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The human mitochondrial transport protein family: Identification and protein regions significant for transport function and substrate specificity

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Abstract

Protein sequence similarities and predicted structures identified 75 mitochondrial transport proteins (37 subfamilies) from among the 28,994 human RefSeq (NCBI) protein sequences. All, except two, have an *E*-value of less than 4e−05 with respect to the structure of the single subunit bovine ADP/ATP carrier/carboxyatractyloside complex (bAAC/CAT) (mGenThreader program). The two 30-kDa exceptions have *E*-values of 0.003 and 0.005. 21 have been functionally identified and belong to 14 subfamilies. A subset of subfamilies with sequence similarities for each of 12 different protein regions was identified. Many of the 12 protein regions for each tested protein yielded different size subsets. The sum of subfamilies in the 12 subsets was lowest for the phosphate transport protein (PTP) and highest for aralar 1. Transmembrane sequences are most unique. Sequence similarities are highest near the membrane center and matrix. They are highest for the region of transmembrane helices H1, H2 and connecting matrix loop 12 and smallest for transmembrane helices H3, H4 and loop 34. These sequence similarities and the predicted high similarities to the bAAC/CAT structure point to common structural/functional elements that could include subunit/subunit contact sites as they have been identified for PTP and AAC. The four residues protein segment (SerLysGlnIle) of loop 12 is the only segment projecting into the center of the funnel-like structure of the bAAC/CAT. It is present in its entirety only in the AACs and with some replacements in the large Ca2+-modulated aspartate/glutamate transporters. Other transporters have deletions and replacements in this region of loop 12. This protein segment with its central location and variation in size and composition likely contributes to the substrate specificity of the transporters.

Keywords: Human; Mitochondria; Transport; Protein; Carrier; Family

1. Introduction

Mitochondria are subcellular structures essential for the aerobic eukaryotic cell. Among biochemical reactions that make them essential is the oxidative phosphorylation of ADP to ATP. Since oxidative phosphorylation requires a mitochondrial membrane potential, it is critically important that the transport of metabolites and other entities across the membrane be tightly controlled. The mitochondrial ADP/ATP carrier and the phosphate transport protein (PTP) are two such transporters that control the flux of the primary oxidative phosphorylation substrates ADP/ATP and inorganic phosphate.

Mitochondria also are responsible for parts of other metabolic pathways critical for the cell and the multicellular organism. Their participation in those pathways requires that metabolites at the interface between cytosolic and mitochondrial matrix reactions be transported in a highly controlled manner across the mitochondrial inner membrane. Human mitochondrial transport proteins have already been identified or implicated for metabolites from metabolic pathways and related functions as diverse as fatty acid

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biosynthesis (citrate) [1] and oxidation (carnitine) [2], the urea cycle (ornithine) [3], the malate-aspartate shuttle for cytosolic NADH oxidation (aspartate/glutamate, oxoglutarate/malate) [4,5], folate metabolism (folate) [6], coupling efficiency of oxidative phosphorylation (H+s) [7–11], CoA or precursor transport [12], reduced glutathione transport (oxoglutarate) [13], precursors for mitochondrial DNA biosynthesis (deoxynucleotides) [14], net import into matrix of ATP [15–17], transport of the methyl group donor S-adenosylmethione [18], gluconeogenesis (dicarboxylate) [19], protein catabolism (glutamate, oxodicarboxylate) [20,21], and metal ion (iron) transport [22]. Initial structural studies identified protein sequence elements that suggested the presence of a mitochondrial transport protein family [23]. Recent reviews have summarized data on human mitochondrial transport proteins [24] and characterized structural elements of 284 mitochondrial transport proteins from eight eukaryotic genomes [25].

We have now thoroughly searched the human genome RefSeq proteins (NCBI) for similarity to mitochondrial transport protein sequences and have found 75 proteins. We have shown that they can be fitted rigorously to the recently published high resolution structure of the bovine ADP/ATP carrier protein subunit/carboxyatractyloside complex [bAAC/CAT (RCSB Protein Data Bank 1OKC)] [26]. In addition, we have identified common sequence/structure elements that will help answer questions such as whether most of these proteins function, similar to the yeast PTP [27,28] and the bovine ADP/ATP carrier [29], as homodimers with the expected subunit/subunit contact site residues. In addition, a four-residue protein segment (FCS) is located at the funnel center of the bAAC/CAT structure. It is absent from many transporters and present in many others with deletions and substitutions and it is likely to play a role in determining transport protein substrate specificity.

2. Materials and methods

The RefSeq proteins of the NCBI human genome database (http://www.ncbi.nlm.nih.gov/genome/guide/human/) was blasted with 23 residue long protein sequences from 12 different regions of 20 human mitochondrial transport proteins (Fig. 1). An *E*-value of 10 was used in this search to emphasize general protein sequence similarities as applicable to common structure elements and to minimize the influence of substrate specific residues on the search.

Protein sequence similarities between two or more sequences was carried out with software of The Biology Workbench (http://workbench.sdsc.edu/).

Threader experiments to identify published protein structures highly similar to structures predicted from protein

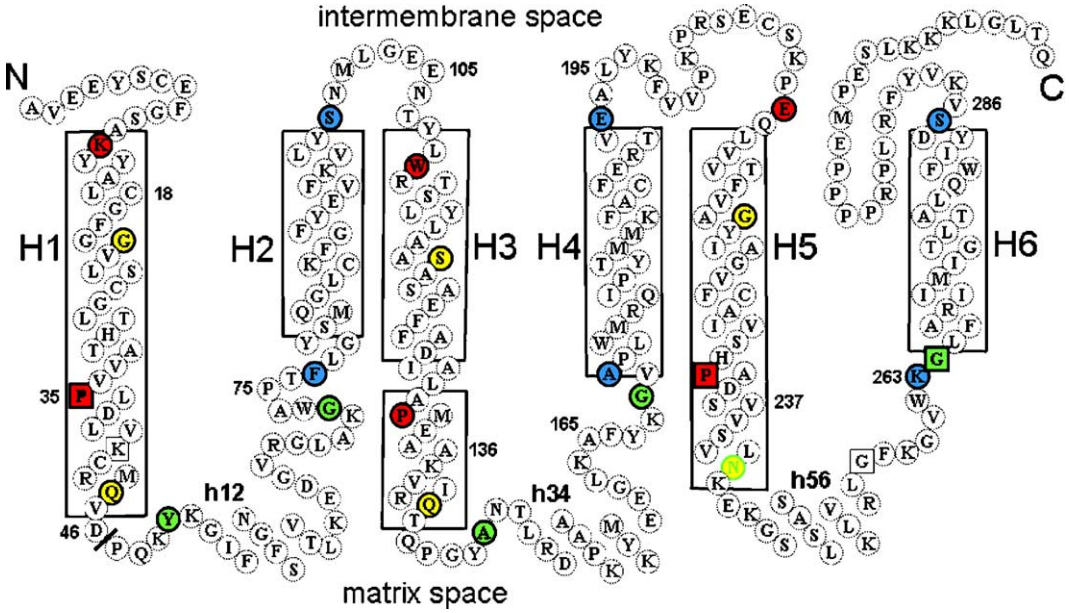

Fig. 1. Topological representation of a subunit of the human mitochondrial phosphate transport protein NP_998776.1. The six transmembrane helices are indicated as H1 to H6. Short helices h12, h34, and h56 lining the matrix surface of the inner membrane are also indicated and their presence is based on the structure of the bAAC/CAT complex. The line separating Asp46 and Pro47 indicates the site from which the four residue segment (FCS) of loop 12 that is present in the ADP/ATP translocases (see Figs. 3 and 4) is absent from PTP and some other transport proteins. 23 residue protein regions H1P (K13 to P35), H3P (W110 to P132), and H5P (E211 to P233); H1Q (G22 to Q44), H3Q (S119 to Q141), H5Q (G220 to N242); h12p (Y50 to G72), h34p (A147 to G169), h56p (N242 to G264); and H2 (F77 to S99), H4 (A171 to E193), H6 (K263 to S285) are indicated by the same color first residue and last residue. The four residues enclosed by a square are most highly conserved among mitochondrial transport proteins.

Table 1
The human mitochondrial transport proteins

SF[a]	NCBI accession number (SLC25 ID)	Function (identified[b])	FCS[c]	Size (rs)[d]	Terminal extensions[e]	Structure[f], similarity comments[g]	Ref.
1*	NP_005975.1 (A1)	citrate	(+)	311	+19/+11	Unique	[1,33]
2*	NP_055067.1 (A15iso1)	ornithine (y)	–	301	+3/+11	Similar to (H1, H3, H5) of A2iso2	[3,34]
2	NP_114153.1 (A2iso2)	ornithine (y)	–	301	+3/+11	Similar to (H1, H3, H5) of A15iso1	[3,35]
3	NP_005879.1 (A3isoa)	phosphate	–	362	+59/+26	Similar to (H1, H5) of XP_497676.1	[36]
3*	NP_998776.1 (A3isob)	phosphate	–	361	+58/+26	Identical to A3isoa (deletion Q54 and replacement of 12 rs within segment D58 to L83) Similar to (H1, H3, H5) of A3isoa; (H1, H5) of XP_497676.1	[36]
3	XP_497676.1	FLJ40434	–	374	+74/+22	Similar to (H1, H5) of A3isoa and A3isob	
4*	NP_001142.2 (A4)	ADP/ATP T1 (y)	+	298	+2/+4	Similar to (H1, H3, H5) of A5, A6, A31, XP_497832.1; (H1, H3) of XP_496859.1; (H5) of XP_498308.1	[37,38]
4	NP_001143.1 (A5)	ADP/ATP T2 (y)	+	298	+2/+4	Similar to (H1, H3, H5) of A4, A6, A31, XP_497832 and XP_496859.1; (H5) of XP_498308	[38,39]
4	NP_001627.1 (A6)	ADP/ATP T3 (y)	+	298	+2/+4	Similar to (H1, H3, H5) of A4, A5, A31, XP_497832.1 and XP_496859.1; (H5) of XP_498308.1	[38,40]
4	NP_112581.1 (A31)	(y)	+	315	+14/+11	Similar to (H1, H3, H5) of A4, A5, A6 (H1, H3) of XP_497832.1 and XP_496859.1	[41]
4	XP_497832.1	ADP/ATP T (similar)	+	362	+66/+4	Similar to (H1, H3, H5) of A4, A5, A6, XP_496859.1; (H1, H3) of A31; (H5) of XP_498140.1	
4	XP_496859.1	ADP/ATP T (similar)	+	348	+52/+4	Similar to (H1, H3, H5) of A5, A6, XP_497832.1; (H1, H3) of A4, A31; (H3, H5) of XP_498140.1	
4	XP_498140.1	ADP/ATP T (similar) (fibroblast)	–	240	+19/−5	No h12, H2; Similar to (H3, H5) of XP_496859.1; (H5) of XP_497832.1	
4*	XP_498308.1	ADP/ATP T (similar) (liver)	+	293	+2/+3	Similar to (H5) of A4, A5, A6	
5*	NP_068605.1 (A7)	UCP1 (y)	(+)	307	+7/+15	Similar to (H1) of A8; (H1) of A9	[7,42]
5	NP_003346.2 (A8)	UCP2 (y)	+	309	+7/+15	Similar to (H1, H3, H5) of A9 (H1) of A7	[8,43–45]
5	NP_003347.1 (A9)	UCP3 L (y)	+	312	+7/+15	Similar to (H1, H3, H5) of A8; (H1) of A7	[9,44–46]
5	NP_073714.1 (A9)	UCP3 S	+	275	+7/−21	No H6; Identical to NP_003347.1 (1 to 275)	[46]
6*	NP_036272.2 (A10)	dicarboxy-late	–	287	0/+10	Unique	[19]
7*	NP_003553.2 (A11)	oxogluta-rate (y)	(+)	314	+15/+11	Unique	[5,13]
8*	NP_003696.2 (A12)	aralar 1 (y)	+	678	+320/+77	Similar to (H1, H3, H5) of A13	[4]
8	NP_055066.1 (A13)	citrin, aralar 2 (y)	+	675	+322/+72	Similar to (H1, H3, H5) of A12	[4]
9*	NP_003942.1 (A14)	UCP5 L	+	325	+34/+5	Similar to (H1, H3, H5) of A30	[10]
9	NP_073721.1 (A14)	UCP5 S, BMCP1	+	322	+31/+5	Identical to NP_003942.1 except deletion of (V23SG) (not part of H1)	[10]
9	NP_001010875.1 (A30)	UCP5, KMPC1	+	291	−1/+5	Similar to (H1, H3, H5) of A14	[47]
10*	NP_689920.1 (A16)	CoA Grave's disease (y)	–	332	+30/+7	Unique	[12]
11*	NP_006349.1 (A17)	ADP/ATP (peroxisomal) (y)	–	307	+3/+16	Unique	[48]
12*	NP_078974.1 (A22)	glutamate 1 (y)	(+)	323	+2/+15	H3 has extralong C-terminal; Similar to (H1, H3, H5) of A18	[20]
12	NP_113669.1 (A18)	glutamate 2 (y)	–	315	+2/−2	H3 has extralong C-terminal; Similar to (H1, H3, H5) of A22	[20]
13*	NP_068380.2 (A19)	deoxynucleotide (y)	+	320	+9/+14	Unique	[14]

(continued on next page)

Table 1 (*continued*)

SF[a]	NCBI accession number (SLC25 ID)	Function (identified[b])	FCS[c]	Size (rs)[d]	Terminal extensions[e]	Structure[f], similarity comments[g]	Ref.
14*	NP_000378.1 (A20)	carnitine/ acylcarnitine (y)	(+)	301	+4/+11	Similar to (H1) of NP_997000.2	[2]
14	NP_997000.2	UCP (liver specific)	–	308	−7/+9	loop12 (short 9 rs) H3 (long 23 rs C-terminal) loop34 (long 23 rs); Similar to (H1) of A20	[49,50]
15*	NP_085134.1 (A21)	oxodicarboxylate (y)	(+)	299	+7/+8	Unique	[21]
16	NP_077008.2 (A23)	ATP-Mg/Pi, APC2, SCaMC-3 (y)	–	468	+179/+9	Similar to (H1, H3, H5) of A24 (H3, H5) of A25 (H3) of XP_496380.1 (H5) of NP_775908.1	[15,16]
16*	NP_037518.2 (A24 iso 1)	SCaMC-1	–	476	+188/+9	Similar to (H1, H3, H5) of A23, A24 iso 2, A25 (H1, H3) of XP_496380.1 (H5) of NP_775908.1	[15,16]
16	NP_998816.1 (A24 iso 2)	ATP-Mg/Pi, APC1 (y)	–	458	+169/+9	Identical to A24 iso 1 (except N-terminal 1 to 61 rs is replaced with a different 1 to 42 rs and in loop 56 (V417 to M399 and A398 insertion)	[15,16]
16	NP_443133.2 (A25 iso a)	SCaMC-2a	–	469	+180/+9	Identical to A25 (except see notes with each iso) Similar to (H1, H3, H5) of A24 (H3, H5) of A23 (H1, H3) of XP_496380.1 (H5) of NP_775908.1	[15–17]
16	NP_001006642.1 (A25 iso b)	SCaMC-2b	–	503	+214/+9	Identical to A25 iso a (segment 1 to 52 rs is replaced with a different 1 to 86 rs)	[15–17]
16	NP_001006643.1 (A25 iso c)	SCaMC-2c, APC3	–	489	+200/+9	Identical to A25 iso a (segment 1 to 53 rs is replaced with a different 1 to 73 rs)	[15–17]
16	NP_001006644.1 (A25 iso d)	SCaMC-2d	–	366	+77/+9	Identical to A25 iso a (deleted 1 to 103 rs) Similar to (H1, H3, H5) of A24, A25; (H1, H3) of XP_496380.1; (H3, H5) of A23; (H5) of NP_775908.1	[15–17]
16*	XP_496380.1		–	561	+272/+9	Similar to (H1, H3) of A24 and A25 (H3) of A23	
16	NP_775908.1	MGC-34725	–	232	−58/+6	No H1, h12; Similar to (H5) of A23, all A24s, all A25s	
17*	NP_001009937.1 (A26 iso a)	AdoMet (y)	–	274	+1/+12	No h12 Unique	[18]
17	NP_775742.2 (A26 iso b)	AdoMet (partial)	–	186	−107/+12	No H1, h12, H2; Identical to A26 iso a (89 to 274 rs)	
17	NP_001009938.1 (A26 iso c)	AdoMet (partial)	–	66	−208/−10	No H1, h12, H2, H4, H5, h56, H6; Identical to A26 iso a (89 to 151 rs) plus EED at its C-terminal (*E*-value 0.019)	
18*	AAD16995.1 (A27)	UCP4	+	323	+13/+9	Unique	[11]
18	NP_004268.2 (A27)	UCP4 (partial plus)	+	134	+13/−170	No H3, h34, H4, H5, h56, H6; Identical to AAD16995.1 (1 to 99 rs) plus 35 rs at its C-terminal (18 of these rs are C-terminal of H2) (*E*-value 0.005)	
19*	AAH76399.1 (A28)	MRS3/4	(+)	364	+66/+15	Similar to (H1, H3, H5) of NP_057696.1	[22]
19	NP_112489.2 (A28)	MRS3/4 (partial)		177	−128/+15	No H1, h12, H2, H3(part); Identical to AAH76399.1 (188 to 364 rs)	
19	NP_057696.1	MRS3/4 (similar)	(+)	347	+39/+21	Similar to (H1, H3, H5) of A28	[51]
19	NP_061049.2		(+)	155	+39/−169	No H3(part), h34, H4, H5, h56, H6; Identical to NP_057696.1 (1 to 148 rs) plus LKAFVWS at its C-terminal (*E*-value 0.019)	
20	NP_689546.1 (A29)	carnitine/acylcarni-tine (swiss only)	(+)	237	+20/−68	No H5(part), h56, H6 Unique	
21*	NP_110407.2	folate (y)	(+)	315	+16/+12	Unique	[6]
22	NP_060345.1	FLJ20551	(+)	304	+21/+8	Similar to (H1, H3, H5) of XP_497724.1 (primary difference between rs 1 to 22 of XP_497724.1 and rs 1 to 26 of NP_060345.1 both outside H1)	[52]
22	XP_497724.1	FLJ20551 (similar)	(+)	300	+17/+8		
23	NP_660348.1		–	341	+6/+51	Unique	[51]
24	NP_055470.1	KIAA0446	–	314	+11/+15	Unique	[52,53]
25	NP_660325.1	CG4995		157	−100/−36	No H1, h12, H2, H6 Unique	[52]
26	NP_057100.1	CGI-69 protein	+	351	−5/+15	Loop 12 (long 42 rs) Unique	[54]
27	NP_997231.1	LOC284723	+	304	0/+12	Loop 23 (short 9 rs) Similar to (H1) of NP_958928.1	[51,52]
27	NP_958928.1	1810012H11RiK and CAH70849 (similar)	+	295	−7/+1	No matching structure h56 and H6; Similar to (H1) of NP_997231.1	[51]

Table 1 (*continued*)

SF[a]	NCBI accession number (SLC25 ID)	Function (identified[b])	FCS[c]	Size (rs)[d]	Terminal extensions[e]	Structure[f], similarity comments[g]	Ref.
28	NP_115691.1	MGC4399	+	321	+5/+9	H1 (long 16 rs); loop12 (long 16 rs) Similar to (H1) of NP_060625.1	[55]
28	NP_060625.1	FLJ10618	+	310	+1/+6	Similar to (H1) of NP_115691.1	[51]
29	NP_061331.2		+	338	+9/+14	H1 (long 19 rs, C-terminal); loop12 (long 26 rs) Unique	[56]
30	NP_872362.1	LOC283130	–	335	−4/+73	No loop12, h12 and no structure for h56, H6 Unique	[51]
31	NP_848621.1	MGC26694	–	318	+27/+9	Unique	
31	XP_209204.2	MGC26694	–	318	+27/+9	Identical to NP_848621 except 39 (S to P) and 312 (L to M)	
32	XP_084000.1		–	307	+34/+4	loop12 (short 12 rs) Similar to (H1, H3, H5) of NP_219480.1 (H3) of XP_372689.2	
32	NP_219480.1		–	297	+24/+4	h12 (short 4 rs); loop12 (short 12 rs); Similar to (H1, H3, H5) of XP_084000.1 (1 to 10 missing from N-terminal; 11 other replacements throughout protein) (H3) of XP_372689.2	
32	XP_372689.2		–	356	+28/+57	Poor h34, H4, H5, H6; Similar to (H1) of XP_084000.1 and NP_219480.1 (*E*-value 0.003)	
33	XP_377034.1		–	307	+21/+13	h34 (longer by 4 rs) Unique	
34	NP_620128.1	BC017169	(+)	418	+93/+8	Poor h34 (8 extra rs between h34 and H4) Unique	
35	XP_372410.2	down in hepatocellular carcinoma	+	298	−3/+33	C-terminal extension of H1, 'poor' fit of H3, h34, H4, H5, h56, H6 (*E*-value 0.005)	
36	NP_055156.1	homolog 1	+	372	+73/−2	Similar to (H5) of XP_497268.1	
37	NP_055157.1	homolog 2	+	303	−6/+1	Unique	

[a] Subfamily (SF) members have at least one transmembrane helix sequence H1 (H1Q), H3 (H3Q), or H5 (H5Q) with similarity *E*-value of less than 1e-03. Bold number with asterisk is subfamily protein that was further analyzed (see Tables 2 and 3, Figs. 2 and 4).

[b] Refers to transported substrate or other identifier. (y), human protein was identified by functional assay.

[c] Center of Funnel Segment (CFS) is protein segment (SerLysGlnIle) in center of funnel structure of bAAC/CAT complex (Fig. 3) (RCSB PDB 1OKC) connecting H1Q and $h_{12}p$ (see Fig. 1) on the matrix side of the membrane. Sequence alignments (mGenThreader) indicated: +, residues present at all six locations; (+), no residue at some locations; −, residues absent from at all six locations.

[d] rs is residues.

[e] Terminal extensions refer to number of residues (N-terminal/C-terminal) extending beyond the similarity lineup between bovine ADP/ATP T1 and the protein (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html).

[f] The *E*-value for all structures with respect to RCSB PDB 1OKC is 4e-05 (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html) except where noted.

[g] Some similarity and structure comments are based on predicted helix content of structure with respect to RCSB PDB 1OKC (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html) and global sequence similarity analysis (http://workbench.sdsc.edu/). h12, h34, h56 refer to the short helices (see Fig. 1). Loop refers to protein segment connecting two transmembrane helices, e.g. loop 23 connects the intermembrane space ends of H2 and H3P.

sequences were carried out with the mGenThreader program (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html).

3. Results

3.1. Identification of the members of the human mitochondrial transport protein family

Twelve different query sequences (Fig. 1) were chosen from the human mitochondrial phosphate transport protein and also from 19 other transport proteins (Table 1). The sequences were chosen to cover different regions of interest of the protein and to include residues rigorously identified as being highly conserved among members of the yeast mitochondrial transport protein family [30]. The sequences were used to blast the human genome protein database (RefSeq protein) of the National Center for Biotechnology Information (NCBI) of the National Institutes of Health. Table 1 shows all the members of the human mitochondrial transport protein family identified in this manner. The proteins of Table 1, when subjected to the mGenThreader program, were all identified with the high resolution structure of the bovine mitochondrial ADP/ATP T1 transporter subunit/carboxyatractyloside complex that has recently become available [26]. The *E*-value with all, except two of them, is less than 4e−05. Two proteins, XP_372689.2 of subfamily 32 and XP_372410.2 of subfamily 35, have *E*-values of 0.003 and 0.005, respectively. Some of the proteins of mass much smaller than the typical 30 kDa also have relatively large *E*-values. They, however, are fragments of another large protein.

3.2. Subfamilies of the human mitochondrial transport proteins

Several different mitochondrial transport proteins are known to catalyze the transport of the same substrates, e.g. ADP and ATP by the ADP/ATP transport proteins NP_001142.2 (A4), NP_001143.1 (A5), NP_001627.1 (A6), and NP_112581.1 (A31). The human genome protein database was blasted with the H1Q, H3Q, and H5Q query

sequences (Fig. 1) of each of the proteins identified in Table 1 with an asterisk. The query peptides were located in the protein sequences by aligning the protein sequence with a structurally well-characterized protein, e.g., bovine ADP/ATP T1 transporter subunit, using the mGenThreader program. Only proteins that showed similarity with at least one of these query sequences at an *E*-value of 1e−03 or less were saved from the search of the human genome proteins. From among these proteins only those that were chosen showed the high degree of similarity in the same sequence element as the query sequence, i.e., search with a H1Q query sequence required the high sequence similarity to be with the H1Q sequence of the new protein(s) and not with the H3Q or H5Q sequences of the new protein(s). Proteins with such sequence similarities were placed into the same transport protein subfamily as the protein from which the 23-residue query sequence had been obtained. Table 1 shows 37 such transport protein subfamilies.

This method used for placing transport proteins into the same subfamily may of course have the consequence that members may have some protein sequence segments with poorer similarity. Table 1 shows, for example, that XP_497676.1 from subfamily 3 is only similar to H1Q and H5Q, but not H3Q, of both A3 iso a and b.

3.3. Major sequence differences among the human mitochondrial transport proteins

Several proteins were identified that either have a significantly larger or smaller mass than the 30 kDa of the typical mitochondrial transport protein. All the larger mass proteins could be identified with an N-terminal extension with a calcium binding site (subfamilies 8 and 16). Almost all proteins with a smaller mass could be identified with partial sequences of 30 kDa mass proteins.

Table 1 (column 7) shows the locations of sequence discrepancies among the proteins. Thus, for example, proteins of subfamily 8 have N-terminal and C-terminal extensions. The N-terminal extensions however are much longer and possess calcium binding sites. The number of residues in the extension was determined by aligning the protein with the bovine ADP/ATP T1 transporter sequence using the mGenThreader program. This alignment of predicted α-helices suggests (see Table 1, column 6) that NP_003696.2 (aralar 1) has a 320-residue N-terminal extension and a 77-residue C-terminal extension. This method for predicting extensions can fail with short protein sequences. Protein NP_001009938.1 (66 residues) from subfamily 17 must first be aligned with the full sequence (NP_001009937.1) (274 residues) followed by the mGenThreader program and the bovine ADP/ATP T1 transporter to identify correctly the sequence shortcomings at its N- and C-terminals (Table 1, column 6).

Proteins from other subfamilies may differ by having protein loops that connect transmembrane helices that are either shorter or longer. Thus, for example, XP_498140.1 from subfamily 4 lacks h12 and H2. Or NP_775908.1 from subfamily 16 lacks H1 and h12. Also in NP_997000.2 from subfamily 14 the loop12 is shorter by 9 residues and loop 34 is shorter by 23 residues than those of the bovine ADP/ATP T1 transporter.

Table 2
Number of subfamilies with protein sequences similar to the query sequence of the indicated protein

Protein (SF[a])	H1P	H2	H3P	H4	H5P	H6[b]	H1Q	H3Q	H5Q[b]	$h_{12}p$	$h_{34}p$	$h_{56}p$[b]
Citrate (1)	0	0	0	0	0	0	4	0	5	6	1	5
Ornithine 1 (2)	0	0	0	0	0	2	1	0	1	3	0	1
Phosphate (3)	0	0	0	0	0	0	1	0	0	1	4	0
ADP/ATP T1 (4)	3	0	0	0	0	0	4	3	2	4	6	9
XP_498308.1 (4)	0	0	0	0	0	0	2	0	0	1	4	4
UCP1 (5)	0	0	0	0	2	2	9	3	2	0	0	6
Dicarboxylate (6)	0	0	0	0	1	2	6	2	5	0	1	1
Oxoglutarate (7)	0	1	2	0	1	4	8	3	3	4	5	2
Aralar 1 (8)	0	1	0	2	0	0	11	3	8	8	0	10
UCP5L (9)	0	2	0	0	0	2	6	2	3	6	2	4
CoA (10)	2	2	3	3	1	2	6	5	7	0	1	0
ADP/ATP (perox.) (11)	0	0	0	0	0	0	4	2	0	0	4	0
Glutamate 1 (12)	0	1	0	4	0	0	13	3	8	5	0	8
Deoxynucleotide (13)	0	0	0	3	1	0	2	1	2	0	1	8
Carnitine (14)	0	0	0	4	0	1	3	0	2	7	8	0
Oxodicarboxylate (15)	1	1	0	0	0	3	9	3	6	3	0	1
SCaMC-1 (16)	2	3	1	3	0	1	4	5	1	0	6	1
XP_496380.1 (16)	0	1	1	3	0	0	5	4	0	0	6	0
AdoMet (17)	0	0	0	2	0	0	11	0	7	0	0	0
UCP4 (18)	1	0	0	1	0	1	7	3	2	2	2	7
MRS 3/4 (19)	0	1	0	0	0	1	7	0	4	0	2	0
Folate (21)	0	0	0	0	0	2	1	2	1	5	8	7

[a] Subfamily (SF); proteins are identified in Table 1 with asterisk.
[b] Results using 23 residue query sequences from the indicated proteins. See Fig. 1 for the query sequences of Phosphate (3) (PTP).

3.4. Protein sequence similarities differ for human mitochondrial transport protein regions

All human genome proteins were blasted with the query sequences (see Fig. 1) from the twenty two proteins shown in Table 2. Only sequence hits with *E*-values of less than 10 and only a single representative protein per subfamily were noted. The results (Table 2) show that while there are 37 subfamilies, the largest number of subfamilies is identified with H1Q from Glutamate 1 (NP_078974.1). Several other results from Table 2 are noteworthy. The transmembrane helices are most unique, i.e., the N-terminal two thirds (Fig. 1) of the odd numbered helices and the C-terminal two thirds of the even numbered helices (Table 2, columns 2 to 7). The even number helices are on average somewhat less unique. The matrix ends or protein regions near the matrix ends of the helices, both odd (H1Q, H3Q, H5Q) and even (h12p, h34p, h56p) show much higher sequence similarities than the transmembrane helices. H1Q peptide sequences show highest similarities.

Fig. 2 shows in more detail the locations, within the query sequences, where residues are most alike among the proteins listed in Table 2. The 23-residue query sequences of the proteins of Table 2 were compared. Two proteins (XP_498308.1, XP_496380.1) of Table 2 that had another member of their subfamily present were not further analyzed. The 23-residue sequences of each of the twelve regions of the 20 proteins were separated into four groups of 5 sequences each. The groupings were based on the degree of sequence similarity. A summary of the comparison of the five most highly similar peptides is shown in the top line of each region in Fig. 2. A summary of the five with lowest similarities are shown at the bottom of each group. Of interest is that in the center of helix 1 (H1P) there is a very well-defined region of sequence similarity. Such sequence similarities in the other helices (H3P, H5P, H2, H4, H6) are not as obvious.

Fig. 2 also shows the 23-residue sequences of the human PTP for each protein region directly above the similarity summary of its group of five sequences, i.e., sequences H1P, H1Q, H4, H5Q, and H6 belong to least similar groups. Red (underlined) residues upon a conservative replacement yield dramatic inhibition of phosphate transport [31]. These replacement mutations can be separated into those that are unique to PTP vs. those that show residue similarity in all other proteins. Among the former is His in the H1P region and among the latter are Asp and Lys in the H1Q region. It is likely that the Asp and Lys of the H1Q region have a role in transport that is common to many of the proteins while His of H1P is unique to one of the substrates being transported by PTP, i.e., phosphate and H+.

Table 3 lists the 20 proteins and the groups of highest (1) and lowest (4) similarity (Fig. 2) that they belong to. The similarity scores for PTP can be read directly from the data shown in Fig. 2. The data show that each protein belongs to at least one group with highly similar sequences in one region of the protein as well as at least one group of lowest similarity in another region of the protein.

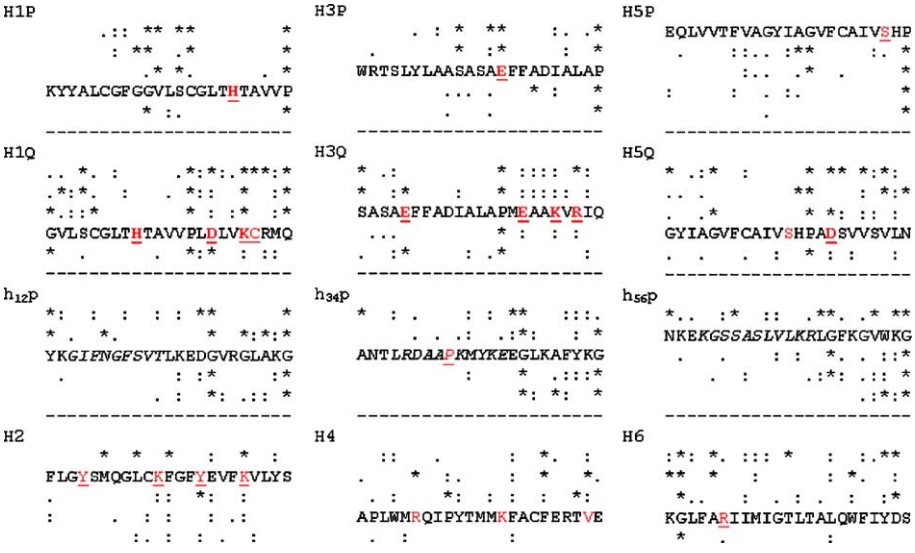

Fig. 2. Amino acid similarities within the twelve protein regions of 20 analyzed transporters (Table 1). For each protein region the five transporters with the largest number of identical/similar amino acids (*identical residue; **:** conservation of strong groups; **.** conservation of weak group) have their similarities summarized in the top line. The second line represents the same analysis for five of the remaining 15 transporters. The third line represents the same analysis for five of the remaining 10 transporters. The fourth line shows amino acid similarities for the remaining five transporters. The sequences of the twelve protein regions of the human PTP (see Fig. 1) are shown directly above the sequence similarities summary line of the group to which they belong, i.e., protein region H1P belongs to the group with lowest similarities and protein region H5P belongs to the group with highest similarities. Residues conserved between human and yeast PTP and that upon a conservative replacement yield transport blocked yeast PTP [31] are red and underlined. This inhibition is most severe for residues indicated in bold. The short helices h12, h34, h56 are indicated in italics.

Table 3
Human mitochondrial transport proteins arranged according to total similarity score

Protein[a] (SF)	H1P	H1Q	$h_{12}p$	H2	H3P	H3Q	$h_{34}p$	H4	H5P	H5Q	$h_{56}p$	H6
ADP/ATP (perox.) (11)	4	4	3	3	1	4	2	4	3	4	4	3
Phosphate (3)	4	4	3	2	3	3	3	4	1	4	2	4
Citrate (1)	3	3	2	4	4	3	2	4	4	1	4	2
Folate (21)	2	4	3	3	4	4	1	4	3	2	3	1
ADP/ATP T1 (4)	3	2	4	1	4	3	2	1	4	4	1	4
Dicarboxylate (6)	3	2	4	2	2	4	2	2	1	4	4	2
Carnitine (14)	2	4	2	4	2	4	4	1	1	3	2	3
Ornithine (2)	2	4	2	4	3	1	3	1	3	2	3	3
AdoMet (17)	2	3	4	1	3	3	2	3	2	1	3	4
CoA (10)	1	3	4	1	3	2	4	3	1	2	4	3
Aralar 1 (8)	4	1	1	3	1	3	3	4	3	1	1	4
SCaMC−1 (16)	1	2	4	2	4	1	1	3	4	2	3	1
UCP4 (18)	3	1	3	2	1	2	4	2	2	3	4	1
Glutamate 1 (12)	1	1	2	3	1	2	4	3	4	1	1	4
Deoxynucleotide (13)	4	3	3	4	3	1	1	1	2	2	1	2
Oxoglutarate (7)	1	2	1	4	1	4	1	1	4	3	2	2
UCP1 (5)	3	2	1	3	2	2	3	2	1	4	1	2
MRS 3/4 (19)	2	3	1	1	4	1	1	3	2	3	2	3
UCP5L (9)	4	1	1	2	2	1	3	2	2	3	2	1
Oxodicarboxylate (15)	1	1	2	1	2	2	4	2	3	1	3	1

[a] Proteins (identified in Table 1 with asterisk) are arranged according to total similarity score. The peroxisomal ADP/ATP transporter at the top of the table has the highest score of 39 and thus overall its sequence similarity is lowest (most unique) with respect to the 19 other proteins. (SF) is the subfamily (see Table 1).

3.5. Protein segment projecting into predicted funnel-like structure of some transport proteins is absent from or altered in others

The structure of the single subunit bAAC/CAT complex (Fig. 3) suggests that the protein segment (SerLysGlnIle) (FCS) that projects into the center of the funnel-like structure must be important to assigning substrate specificity to these transport proteins. This segment is present in the other proteins of subfamily 4 (ADP/ATP transporters) (Fig. 4) with the exception of XP_498140.1, the subfamily 4 member with most of helices h12 and H2 missing. Fig. 4 shows the detailed sequences that neighbor FCS of all members of subfamily 4. Transport has been demonstrated only with the NP members and the four-residue FCS of these are identical. XP_498308.1 has a FCS with replacements (SerMetProMet). Other replacements (Fig. 4) in the four XP members of subfamily 4 also may affect transport properties of these transporters significantly.

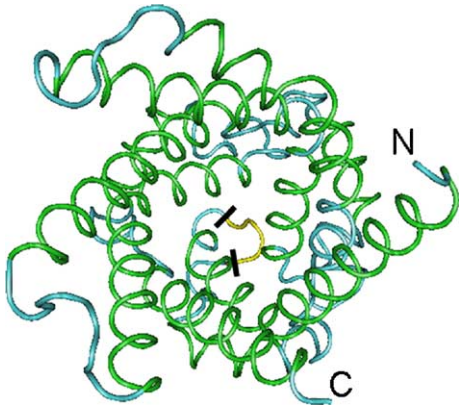

Fig. 3. Four residue protein segment (SerLysGlnIle) that projects into the center of the funnel of the structure of the bovine ADP/ATP T1 subunit/carboxyatractyloside complex [26]. The segment (yellow) is located between the bars. Helical regions of the protein are in green. The N-terminal (N) and the C-terminal (C) are indicated.

The sequences of the other proteins listed in Table 1 were analyzed for the presence of residues in FCS. Only proteins of a few subfamilies possess the complete segment and then with substitutions or deletions, i.e., subfamilies 5 (UCP1 has a single residue deletion), 8, 9, 13, 18, and other subfamilies (26, 27, 28, 29, 35, 36, 37) with unknown functions. Other proteins with partial FCS segments are indicated in Table 1 and Fig. 4. Proteins that do not possess this segment, like PTP (subfamily 3) (see Figs. 1 and 4), are also noted in Table 1 (column 4).

4. Discussion

With the availability of the human genome, it has become of great interest to identify the whole human family of mitochondrial transport proteins. While uncertainty remains as to the total number of proteins coded for by the human genome [32], the database of human genome proteins available through NCBI contains the sequences of 28,994 proteins.

The identification of the human mitochondrial transport proteins and their functions will lead to a better understanding of cellular metabolism and of metabolic diseases associated with them. The availability of all members of this family will

Fig. 4. Aligned protein sequences of the region near the four residue segment (FCS) of the twenty (Table 1) analyzed proteins and all members of subfamily 4. The FCS (SerLysGlnIle) is underlined in the sequence of the bovine ADP/ATP T1 carrier (bADP/ATP T1) of the single subunit structure bAAC/CAT. The C-terminal end of helix H1 and the short helix h12 of the connecting loop 12 are shown in red. Other predicted helical elements are also shown in red. Predictions are based on mGenThreader program (http://bioinf.cs.ucl.ac.uk/psipred/psiform.html) results.

also help unravel the molecular mechanism of transport since some regional protein sequences are present in only a select group of transport proteins. There are no regional protein sequences that have a dominating similarity among all members of this transport protein family. Regional sequence similarities, as discussed earlier, are highest near the matrix side of the mitochondrial membrane. This suggests that this is a protein region, as suggested from the narrow part of a funnel-like structure of the single subunit bAAC/CAT complex, important for the mechanics of transport rather than part of the substrate specific translocation path. Support for this comes also from the locations of conservative replacement mutations that strongly inhibit transport catalyzed by the PTP (Fig. 2). Regional sequence similarities are also highest in the center of H1P which is a subunit/subunit contact site for the yeast PTP homodimer [27]. A bovine ADP/ATP T1 subunit/subunit contact site has been established between Cys residues of the short h12 helices (residues marked with italics in h12p of Fig. 2) [29]. Sequence similarity is not so high for the short helix h12 regions of all members of this family, yet this could be an example where a h12 residues subunit/subunit contact site is more likely for a subset of transport proteins with high sequence similarity in this region, e.g. proteins of subfamilies 5, 7, 8, 9, and 19 (Table 3).

Table 1 shows for the first time the members of the human mitochondrial transport protein family. While most of these proteins have the traditional mass of around 30 kDa per subunit and some have N-terminal extensions with calcium binding sites, a few are much smaller. These proteins are of interest since it is not clear how they assemble in the membrane to catalyze transporter activity. At least one of them (NP_112489.2 with 177 residues, i.e., the C-terminal half of AAH76399.1, and of subfamily 19) has been shown to be expressed in mitochondria [22]. NP_061049.2 also of subfamily 19 consists of 155 residues, 148 of them are the N-terminal residues of NP_057696.1, a protein similar to MRS3/4 (AAH76399.1). It remains to be shown what the catalytic functions of these C-terminal and N-terminal half proteins are.

Only 21 of the human mitochondrial transport proteins have been functionally identified (Table 1). Future experiments should answer questions like: how do proteins with similar sequences catalyze the transport of such a variety of substrates? And why do members of this transport protein family catalyze the transport of these particular substrates?

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