differed by the presence of a 1.1 kb insert (HSJ1a; HSJ1b). The presence of the insert results in a *C*-terminal amino acid sequence alternative to that of the predicted protein, which is longer by 74 amino acids. The sequences are shown in Fig. 1(*a*) below.

However, the extra 1.1 kb of sequence in HSJ1b could possibly start with a 'GT' and end with an 'AG'. These are the highly conserved intron splice donor and acceptor sites. In addition, seven of the bases in the sequence surrounding the 'GT' match the 9-base intron splice consensus sequence, and the sequence immediately preceding the 'AG' is very pyrimidine-rich, again typical of a splice acceptor site. So it is possible that the insert in HSJ1b represents an unspliced intron which has not been efficiently removed.

Sequence similarity of HSJ1a and HSJ1b to DnaJ

A search of 26651 protein sequences in the National Biomedical Research Foundation Protein Database with both HSJ1a and HSJ1b derived putative amino acid sequences using the FASTA algorithm revealed interesting areas of similarity. The strongest similarity detected was to the *E. coli* heat-shock protein DnaJ (Bardwell *et al.*, 1986; Ohki *et al.*, 1986), which showed 45.8% identity in a 107-amino-acid overlap at the *N*-termini of both polypeptides; furthermore, many of the changes are semi-conservative. This similarity was shared by several other bacterial DnaJ amino acid sequences, including *Caulobacter crescentus* (Gomes *et al.*, 1990), *Mycobacterium tuberculosis* (Lathigra *et al.*, 1988) and *Bacillus subtilis* (Wetzstein *et al.*, 1990). A yeast protein NPL1/Sec63 (Sadler *et al.*, 1989) also shared this region of sequence identity. The similarity of HSJ1a and HSJ1b to these other proteins *C*-terminal of this region is low. A comparison of the HSJ1a/b and the *E. coli* DnaJ predicted amino acid sequences using the PCGENE program PCOMPARE indicated that the similarity was significant.

Recently described yeast homologues of DnaJ, SCJ1 (Blumberg & Silver, 1991) and YDJ1 (Caplan & Douglas, 1991) proteins are similar to DnaJ over their entire lengths. Another recently described yeast DnaJ homologue, SIS1 protein (Luke *et*

(a)

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                                    -26 CCCGCCTGACGACTGACCAGTTGCC
  1 ATGGCATCCTACTACGAGATCCTAGACGTGCCGCGAAGTGCGTCCGCTGATGACATCAAG
  1 METAlaSerTyrTyrGluIleLeuAspValProArgSerAlaSerAlaAspAspIleLys

 61 AAGGCGTATCGGCGCAAGGCTCTCCAGTGGCACCCAGACAAAAACCCAGATAATAAAGAG
 21 LysAlaTyrArgArgLysAlaLeuGlnTrpHisProAspLysAsnProAspAsnLysGlu

121 TTTGCTGAGAAGAAATTTAAGGAGGTGGCCGAGGCATATGAAGTGCTGTCTGACAAGCAC
 41 PheAlaGluLysLysPheLysGluValAlaGluAlaTyrGluValLeuSerAspLysHis

181 AAGCGGGAGATTTACGACGGCTATGGCGGGGAAGGGCTGACAGGGACAGGAACTGGCCCA
 61 LysArgGluIleTyrAspArgTyrGlyArgGluGlyLeuThrGlyThrGlyThrGlyPro

241 TCTCGGGCAGAAGCTGGCAGTGGTGGGCCTGGCTTCACCTTCACCTTCCGCAGCCCCGAG
 81 SerArgAlaGluAlaGlySerGlyGlyProGlyPheThrPheThrPheArgSerProGlu
                Eco RI
301 GAGGTCTTCCGGGAATTCTTTGGGAGTGGAGACCCTTTTGCAGAGCTCTTTGATGACCTG
101 GluValPheArgGluPhePheGlySerGlyAspProPheAlaGluLeuPheAspAspLeu

361 GGCCCCTTCTCAGAGCTTCAGAACCGGGGTTCCCGACACTCAGGCCCCTTCTTTACCTTC
121 GlyProPheSerGluLeuGlnAsnArgGlySerArgHisSerGlyProPhePheThrPhe

421 TCTTCCTCCTTCCCTGGGCACTCCGATTTCTCCTCCTCATCTTTCTCCTTCAGTCCTGGG
141 SerSerSerPheProGlyHisSerAspPheSerSerSerSerPheSerPheSerProGly

481 GCTGGTGCTTTTCGCTCTGTTTCTACATCTACCACCTTTGTCCAAGGACGCCGCATCACC
161 AlaGlyAlaPheArgSerValSerThrSerThrThrPheValGlnGlyArgArgIleThr

541 ACACGCAGAATCATGGAGAACGGGCAGGAGCGGGGTGGAAGTGGAGGAGGATGGGCAGCTG
181 ThrArgArgIleMetGluAsnGlyGlnGluArgValGluValGluGluAspGlyGlnLeu

601 AAGTCAGTCACAATCAATGGTGTCCCAGATGACCTGGCACGTGGCTTGGAGCTGAGCGGT
201 LysSerValThrIleAsnGlyValProAspAspLeuAlaArgGlyLeuGluLeuSerArg

661 CGCGAGCAGCAGCGGTCAGTCACTTCCAGGTCTGGGGGCACTCAGGTCCAGCAGACCCCT
221 ArgGluGlnGlnProSerValThrSerArgSerGlyGlyThrGlnValGlnGlnThrPro

721 GCCTCATGCCCCTTGGACAGCGACCTCTCTGAGGATGAGGACCTGCAGCTGGCCATGGCC
241 AlaSerCysProLeuAspSerAspLeuSerGluAspGluAspLeuGlnLeuAlaMetAla

781 TACAGCCTGTCAGAGATGGAGGCAGCTGGGAAGAAACCCGCAGGTGGGCGGGAGGCACAG
261 TyrSerLeuSerGluMetGluAlaAlaGlyLysLysProAlaGlyGlyArgGluAlaGln

841 CACCGACGGCAGGGGCGCCCAAGGCCCAGCACCAAGATCCAGGCTTGGGGGGGACCCAGG
281 HisArgArgGlnGlyArgProArgProSerThrLysIleGlnAlaTrpGlyGlyProArg

901 AGGGTGCGAGGGGTGAAGCAACCAAACGCAGTCCATCCCCAGAGGAGAAGGCCTCTCGCT
301 ArgValArgGlyValLysGlnProAsnAlaValHisProGlnArgArgArgProLeuAla

961 GCCTCATCCTCTGAACACCGGGCCCAACCTGATCTGATCCAGATCTTGACTGGGGGTCT
321 AlaSerSerSerGluHisArgAlaGlnProAspLeuIleGlnIleLeuThrGlyGlySer

1021 GACTCACTGTGGGAAGAGAAGAGGGAGTATCCTGAGTTGTAGGAACTGCTTTCCAACTC
341 AspSerLeuTrpGluGluLysArgGlyValSer * 351

1081 CAAGCTCCCTCCACAAGTTTCCCTCCCCAGGCCCCCCACACCCCAGTGTGGACTTGGGAT
1141 TTGCTGTGCTCAGCCCAGGGCTGATAGGTCCCTGGTGAAGCCCAGGGTGGGGGGTGTCAG

1201 GGCAGTGGAGGGGCCCGAGGAGCCAGGTTGCATTTATTGGATGGGGAGCTCCAAGGGGCA
1261 TTAGTGGTTTGGGCTGGGCTTTTGTGCCCTGGTACTCTGCCACCTGTGTTGCTGATGGTG
1321 TCAAGGAAGGAGGACTTGGCCTAGGGTTGTCTGAGCCGGAGCCGGCAGCTCCACTGGAGA
1381 GCAGTGCAGGCAGAGTGGAGCCTCCTGCTCTCCTGGACCAGCTGCAGACCCCCAACCCTG
1441 GTTTCTGTGCCATGTTGCGCTCTGACCGTCTCTGTTGCTTCTCTTCTGGTGTTGCTTCTC
1501 CTCCCTCCCATTCTCTCTGCAACTCCTGCGGGGCGCATCGCTTGCTTTCACTGCCGTCTGG
1561 CTAGGACTCCCTTCTTCCTTCCTTCCCCGAGAAGGCCTCAATGTGGCGAGGAAGATGCTG
1621 GGGCCCGGTAGGGCTGTGAGATCTTCTGGGGAGGCTAGCCGGGGTGGGGCGGGAGCCTCTCA
1681 GCTGTCCAGATTCAGAACTGGAGCCCACTCCTCCTCCCTCTCGTTGCCTCAGCCCTGCCC
1741 TCACCCTCAGACTAGGCAGAGGTGAGGCTGGCTCACCCTGAAGAGGTGGATAGGAGGGG
1801 ACTGCACCCATACTGCTTCCCTACCACAAATCAGGGCTCAGGGAGAGGCCATGCGGCAGC
1861 CCAGGTCTGCATGCTGAGCCCCATCCTCCACAGCTTGCCGCTGACGCTCTCTCCTGTCAC
1921 CCGGCCCCTGCTCTCTCCCCAGATGTGTTCTGAGCTGGATGCCGGGTTCCAGAATCGCTG
                                AspValPhe * 277
1981 CACAGTTCCAACAGGACAGCGCCTTCCCCCATGCGCTGGGAGGGGACCCTCCATTTCTCC
2041 CCCTCACCCATGCTGAGTGTAGAGCCCGGGCCTGGGTGGCGGGTGGGGGCCGGGTGGGAG
2101 GTGGCAGTAGTCTTAGCCTGTGCACTCTCTTCCTTGGGTGTTTGGTGCTGGCTCCTGGGG
2161 ACTACAAATCCCAGAGTGCGGTGTGCCCGGCCTCATTTCTGATAGATCCCGCTTGGGGGA
2221 GGTGGTGTATGGTTACGGAGCTGTGCATCTTGGGACATGTAGTAGCCCAGGTCTTGTCAC
2281 TCGCTGTGAGATGGGGAGATTTTGTCTTTGATTTATCCCTGTAGGGCTGGCAGGGTTGT
2341 AGATGAAGGGGGAATGATCTGAGCCTTGGTTCCCCTGACACGTCTTGCTAGCCCCAGGGT
2401 TAGAGTGGGCAGGGCAGAGCCGCGCAGCACCTGGGAGCGGTACCTTCCCTTGGGCAGCC
2461 TGGGGTCCAGGAACAAGCCAGGGCGAGTGCCATGTCTGCCTGAGCAGGGTGTGGCCCCA
2521 GAAAGCTGAGGAGTGTGGGCTGGCAGAGAGCTTCGAGGGCAAGGCCACCCGCGGGGGGGT
2581 GTGTGTGGTGGGGCTTGGCATGTGATGGCAGCTCCAGCTCCAGGCATGCCGCTGCTTGTA
2641 TGGCTTTCTTTGGCCTCTGACCCTGCTGCCCATTCTTTCCAACATCACAGATGAACTGCC
2701 TCTCCTCCTCCCTGCCTGGGGAGCCCAGTGGCCAGGGAGGGAGTGGTGGAGCCAGTCGCT
2761 GTAACACTGAGCCTCAGAGACGAACCAAAACCAGCTGGGCTGAGCTCAGATCCAGGGGGA
2821 AGAAATGCTGGAAGTCAATAAAACTGAGTTTGAG 2854
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(b)

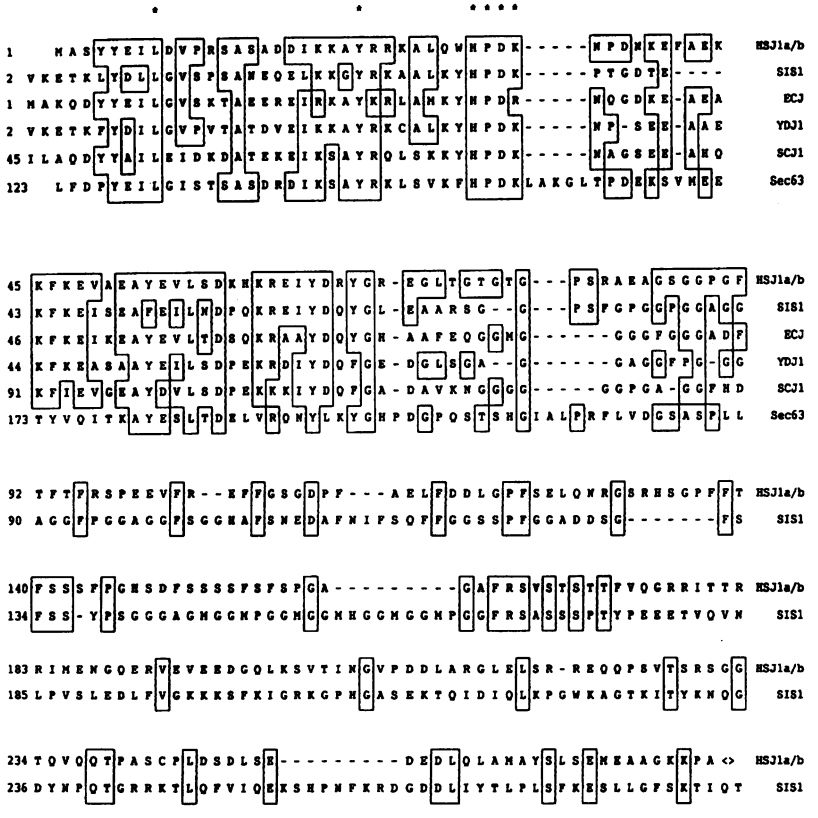

Fig. 1. (*a*) Nucleotide sequence and predicted amino acid sequence of HSJ1a and HSJ1b and (*b*) comparison of the predicted amino acids of HSJ1a and HSJ1b (residues 1–274) with the amino acid sequences of the other members of the DnaJ 'family' [SIS1 (residues 2–287), *E. coli* (ECJ) DnaJ (residues 1–90), yeast YDJ1 (residues 2–85), SCJ1 (residues 45–135) and Sec63 (residues 123–224)]

HSJ1b (351 amino acids) possesses a 1.1 kb insert, which is underlined; this results in an alternative *C*-terminal amino acid sequence from HSJ1a; the *C*-terminal amino acid sequence (three residues) of HSJ1a (277 amino acids) is shown after the insert sequence. The original clone HSJ1′ that was isolated lacked the insert, contained a polyadenylation signal and began at the *Eco*RI site at nucleotide 313. '*' Marks the termination codons

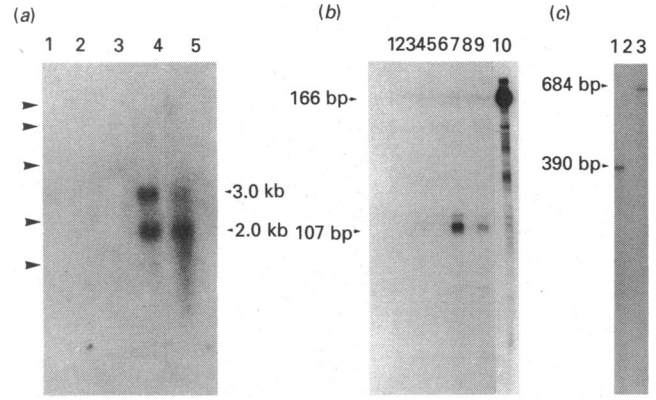

Fig. 2. (*a*) Northern-blot analysis of 5 μg of polyadenylated RNA extracted from human tissues, (*b*) RNAase-protection analysis of total RNA extracted from human tissues and (*c*) RNAase-protection analysis of the 5′ end of HSJ1a

(*a*) 1, liver; 2, kidney; 3, skeletal muscle; 4, brain frontal cortex; and 5, brain hippocampus. RNA molecular-mass markers (BRL) are arrowed on the left (from the top: 9.5, 7.5, 4.4, 2.4 and 1.4 kb). Two bands, of sizes 2.0 and 3.0 kb, were present only in the brain-derived RNA. (*b*) 1, tRNA control; 2, liver; 3, kidney; 4, skeletal muscle; 5, testes; 6, spleen; 7, brain frontal cortex; 8, brain cerebellum; 9, brain hippocampus; and 10, no-RNAase control. The band at 107 bp is specific for HSJ1b; the signal was only strongly detected in the frontal cortex and hippocampus RNA preparations, with very weak signals from cerebellum, skeletal muscle and spleen. (*c*) 1, Brain frontal-cortex; 2, tRNA control; and 3, no-RNAase control. Only one band was seen at the expected size of 390 bp, indicating that there is no alternative splicing in this part of the mRNA.

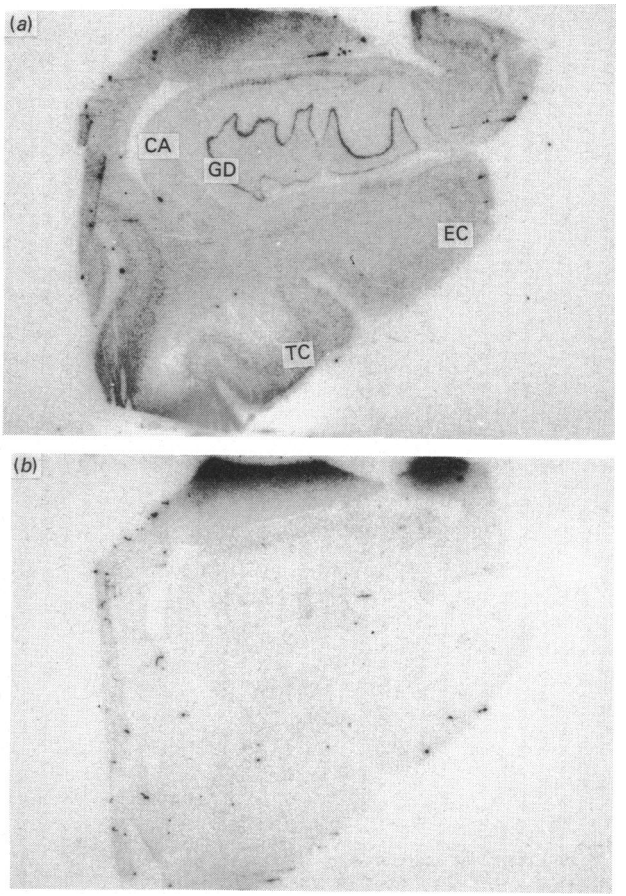

Fig. 3. Hybridization *in situ* on human hippocampus and the adjacent temporal cortex (non-Alzheimer case) with (*a*), an anti-sense ^{32}P-labelled riboprobe derived from HSJ1a and (*b*) ^{32}P-labelled sense riboprobe as control

The signal was stronger in the neuronal layers, i.e. the dentate gyrus (GD), the pyramidal layers of Ammon's horn (CA), the entorhinal cortex (EC) and the temporal cortex (TC). The intensity of hybridization showed discrete regional variations; it was stronger in the dentate gyrus, the entorhinal cortex and the temporal cortex than in the CA1 sector of Ammon's horn. These data do not discriminate between potential differential distributions of HSJ1a and HSJ1b.

al., 1991) is similar to bacterial DnaJ proteins in the *N*-terminal third and *C*-terminal third of the proteins, but not in the central third of the proteins. This central region of SIS1 shows some similarity to HSJ1a and HSJ1b which is not shared by the other members of the DnaJ 'family'. The alignment and identity of HSJ1a/b proteins to the *E. coli* DnaJ protein and yeast homologues is shown in Fig. 1(*b*).

The search also highlighted several other proteins of potential interest. The large and small T-antigens of the budgerigar-fledgling-disease virus (Rott *et al.*, 1988) also showed a high degree of similarity to the predicted HSJ1a and HSJ1b amino acid sequences at the *N*-termini (43.8% identity in a 48-amino-acid overlap). The same region in HSJ1a and HSJ1b also showed some similarity to the amino acid sequence of the polyomavirus JC large and small T-antigens (Frisque *et al.*, 1984) (32.6% identity in a 43-amino-acid overlap). Within this region of sequence similarity, six amino acids are absolutely conserved between the viral T-antigens of simian virus 40 (Fiers *et al.*, 1978) and polyomavirus (Yang & Wu, 1979; Pawlita *et al.*, 1985), and these same residues are conserved in HSJ1a and HSJ1b and the other eukaryoic DnaJ homologues; this includes a four-residue sequence of His-Pro-Asp-Lys.

The predicted amino acid sequence of the extra 74 amino acids encoded by HSJ1b does not show any significant similarity to any other proteins in the database.

Analysis of HSJ1 expression

Northern-blot analysis of human brain polyadenylated RNA with the HSJ1′ DNA detected two bands of sizes 3.0 and 2.0 kb; there was no signal detected in polyadenylated RNA from other tissues (Fig. 2*a*). The distribution of the mRNA within the brain appears to be dependent upon area, the hippocampus having a reduced signal of the 3 kb message relative to the frontal cortex; however, this may be due to an increased degradation of the hippocampal RNA. We detected no qualitative difference between Alzheimer's-disease-patient and control brain by Northern-blot analysis. The probe also detected two species of mRNA of approximately the same size in rat brain when washed at high stringency (results not shown).

of HSJ1a and HSJ1b. A rabbit polyclonal antiserum was raised to a synthetic peptide (peptide 15) corresponding to the doubly underlined sequence (amino acids 250 to 262). (*b*) Boxed residues are identical between HSJ1a and HSJ1b and the other protein homologues; amino acid residues are numbered on the left. The splice junction in HSJ1a/b is marked '< >'; there is no similarity to the other DnaJ homologues or other proteins in the isoform-specific sequences. Residues which are identical between the all eukaryotic DnaJ homologues and all the T-antigens of simian virus 40 and polyoma virus are marked '*'. Similarity between HSJ1a and HSJ1b and DnaJ was identified using the University of Wisconsin Genetics Computing Group computer program FASTA (Devereux *et al.*, 1984); dashes have been introduced to optimize alignments.

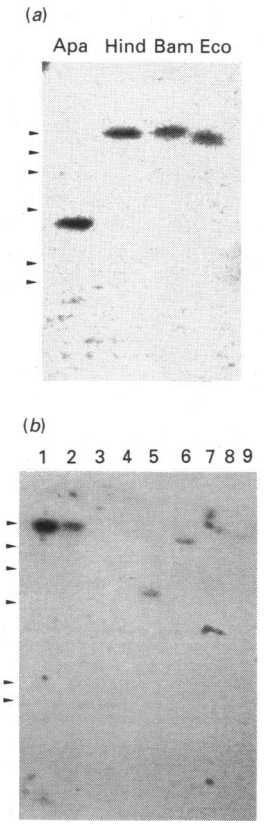

Fig. 4. Southern-blot analysis using HSJ1′ as a probe

(*a*) Human genomic DNA digested with *Apa*I (Apa), *Hin*dIII (Hind), *Bam*HI (Bam) and *Eco*RI (Eco). Molecular-mass markers are arrowed on the left (from the top: 23.1, 9.4, 6.5, 4.4, 2.3 and 2.0 kb). (*b*) 'Zoo blot' (Clontech) of *Eco*RI-digested genomic DNA from: 1, human; 2, monkey; 3, rat; 4, mouse; 5, dog; 6, cow; 7, rabbit; 8, chicken; 9, yeast. Molecular-mass markers were as in (*a*).

RNAase-protection analysis using a riboprobe generated from a deletion mutant which possesses the insert splice junction (Fig. 2*b*) demonstrated that the insert is specific to a genuine RNA present in brain and does not appear to be an intron. The probe also detected a smaller band specific to HSJ1a mRNA (result not shown). The results also demonstrated that the expression of HSJ1a and HSJ1b is restricted almost exclusively to brain, with human frontal-cortex and hippocampus containing the highest levels of mRNA, but with only low levels in the cerebellum. Weak signals were found in RNA from skeletal muscle and spleen. RNAase-protection analysis also showed that there is no variation in the coding region at the 5′ end of the mRNA (Fig. 2*c*).

Hybridization *in situ* with HSJ1

Hybridization studies *in situ* on sections of human brain localized HSJ1a/b expression to the neuronal layers in the hippocampus and temporal cortex (Fig. 3), and the intensity of hybridization showed discrete regional variations. The signal was stronger in the dentate gyrus, the entorhinal cortex and the temporal cortex than in the CA1 sector of Ammon's horn. No signal was detected with the sense probe. The results did not discriminate between potential differential distributions of HSJ1a and HSJ1b. The areas showing a strong signal are some of those that are susceptible to the development of neurofibrillary tangles in Alzheimer's disease.

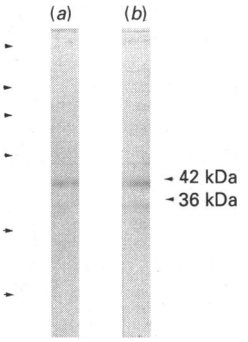

Fig. 5. Western-blot analysis on human brain homogenate (*a*) affinity-purified antiserum to peptide 15 (Fig. 1*a*) and (*b*) affinity-purified antiserum raised to HSJ1′–β-galactosidase fusion protein

Two bands were labelled at 36 and 42 kDa; other faint bands seen could not be removed by absorption with purified HSJ1′–β-galactosidase fusion protein. The positions of the molecular-mass markers (Amersham Rainbow) are arrowed on the left (from the top: 200, 97.5, 69, 46, 30 and 21.5 kDa).

Southern blotting

Preliminary Southern-blot analysis of human genomic DNA with HSJ1′ as a probe suggested that *HSJ1* was a highly conserved, single-copy, gene (J. Hardy & A. Ultsch, personal communication). A repeat of this experiment is shown in Figs. 4(*a*) and 4(*b*). The HSJ1′ probe only detects single bands in human genomic DNA digested with *Apa*I, *Hin*dIII, *Bam*HI and *Eco*RI (Fig. 4*a*). The HSJ1′ part of the gene appears to be highly conserved in mammals. An HSJ1′ probe detects a single band in *Eco*RI-digested genomic DNA from human, monkey, dog, cow and rat when washed at high stringency [0.1 × SSC/0.1% (w/v) SDS at 65 °C], suggesting that the HSJ1′ part of the sequence is highly conserved in these species (Fig. 4*b*).

Western blotting

Several polyclonal antisera were raised to synthetic peptides corresponding to various sequences in the non-conserved region of HSJ1a and HSJ1b (Fig. 1*a*) or the HSJ1′-β-galactosidase fusion protein. These antisera, which would not discriminate between HSJ1a and HSJ1b, labelled two bands on Western blots of human brain homogenates with apparent molecular masses of approx. 36 and 42 kDa, which is in reasonable agreement with the predicted molecular masses from the amino acid sequences of 32 and 38 kDa respectively (Fig. 5), suggesting that both HSJ1a and HSJ1b are genuine mRNAs which are translated into proteins. Post-translational modification of the two protein isoforms HSJ1a and HSJ1b may account for the apparent molecular masses measured from Western blots, being higher than the predicted molecular masses. However, transcription *in vitro* followed by translation *in vivo* of the HSJ1a cDNA produced a protein of 36 kDa that is also recognized by these antibodies (result not shown) and this suggests that there is no extensive post-translational modification of the isoforms detected on Western blots, but rather that their somewhat anomalous electrophoretic mobility may be conformational.

DISCUSSION

We have described two isoforms of a human homologue, which we have designated 'HSJ1a' and 'HSJ1b', of the bacterial DnaJ and yeast Sec63, SCJ1, YDJ1 and SIS1 heat-shock-protein family members. We have shown that the isoforms are prefer-

entially expressed in the brain and, within the brain, in the neuronal layers. Antibodies raised to a synthetic peptide or a fusion protein derived from HSJ1′ recognize two bands of approximately the correct molecular mass in human brain homogenates.

The similarity between HSJ1a and HSJ1b and DnaJ is restricted to the *N*-terminal region of the molecule, which is the region of highest sequence similarity between the *E. coli* and yeast proteins. The HSJ1a/b sequences contain a glycine-rich region (residues 69–92 of HSJ1a and HSJ1b) in the position corresponding to the glycine-rich domain of DnaJ which has been proposed to separate functional domains (Sadler *et al.*, 1989). The similarity of HSJ1a and HSJ1b to DnaJ *C*-terminal in this region is low and does not include a region of cysteine repeats occurring in DnaJ; this region is conserved in SCJ1 and YDJ1, hence they may be true DnaJ homologues. In contrast, SIS1, HSJ1a and HSJ1b do not possess this domain, but do have other small regions of sequence identity, which suggests that these latter three proteins may be more related in function. The significance of the greater similarity of HSJ1a/b to SIS1 is unclear at present.

The apparent sequence conservation of the *N*-terminal domains between bacterial DnaJ and the eukaryotic homologues suggests a conservation in function; this might be an interaction with the DnaK/Hsp70 proteins. Computer-based secondary-structure predictions for this domain suggest that it may form two α-helices separated by a flexible domain, almost a 'pincer' or 'J finger and thumb'. This may be the region of the DnaJ protein that binds to DnaK and accelerates the rate of ATP hydrolysis (Liberek *et al.*, 1991). The hypothesis that the *N*-terminal domain mediates an interaction with Hsp70 is consistent with the conservation of the His-Pro-Asp-Lys motif within the viral T-antigens. Several studies have shown that Hsp70 apparently interacts with a number of early viral gene products, including the simian-virus-40 large T-antigen (Sawai *et al.*, 1989; May *et al.*, 1991) and the polyoma middle T-antigen (Walter *et al.*, 1987).

Although the functions of the *E. coli* DnaJ protein and the yeast homologues are not well characterized, they are thought to act in protein assembly, disassembly and targeting. So it is possible that the HSJ1 proteins may be involved in mediating protein–protein dissociations. The two isoforms may act to 'target' different proteins to the normally 'promiscuous' Hsp70. The possibility of a differential distribution of HSJ1a and HSJ1b, however, remains to be addressed. It will be necessary to determine the functions of HSJ1a and HSJ1b in order to understand why neurons apparently express relatively high levels of these proteins and whether they have a role in responding to pathogenic insults in Alzheimer's disease.

There have been numerous reports demonstrating that stress-response proteins and mRNAs are elevated in neurons that have been subjected to stress by ischaemia (Nowak *et al.*, 1990), excitotoxins (Uney *et al.*, 1988) and in neurodegenerative conditions (Mori *et al.*, 1986; Leigh *et al.*, 1988). We have so far been unable to produce convincing immunohistochemical staining of human brain including Alzheimer's-disease-patient brain in spite of testing a range of fixation and processing conditions. The HSJ1a and HSJ1b proteins are not particularly abundant as assessed by Western blotting, but the lack of immunohistochemical staining is more likely to reflect a problem of epitope conformation or availability to our present antisera. We have therefore been unable to demonstrate a possible role for HSJ1 in the pathology of Alzheimer's disease, and the isolation of the original cDNA using an antiserum to PHF may have been serendipitous, but further immunohistochemical studies are needed.

Note added in proof (received 27 February 1992)

Another human homologue, HDJ1, has recently been described (Raabe & Manley, 1991).

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