The mitochondrial TIM22 preprotein translocase is highly conserved throughout the eukaryotic kingdom

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Abstract The Mohr–Tranebjaerg syndrome (MTS), a neurodegenerative syndrome characterized by progressive sensorineural hearing loss, dystonia, mental retardation and blindness, is a mitochondrial disease caused by mutations in the deafness/dystonia peptide 1 (DDP1) gene. DDP1 shows similarity to the yeast proteins Tim9, Tim10 and Tim12, components of the mitochondrial import machinery for carrier proteins. Here, we show that DDP1 belongs to a large family of evolutionarily conserved proteins. We report the identification, chromosomal localization and expressional analysis of six human family members which represent further candidate genes for neurodegenerative diseases.

Key words: Mitochondrial inner membrane translocase TIM22; Deafness/dystonia peptide 1/Tim10 protein family; Mohr–Tranebjaerg syndrome

1. Introduction

The recent description of a novel pathomechanism leading to a mitochondrial disease is based on the homology of the affected protein DDP1 to a family of conserved yeast components involved in mitochondrial preprotein import [3]. The gene encoding deafness/dystonia peptide 1 (DDP1) is mutated in the Mohr–Tranebjaerg syndrome (MTS), a rare X-linked neurodegenerative syndrome characterized by progressive sensorineural hearing loss, dystonia, mental retardation and blindness [1]. Until recently, the cellular localization and function of the 11 kDa deafness/dystonia peptide was unknown [1]. The putative function of DDP1 was recently discovered based on its similarity to Tim9, Tim10 and Tim12, components of the TIM22 machinery for import of nuclear encoded mitochondrial inner membrane proteins [2–5].

Import of nuclear encoded precursor proteins into the mitochondria is mediated by a general translocase in the outer membrane, the TOM complex, which cooperates with two distinct translocases in the mitochondrial inner membrane, the TIM23 and the TIM22 complex [6,7]. The TIM23 complex mediates the import of preproteins with a positively charged matrix targeting signal while the TIM22 complex mediates the import of integral inner membrane proteins which do not carry a matrix targeting signal. These are members of the mitochondrial carrier family such as the ADP/ATP carrier, the inorganic phosphate carrier but also other hydrophobic membrane proteins with internal targeting signals. For import of carrier proteins, the TIM22 complex interacts with three small proteins of the mitochondrial intermembrane space, Tim9, Tim10 and Tim12 which are organized in two distinct hetero-oligomeric 70 kDa complexes [2,8]. Tim9, Tim10 and Tim12 are structurally related zinc finger proteins [4] that contain a conserved Cys_4 zinc finger motif. The TIM9·10 complex appears to contain three molecules of Tim9 and three molecules of Tim10 [2,9] and the TIM9·10·12 complex might be composed of three molecules of Tim9, two molecules of Tim10 and one molecule of Tim12 [2,9]. The TIM9·10·12 complex is firmly associated with the membrane-integrated components of the TIM22 complex whereas the TIM9·10 complex is not associated with mitochondrial membranes [2,9]. The TIM22 complex cooperates with both the TIM9·10 and the TIM9·10·12 complex which sequentially interact with hydrophobic precursors and maintain them in an insertion-competent conformation [10–13]. The insertion of the hydrophobic preproteins into the inner membrane is mediated by Tim22 in a reaction that requires the inner membrane potential, $\Delta\Psi$ [14].

Saccharomyces cerevisiae encodes two further proteins, Tim8 and Tim13, which are structurally related to Tim9, Tim10 and Tim12 [3]. These proteins also contain a Cys_4 motif and are therefore likely also zinc finger proteins. The precise function of Tim8 and Tim13 is not known. Both proteins are localized in the intermembrane space and they are organized in hetero-oligomeric 70 kDa complexes [3]. However, import of carrier proteins is not affected in cells harboring disrupted alleles of TIM8 and TIM13. Thus, they are not essential for import of these precursors. Yet, a fraction of Tim8 and Tim13 appears to be in a complex with Tim9 and Tim10 as Δ*tim8* yeast strains display synthetic lethality with a temperature sensitive mutant allele of TIM10, suggesting a functional interaction of these components [3].

DDP1, the human Tim8 homologue, and DDP2, a second predicted protein highly similar to DDP1 [1], are human members of this novel family of small zinc finger proteins. Here, we show that the small Tim proteins belong to an evolutionarily conserved protein family. In particular, we report the identification, chromosomal localization and expressional analysis of the human homologues of components of the yeast

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TIM22 translocase which represent further candidate genes for human neurodegenerative diseases.

2. Material and methods

2.1. cDNA isolation

Human EST databases were searched for DDP/Tim10-related sequences using the TBLASTN option of the BLAST division of NCBI. EST contigs corresponding to the human cDNAs for Tim8, Tim9, Tim10 and Tim13 were identified. The assembled EST contigs are mainly represented by the following 'tentative human contigs' (THCs) accessible via TIGR (http://www.tigr.org/): THC221395 (hTim9), THC256243 (hTim10a), THC214970 (hTim10b), THC219996 (hTim13), THC260031 (DDP1), THC258076 (DDP2) and THC258075 ('hDDP2-like'). EST clones falling into the same cluster are represented by the UNIGENE IDs Hs.108527 (hTim9), Hs.109571 (hTim10a), Hs.54943 (hTim10b), Hs.76086 (hTim13), Hs.7499 (DDP2 and 'DDP2-like') and Hs.125565 (DDP1). The hTim22 cDNA is based on EST sequences represented by UNIGENE ID Hs.87595. Downloaded ESTs were assembled using the DNAstar software for PC and edited manually. The coding sequences of the deduced cDNAs were additionally confirmed by direct sequencing of selected EST clones using vector-specific primers.

2.2. Radiation hybrid (RH) mapping

Mapping data were available for TIM10a, TIM13, TIM9, DDP2 and TIM22 through the EST mapping consortium. The TIM13 gene has previously been mapped as part of the human LMNB2 gene locus (accession no. M94363). RH mapping was performed to locate TIM10b and, additionally, to confirm map locations of TIM10a and TIM9. The GeneBridge4 RH panel (HGMP Ressource Center, Cambridge) was screened by PCR using combinations of intron-specific and exon-specific primers according to the manufacturer's protocol.

2.3. Northern blot analyses

Adult human tissues Northern blots, containing 2 μg per lane of poly(A)$^+$ RNA, were purchased from Clontech® and hybridized using standard protocols. Hybridization probes specific for coding regions of DDP1, DDP2, hTim9, hTim10a, hTim10b and hTim13 were generated by PCR on human first strand cDNA and ^{32}P-labeled using the ReadyPrime kit (Pharmazia®). The same blot was re-hybridized with a β-actin probe in order to normalize the intensity of the specific hybridization signal to that of β-actin.

2.4. Sequencing of TIM13 in the DFNB15 family

Using genomic DNA from affected and unaffected members of the DFNB15 family as template, exons 1, 2 and 3 of the TIM13 gene (ppv1 gene) were amplified as two non-overlapping fragments using gene-specific primers according to the published ppv1 sequence. To exclude major deletions, a 1.6 kb fragment encompassing the entire TIM13 gene including major 5′- and 3′-untranslated region (UTR) sequences was additionally amplified from all family members. The PCR fragments were purified (Qiagen®) and subjected to direct sequencing using the BIG-DYE termination kit (ABI-Perkin-Elmer®).

2.5. Primers

Oligonucleotide primer sequences for amplification of DDP1/hTim8a, DDP2/hTim8b, hTim9, hTim10a, hTim10b and hTim13 fragments and RH data vectors are available from the authors on request.

3. Results and discussion

3.1. Identification of the human homologue of Tim22

TBLASTN searches in human EST databases identified over 50 ESTs with similarity to yeast Tim22, a central component of the membrane-integrated portion of the TIM22 translocase. The assembled cDNA contig of 1669 bp encodes a predicted protein of 194 amino acid residues. The protein sequences of hTim22 and yTim22 exhibit an overall identity of 29% (similarity 41%) (Fig. 1). In yeast, Tim22 is in a complex with the integral inner membrane protein Tim54. No human homologue of Tim54 could be identified in the EST databases. Interestingly, no TIM54 gene could be identified in the genome of *Caenorhabditis elegans*, while Tim54 homologues appear to be expressed in a number of different fungi (Prokisch, personal communication).

3.2. Identification and characterization of human cDNAs encoding members of the DDP/Tim10 protein family

In *S. cerevisiae*, Tim22 interacts functionally and physically with small, related zinc finger proteins of the mitochondrial intermembrane space. Homology searches identified novel human open reading frames (ORFs) encoding putative zinc finger proteins characterized by a Cys_4 motif. Based on their sequence similarity to the small Tim proteins of yeast, they were classified as members of the DDP/Tim10 protein family (Fig. 2). Three human cDNA contigs encoding putative homologues of yeast Tim9, 10 and 12 were found. Based on phylogenetic analysis, two sequences, hTim10a and hTim10b, may represent orthologues of yeast Tim10 and Tim12, while the third sequence may encode an orthologue of yeast Tim9 (Fig. 3). The cDNA sequences of hTim10a and hTim10b are 658 bp and 1468 bp in length and were derived from two distinct, non-overlapping EST contigs, THC256243 and THC214970. Both contigs contain a single ORF encoding predicted proteins of 90 and 103 amino acid residues.

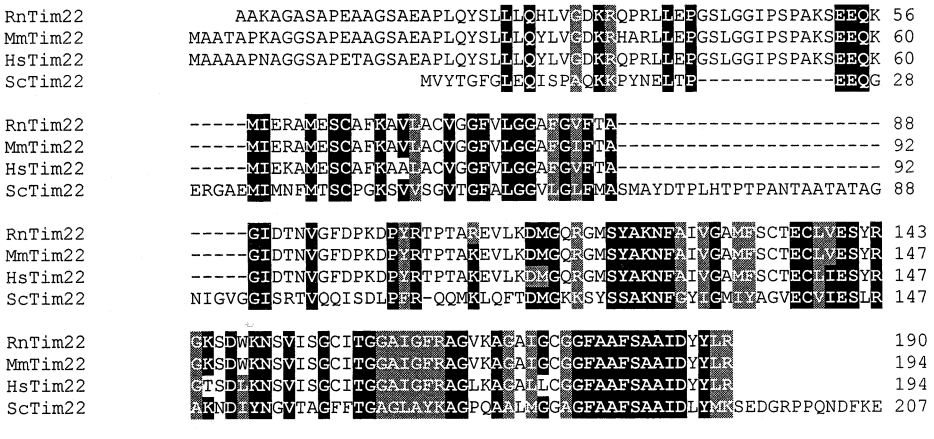

Fig. 1. Protein alignment of yeast Tim22 with the human, mouse and rat Tim22 homologues. Identical and similar residues are indicated.

Name	Organism	Motif	Aa	Accession
Tim9	*H.sapiens*	LGTYNKLTETCFLDCVKD__FTTREVKPEETTCSEHCLQKYLKMTQRISMRFQE	89	AF150100
Tim9	*M.musculus*	LGTYNKLTETCFLDCVKD__FTTREVKPEEVTCSEHCLQKYLKMTQRISVRFQE	89	AF150101
Tim9	*Da.rerio*	LGTYNKLTENCFMDCVKD__FTTREVKPEETTCSESCLQKYLKMTQRISMRFQE	84	AF150107
Tim9	*C.elegans*	LTVYNTLSERCFNACARD__YTTSTLTKDEGSCVSQCIDKQMLVNRRFMLVFAE	111	AF150109
Tim9	*S.cerevisiae*	MRLYSNLVERCFTDCVND__FTTSKLTNKEQTCIMKCSEKFLKHSERVGQRFQE	87	AF093244
Tim9	*A.nidulans*	MTMYSKLVQRCFDDCVND__FTTKSLISREEGCVMRCVDKFMKGSQRLNERFQE	90	AF150110
Tim9	*D.melanogaster*	FTLYNKVTELCFSRCVDN__LSQRDLGGHEDLCVDRCVTKFARFNQNMMKVYVD	117	AF150104
Tim9	*Ar.thaliana*	LRMYNSLVERCFXDCFDS__FXRKXXQKQEETCVMRCAEKFLKHTMRVGNRFSE	93	AF150111
Tim9	*Me.crystallinum*	LRMYNNLVERCFTDCVDS__FRRKTLDKQEETCVKRCAEKFLKHSMRVGLRFAE	93	AF150112
Tim9	*O.sativa*	LRMYNSLVERCFTDCVDT__FRRKTLDKQEESCVRRCAEKFLKHSMRVGMRFAE	93	AF150113
Tim10a	*H.sapiens*	ADMYNRMTSACHRKCVPP_HYKEAELSKGESVCLDRCVSKYLDIHERMGKKLTE	90	AF150089
Tim10a	*M.musculus*	ADMYNRMTSACHRKCVPP_HYKEAELSKGESVCLDRCVSKYLDIHERMGKKLTE	90	AF150090
Tim10a	*R.norvegicus*	ADMYNRMTSACHRKCVPP_HYKEAELSKGESVCLDRCVSKYLDIHERMGKRLTE	90	AF150091
Tim10b	*H.sapiens*	LLVYNRMTELCFQRCVPS__LHHRALDAEEEACLHSCAGKLIHSNHRLMAAYVQ	103	AF150105
Tim10b	*M.musculus*	LLVYNRMTELCFQRCVPS__LHHRALDAEEEACLHSCAGKLIHSNHRLMAAYVH	100	AF150103
Tim10b	*R.norvegicus*	LLVYNRMTELCFQRCVPS__LHHRALDAEEEACLHSCAGKLIHSNHRLMAAYVH	100	AF150106
Tim10a	*C.elegans*	SDMYRRMTNSCQAKCIAT_AFRESELTKGEAVCLDRCVAKYLDVHEKLGKRLTS	86	AF150094
Tim10b	*C.elegans*	LTQYNLVAEQCFNSCVNE__FGSRTVSGKEESCANNCLDKFLKMTQRVSQRFQE	90	AF150109
Tim10	*Ci.intestinalis*	ADMYNRMTSSCHKKCIST_RYDTGDLEKGEAVCIDRCVAKYLDIHEQIGKKLTE	115	AF150095
Tim10	*D.melanogaster*	SDLYNRMTNACHKKCIPP_RYSESELGKGEMVCIDRCVAKYLDIHEKIGKKLTA	92	AF150092
Tim10	*Bo.mori*	SDMYNRLVSACHRKCIPI_KYHEPELGKGESVCLDRCVAQYLDVHERIG.	70	AF150098
Tim10	*S.cerevisiae*	TDMFNKLVNNCYKKCINT_SYSEGELNKNESSCLDRCVAKYFETNVQVGENMQK	93	U10555
Tim12	*S.cerevisiae*	CSTFNNILSTCLEKCIPHEGFGEPDLTKGEQCCIDRCVAKMHYSNRLIGGFVQT	109	P32830
Tim10	*A.nidulans*	TDMFNRLSESCSKKCIPN_DYREGDLNKGESVCLDRCVGKFFEVNIKVSEKMQG	93	AF150097
Tim10	*Ar.thaliana*	VELFNKLAQTCFNKCVDK_RYKEAELNMGENSCIDRCVSKYWQVNGMVGQLLSA	83	AF150093
Tim10	*L.esculentum*	VEMFNKLTHTCFKKCVEN_KYKDSELNMGENSCIDRCVSKYWQVTNLVGTLLGN	81	AF150096
Tim10	*P.taeda*	VELFNKLTKTCFDKCIER_RYKESELNMGECSCVDRCVAKYWQVTSIVGQLLGS	84	AF144706
Tim8	*C.elegans*	TEQVHTLTGRCWDVCFADYRP_PSKMDGKTQTCFQNCVNRMIDASNFMVEHLSK	83	AF150086
Tim8a	*H.sapiens*	QQLVHQMTELCWEKCMD__KP_GPKLDSRAEACFVNCVERFIDTSQFILNRLEQ	97	U66035
Tim8a	*M.musculus*	QQLVHQMTELCWEKCMD__KP_GPKLDSRAEACFVNCVERFIDTSQFILNRLEQ	97	AF150081
Tim8a	*R.norvegicus*	QQLVHQMTELCWEKCMD__KP_GPKLDSRAEACFVNCVERFIDTSQFILNRLEQ	97	AF150082
Tim8b	*H.sapiens*	TAQVHHFMELCWDKCVE__KP_GNRLDSRTENCLSSCVDRFIDTTLAITSRFAQ	83	AF150087
Tim8b	*M.musculus*	TAQVHHFMELCWDKCVE__KP_GSRLDSRTENCLSSCVDRFIDTTLAITGRFAQ	83	AF196314
Tim8b	*R.norvegicus*	TAQVHHFMELCWDKCVE__KP_GSRLDSRTENCLSSCVDRFIDTTLAITGRFAQ	83	AF196315
Tim8	*D.melanogaster*	NAQIHEFNEICWEKCIG__KP_STKLDHATETCLSNCVDRFIDTSLLITQRFAQ	88	AF142424
Tim8	*Ss.pombe*	QQAIHQFTSTCWPKCIGNI___GNKLDKSEEQCLQNCVERFLDCNFHIIKRYAL	98	AF143537
Tim8	*S.cerevisiae*	QMSIHQFTNICFKKCVESVN__DSNLSSQEEQCLSNCVNAFLDTNIRIVNGLQN	87	YJR135wa
Tim8	*N.crassa*	QGQTHALTDSCWKKCVTSPI_KTNQLDKTEAVCMADCVERFLDVNLTIMAHVQK	92	AF142423
Tim8	*Ar.thaliana*	NEMVSKMTSVCWDKCITSA_P_GSKFSSSESSCLTHCAQRYMDMSMIIMKRFNS	77	AF150083
Tim8	*Ma.domesticus*	NEMVGKLTNVCWDKCITGT_P_GSKFSSSESACLANCARRYLDMSMIIM	71	AF150084
Tim13	*H.sapiens*	QELLQRMTDKCFRKCIGK__P_GGSLDNSEQKCIAMCMDRYMDAWNTVSRAYNSR	95	AF144700
Tim13	*R.norvegicus*	QELLQRMTDKCFRKCIGK__P_GGSLDNSEQKCIAMCMDRYMDAWNTVSRAYNSR	95	AF144701
Tim13	*M.musculus*	QELLQRMTDKCFRKCIGK__P_GGSLDNSEQKCIAMCMDRYMDAWNTVSRAYNSR	95	AF144702
Tim13	*Br.malayi*	QNMITDXSERCLTKCITX__P_GSALSXTERQCLQRCMDRFMETYXLASQXLQNR	98	AF144705
Tim13	*C.elegans*	QNLVTDISEKCTNKCITA__P_GSSLASGEKQCLQRCMDRFMESWNLVSQTLQHR	108	AF144704
Tim13	*S.cerevisiae*	TELVNKISENCFEKCLTS__P____YATRNDACIDQCLAKYMRSWNVISKAYISR	105	G7157
Tim13	*Ss.pombe*	GELISKINENCFDRCIPE__P_GSTFDPNEKSCVSKCMERYMDAWNIVSRTYISR	95	AF143538
Tim13	*A.thaliana*	EELIETLRTKCFDRCVTX__P_GSSLGGSESSCISRCVERYMEATAIISRSLFTQ	87	AF144703
Tim13	*O.sativa*	QEFLETVGNKCFAKCVTK__P_GSSLSGSESSCISRCVDRYIEATGIVSRXLFXS	84	AF150080

Fig. 2. Sequence alignment of zinc finger domains of members of the DDP/Tim10 protein family. The $\underline{CX_3C}$-X_{14-17}-$\underline{CX_3C}$ zinc finger domains and flanking regions (49–54 amino acid residues) of small Tim proteins from different species were aligned using MegAlign program, DNAstar for PC (Gap weigth = 3, gap length penalty = 10). Entire lengths of proteins (in amino acid residues) and GenBank accession numbers are indicated. Genera are: A., *Aspergillus*; Ar., *Arabidopsis*; B., *Brassica*; Bo., *Bombyx*; Br., *Brugia*; C., *Caenorhabditis*; Ci., *Ciona*; Da., *Danio*; D., *Drosophila*; H., *Homo*; L., *Lycopersicon*; Ma., *Malus*; Me., *Mesembryanthenum*; M., *Mus*; N., *Neurospora*; O., *Oryza*; P., *Pinus*; R., *Rattus*; S., *Saccharomyces*; Ss. *Schizosaccharomyces.*

hTim10b is highly similar to mouse and rat Tim10b (AF150103 and AF150106). A previously published cDNA sequence encoding *Rattus norvegicus* FxC1 (fracture callus 1) [15] is identical to the rat Tim10b cDNA but lacks the 5′-region. FxC1 therefore represents an N-terminally incomplete Tim10b protein sequence.

The human cDNA (THC221395) with similarity to yeast Tim9 is 1027 bp in length and contains a single ORF encoding a predicted protein of 89 amino acid residues.

A previously described gene (ppv1) located within the 3′-UTR of the human lamin B gene (LMNB2) encodes the putative human homologue of yeast Tim13 [16]. The predicted

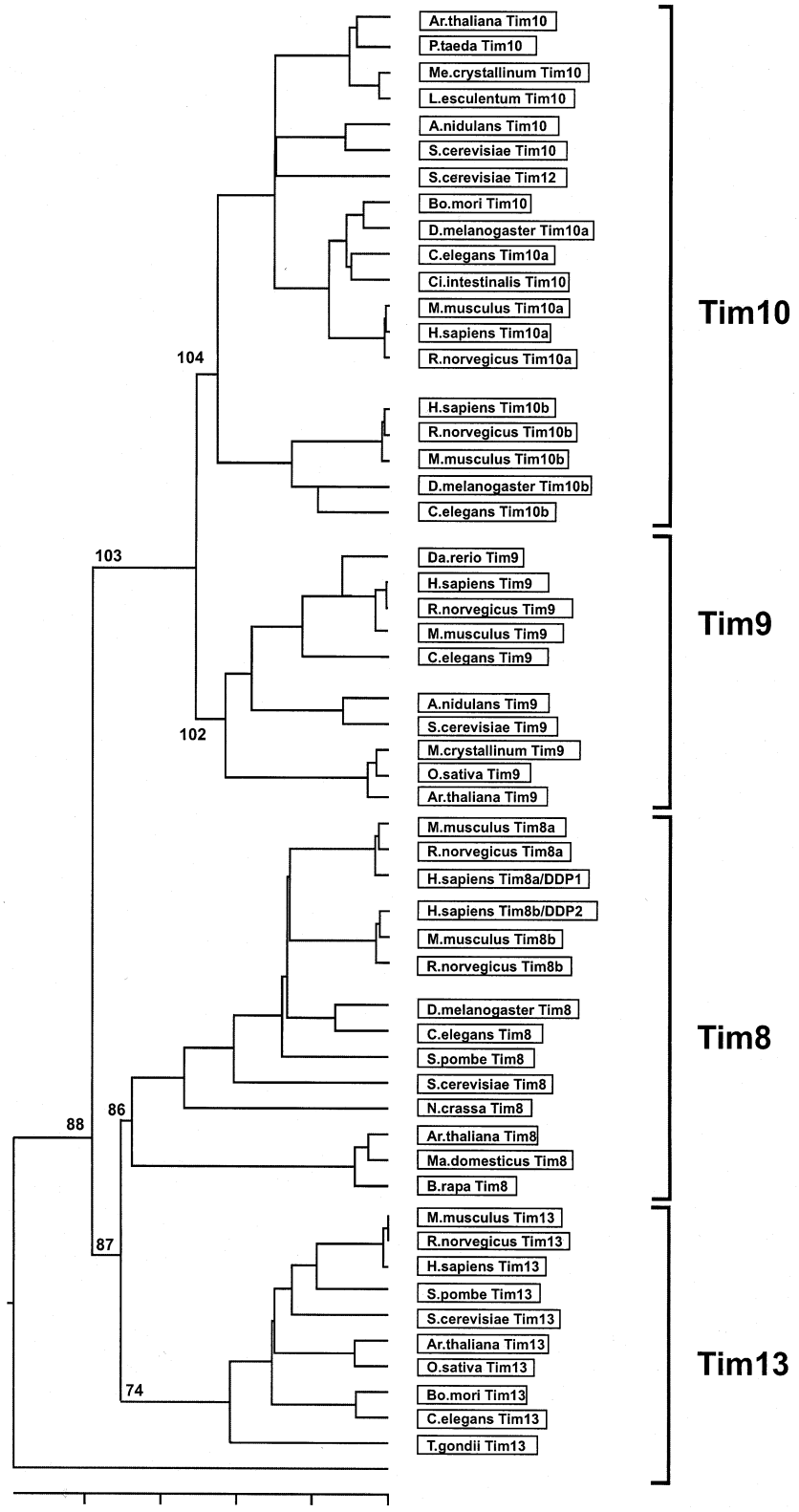

Fig. 3. Dendrogram generated by alignment of members of the DDP/Tim10 protein family. The DDP/Tim10 family consists of two major groups which divide in two subgroups: Tim9/Tim10 and Tim8/Tim13. The length of the branches is proportional to the degree of sequence divergence. The dendrogram was generated using the entire sequences aligned by the Clustal algorithm with default parameters (MegAlign program, DNAstar for PC).

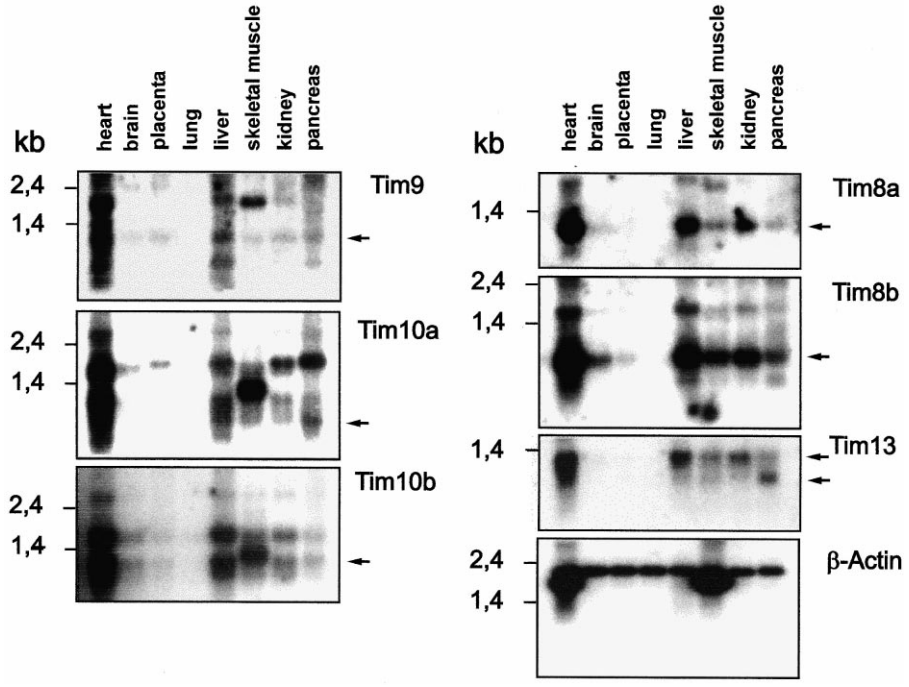

Fig. 4. Northern blot analyses on multiple-tissue mRNA blots were performed using radiolabeled cDNA probes. Transcript sizes of 1.2 kb (DDP1), 0.8 kb (DDP2), 1.2 kb (hTim9), 1.0 kb (hTim10a), 1.35 kb (hTim10b) and 1.3 kb/1.0 kb (hTim13) were detected. Blots were exposed for 1–2 days, the hTim10a-specific blot was exposed for 6 days.

ppv1 protein of 95 amino acid residues is classified as member of the DDP/Tim10 protein family based on its characteristic Cys_4 motif and was, therefore, renamed hTim13 (Fig. 2). A second THC contig (THC196963) covered by only two ESTs (AA071111 and AA084879) gives rise to a protein which differs in five amino acid residues from hTim13 (not shown). These two ESTs are derived from a neuroepithelial library which has been assigned to be contaminated with mouse-derived clones. Since the two EST sequences are identical with the cDNA sequence of mouse Tim13, it is unlikely that AA071111 and AA084879 encode a second human Tim13 homologue.

A number of human ESTs correspond apparently to the previously described genes DDP1 and DDP2 [1,3] which encode small proteins of 97 and 83 amino acid residues, respectively, which both contain the characteristic Cys_4 motif (Fig. 2). Analysis of DDP2-specific EST clones revealed that the amino acid sequence predicted from the assembled EST contig (THC258076) differs in the N-terminal portion from the published protein sequence [3]. We confirmed this by sequencing of three independent EST clones.

A second 'DDP2-like' EST contig could be assembled (THC258075) differing from the authentic DDP2 cDNA by an insertion of 333 bp between codon 28 and codon 29 and by a number of nucleotide exchanges upstream of this insertion. The 333 bp insertion is located exactly at the exon/intron splice site (CAG-GTG) of the DDP2 gene but shows no similarity to the genomic intron sequence of the authentic DDP2 (data not shown). The 'DDP2-like' ESTs may therefore originate from a duplicated gene. The 333 bp insertion contains no ORFs. Translation initiation from an ATG corresponding to codon 33 of authentic DDP2 could give rise to an N-terminally truncated polypeptide containing the Cys_4 motif. As the 333 bp insert contains out of frame ATG codons, it is unclear whether the 'DDP2-like' cDNA is translated.

A pseudogene of DDP2 was identified in a genomic DNA library. The pseudogene shows 97% identity with the DDP2 ORF. The genomic sequence lacks the DDP2 translation initiation codon, does not contain introns but contains a poly(A) tail. This suggests that the pseudogene resulted from re-integration of a processed DDP2 mRNA into the genome.

Table 1
Members of the human DDP/Tim10 gene family

Human sequence	Yeast homologue	Transcript size (bp)	Protein size (aa)	Polymorphic marker	Chromosomal location	Accession number	References
Tim8a/DDP1	Tim8	1 169	97	DXS6724	Xq22.1	U66035	Jin et al. [1]
Tim8b/DDP2	Tim8	823	83	D11S1347-D11S939	11q22.1q22.2	AF150087	This study
Tim9	Tim9	1 027	89	D14S290-D14S274	14q21	AF150100	This study
Tim10a	Tim10, Tim12	658	90	D11S1361-D11S913	11p11–p12	AF150089	This study
Tim10b	Tim10, Tim12	1 469	103	D11S909-D11S4194	11p15.3–p15.5	AF150105	This study
Tim13	Tim13	763	95	pTEL-D19S413	19p13.1–19p13.3	AF144700	This study
Pseudo-DDP1	–	1 067	–	D2S156-D2S376	2q22–q23	U66034	Jin et al. [1]
Pseudo-DDP2	–	–	–	–	Xq27.2	AC002407	Chen et al. [17]

3.3. The small Tim proteins belongs to an evolutionary conserved family

TBLASTN searches in available EST and genomic databases resulted in the identification of 44 new ORFs throughout the kingdom of eukaryotes with similarity to the small Tim proteins of yeast (Fig. 2). Thus, homologues of the small Tim proteins appear to be expressed in all eukaryotic organisms. All ORFs encode putative zinc finger proteins of about 10 kDa containing a Cys_4 motif with the consensus CX_3-C-X_{14-17}-CX_3C (Fig. 2). A phylogenetic analysis based on the alignment of the proteins assigned the sequences to two major groups, each of which divide into two subgroups (Fig. 3). The significance of the groups and subgroups was assessed by bootstrap analyses. One group is represented by the essential yeast components Tim9, Tim10 and Tim12 while the other group contains the non-essential yeast components Tim8 and Tim13. Yeast Tim10 and Tim12 are closely related and fall in one subgroup, while Tim9, Tim8 and Tim13 fall into independent subgroups (Fig. 3). The four subgroups of the protein family differ in their sequence composition and in the length of the zinc finger between the two conserved cysteine pairs (Fig. 2).

Apparently, all organisms encode at least one member of each subgroup. Six putative members of this protein family seem to be expressed in mammals. The Tim8/13 group contains three mammalian homologues, two belong to the Tim8 subgroup and one falls into the Tim13 subgroup. The Tim9/10 group also contains three mammalian members. Although the assignment to the subgroups is not unambiguous, one member appears to belong to the Tim9 subgroup and two to the Tim10 subgroup (Fig. 3).

3.4. Mapping of human TIM genes

The rapid and ongoing efforts of the international EST mapping consortium result in a rising number of mapped human ESTs. Corresponding to EST data, TIM10a maps between D11S1361 and D11S913 (11p11.1–q12), DDP2 between D11S1347 and D11S939 (11q22.1–q22.2), TIM9 between D14S290 and D14S274 (14q21) and TIM13 between pTEL and D19S413 (19p13.3) (Table 1). The latter position is in accordance with the map location reported for the LMNB2 gene [16]. The human TIM22 gene maps to chromosome 17p13. None of the ESTs corresponding to TIM10b contained sequence-tagged sites. Using RH mapping, we localized TIM10b to chromosome 11q15.3–q15.5. We additionally confirmed the chromosomal locations of TIM10a and TIM9 gene by RH mapping. The DDP2 pseudogene was found to be contained on BAC clone bWXD177 (AC002407) which has been sequenced during the X-chromosome sequencing project (Center for Genetics in Medicine, Washington University, http://www.ibc.wustl.edu/) and maps to chromosome Xq27.2.

3.5. Expression analysis of human genes encoding small Tim proteins

Human genes encoding members of the DDP/Tim10 protein family were analyzed for their expression patterns in adult human tissues. All members of the human DDP/Tim10 family appear to be ubiquitously expressed with highest steady-state levels in heart, liver, skeletal muscle and kidney (Fig. 4). The transcript sizes were determined as 1.2 kb for DDP1, 0.8 kb for DDP2, 1.2 kb for hTim9, 1.0 kb for hTim10a, 1.35 kb for hTim10b and 1.3 kb for hTim13. A second hTim13 transcript of 1.0 kb was detected in pancreas. hTim10a is expressed at significantly lower levels than hTim9 and hTim10b, specific signals being detected only after long exposition of Northern blots. In yeast, Tim12 is more than one order of magnitude less abundant than Tim9 and Tim10. yTim12 mediates association of the TIM9·10·12 complex with Tim22 in the inner membrane while the TIM9·10 complex is not membrane-associated [2]. Whether hTim10a exerts a similar function as yTim12 remains to be established.

3.6. Small Tim proteins and neurodegeneration

Mutation of DDP1/hTim8a was shown to be causative for the MTS [1,3]. Since the DDP/Tim10 family is highly conserved, the other members of the DDP/Tim10 family are also candidate genes for neurodegenerative disorders associated with deafness, dystonia, optic atrophy or mental deterioration. As yTim13 forms a complex with yTim8, hTim13 could interact with DDP1/hTim8a in human mitochondria. Thus, hTim13 appears to be a likely candidate gene for a MTS-like disorder. We noticed that the chromosomal localization of TIM13 (19p13.3) corresponds to a disease locus associated with an autosomal recessive and non-syndromic form of deafness in an Indian family (DFNB15) [17]. However, sequencing of the TIM13 gene in affected as well as in unaffected members of the DFNB15 family revealed no pathogenic mutations (data not shown). This excludes TIM13 as the underlying disease gene for the DFNB15-associated inherited hearing loss. Chen and co-workers identified a second DFNB15 locus on 3q21.3–q25.2. No member of the human DDP/Tim10 gene family maps to chromosome 3. Therefore, it is unlikely that this form of hearing loss is caused by a defect in members of the DDP/Tim10 family.

Clinical phenotypes associated with mutations of DDP1 have been shown to range from apparent non-syndromic forms of deafness to complex neurodegenerative syndromes including deafness, dystonia, mental deficiency and blindness [1]. In the original Norwegian family with MTS described first in 1960 [18], a non-syndromic recessive and progressive form of deafness was reported. The re-investigation of this family 35 years later revealed the deafness as part of a more complex syndrome that includes cortical blindness, dystonia and mental deficiency. Moreover, mutations in DDP1 might also be responsible for related clinical phenotypes such as Jensen syndrome which is characterized by optico-acoustic nerve atrophy with dementia [19]. The underlying pathomechanism common to these diseases might be degeneration of the central nervous system, in particular basal ganglia and corticospinal tract due to a deficiency of a subclass of inner membrane proteins.

Clinical features of MTS resemble typical defects of mitochondrial respiration and oxidative phosphorylation [20]. Yet, the underlying mechanism cannot be deduced from yeast strains harboring single and double deletions of TIM8 and TIM13. Those retain respiratory competence and show no effect on the biogenesis of the ADP/ATP carrier and of the phosphate carrier [3]. As yeast encodes 35 different carrier proteins [21], it is possible that Tim8 promotes the import of carrier proteins whose inactivation has no phenotype in yeast [5].

MTS is a progressive neurodegenerative disorder which affects post-mitotic tissues. Since the TIM8 is not an essential gene in yeast, mutations in DDP1/TIM8a in humans could

cause subtle defects which are recognized as MTS only upon accumulation over time.

Knowledge about the human homologues and their function in import pathways will give important insights into the pathomechanisms of MTS and will facilitate the identification of further disease genes responsible for mitochondrial disorders.

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