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Review

New mitochondrial carriers: an overview

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Abstract. The transport of metabolites, nucleotides and cofactors across the mitochondrial inner membrane is performed by members of mitochondrial carrier family (MCF). These proteins share marked structural features that have made feasible the functional characterization of numerous MCs in the last years. The MCs responsible for transport activities in mitochondria known for decades such as glutamate uptake or ATP-Mg/Pi exchange have recently been identified as well as novel carriers such as those involved in S-adenosylmethionine or thiamine pyrophosphate uptake. Here, after a brief review of the novel data on structural characteristics and import mechanisms of MCF members, we present an exhaustive compilation of human MC sequences, including previously characterized carriers, together with their respective *Saccharomyces cerevisiae* orthologues, ordered according to the phylogenetic analysis of el Moualij and co-workers [Yeast (1997) 13: 573–581]. We have detected the existence of at least 49 human MC sequences, including those of yet unknown function. An overview of novel MCF members functionally characterized in recent years in mammals and in yeast genomes is presented.

Key words. Mitochondrial transport; import process; phylogenetic analysis; gene duplication; mitochondrial calcium; mitochondrial metabolism.

Introduction

Mitochondria perform a variety of energy-generating processes such as oxidative phosphorylation, the citric acid cycle and, in higher eucaryotes, β-oxidation of fatty acids. Parts of urea cycle, the biosynthesis of heme and certain amino acids are also carried out in mitochondria. These metabolic activities require the rapid and highly specific exchange of molecules between the cytosol and the mitochondrial matrix space. In eukaryotic cells, members of the mitochondrial carrier family (MCF) facilitate many of the transport steps through the inner mitochondrial membrane, connecting cytoplasmic and matrix functions (reviewed in [1–3]).

The analysis of first known amino acid sequences from mitochondrial carriers (MCS), those of bovine ATP/ADP carrier [4] and uncoupling protein (UCP) [5], highlighted the conserved features of this protein family. The mitochondrial carriers have a tripartite structure made up by three homologue repeats of about 100 amino acids in length, probably generated by ancient duplication events [4]. Each repeat is formed by two transmembrane α-helical domains connected by a long hydrophilic loop which contains the characteristic mitochondrial signature Px(D/E)xh(K/R)x(R/K)$x_{20–30}$(D/E)Gx_4a(K/R)GRG (h is hydro-

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phobic and a is aromatic) [6]. Recent structural work has shown that adenine nucleotide translocase and, possibly, all mitochondrial carriers form a helical bundle with threefold symmetry that could function as monomers in the membrane [3, 7]. MCs are not exclusive of mitochondria since members of the MCF have been found in peroxisomes [8–10], and hydrogenosomes [11].

In recent years, this common structure has facilitated the identification of mitochondrial carrier sequences from *Saccharomyces cerevisiae, Arabidopsis thaliana* or human genome databases [2, 12–17]. The wide range of available genomic tools has made it possible to overcome the difficulty that the relatively low abundance of mitochondrial carriers represented for their purification and functional characterization. In this way, the initial analysis of the S. cerevisiae genome revealed that yeast MCF is composed of 35 members, suggesting the existence of at least 27 functionally distinct carriers [12]. Sequence availability has facilitated the discovery of transported substrates after carrying out controlled transport assays with carriers overexpressed in Escherichia coli or yeast and reconstituted in proteoliposomes [17]. This strategy allowed the initial identification of the mitochondrial carriers for dicarboxylates, succinate/fumarate, oxaloacetate/sulfate or oxodicarboxylates [18–21] and, more recently, the mitochondrial transporters for aspartate/glutamate, glutamate, GDP/GTP, S-adenosylmethionine, thiamine pyrophosphate or ATP-Mg/Pi [22–29] and the peroxisomal adenine nucleotide transporter [8–10]. Other approaches, mainly using yeast strains deficient for MC genes, have made it possible to infer the proteins involved in mitochondrial uptake of flavin nucleotides and coenzyme (CoA) [30, 31] or in iron accumulation [32].

The aim of this review is to summarize recent developments related to MCs with an emphasis on the new carriers functionally identified in the last years. A more global description of metabolic functions and physiological implications of yeast and human MCs has been already discussed in recent reviews [2, 3]. Likewise, new aspects of MC structure and import to mitochondria have been introduced [7, 33, 34]. In order to get a better understanding of the relationship between members of MCF, a compilation of previously characterized human carriers together with their respective *S. cerevisiae* orthologues has been elaborated, and the putative human MCs that remain still unclassified have been included.

Structure and import of MCs

The structure of ADP/ATP carrier (AAC or ANC for adenine nucleotide carrier), the most studied MC, was the first to be unravelled at high resolution. It was recently explored by two-dimensional electron microscopy [33] and X-ray crystallography [7]. In both studies, to overcome the difficulty that conformational changes involve in crystallization, stabilization of one conformation by co-crystallizing with a specific inhibitor, atractyloside (ATR) or carboxyatractyloside (CART), was performed [7, 33]. ATR and CART are known to block nucleotide translocation from the intermembrane space, and therefore the structure in the presence of these inhibitors could represent the conformation of AAC that is ready to bind ADP from the intermembrane space [7].

The AAC structure solved in both surveys is consistent with a bundle of threefold symmetry with six transmembrane α-helices tightly closed toward the matrix and a cavity opened toward the intermembrane space [7, 33, 34]. The cavity extends deeply into the protein and ends in a narrow pit only 10 Å from the matrix surface, toward which the carrier is closed. The three loops connecting the transmembrane domains on the matrix side are organized as small amphipathic helices parallel to the membrane surface [7, 34]. The analysis of residues located in the cavity hints at the mechanism of binding of adenine nucleotides. First, tyrosines and positively charged residues present at the cavity can attract and orient the nucleotide toward the bottom of the cavity, where a ring formed by highly conserved residues among AACs could function as a selectivity filter for the transported molecules [7, 34]. Then, the nucleotide faces the acidic and basic residues from the Px(D/E)xx(K/R) motif that seal the cavity due to the proline residues of MCF motifs which are responsible for inducing sharp kinks in the odd-numbered α-helices [34]. The residues of the MCF signature interact electrostatically and participate in holding together the odd-numbered α-helices in the bundle [7]. Nucleotide binding could compete with the electrostatic interactions rearranging the interaction network, thus favouring the opening of the central pore [3]. The existence of charge-pair networks involving mitochondrial carrier signature has been already predicted from analysis of second-site revertants in yeast [13]. Because the Px(D/E)xx(K/R) sequence is characteristic of all mitochondrial carriers, the opening mechanism, triggered by the binding of transported metabolites, could be extended to other transporters.

Despite the fact that most biochemical and biophysical data support the idea that MCs function as homodimers [35–36], the mentioned structural analysis indicates that AAC could function as a monomer [3, 7, 33, 34]. A projection structure obtained from two-dimensional crystals of yeast AAC3 showed them arranged in symmetrical homodimers, with the translocation pore for adenine nucleotides lying in the center of each monomer [3, 33]. The central cavity could in principle be large enough to bind two adenine nucleotides, depending on the orientation of the substrates [3, 33], opening the possibility that

ADP/ATP exchange could be carried out by a single carrier molecule.

A variation of the typical MC structure is exhibited by a subset of MCs which show bipartite structure, with a long hydrophilic amino extension harbouring EF-hand calcium-binding domains (calcium-binding mitochondrial carriers, CaMCs) [37]. Two kinds of carriers capable of binding calcium have been identified, with unrelated N-termini sequences of about 200 amino acids (short CaMC, SCaMC) (fig. 1A) or about 350 amino acids (aralar1 and citrin, the aspartate glutamate carriers) [28, 29, 37–39]. The effect of the N-terminal extension on the global structure is actually unknown, but a reasonable proposal is that it might control the accessibility of metabolites to the central cavity of the carriers. As the EF-domains face the intermembrane space, transport activity can be regulated by cytosolic calcium (fig. 1A) [22, 40]. Interestingly, these N-extensions appear to be important in selecting the mechanism for translocation across the mitochondrial intermembrane space of the CaMC proteins (fig. 1B) [41, 42].

The mechanisms of import of MCF precursor proteins are now starting to be understood and have been discussed in detail in recent reviews [43, 44]. The outer mitochondrial membrane contains a single translocase of the outer membrane (TOM) for the passage of polypeptides. Imported proteins pass through the TOM complex to the intermembrane space, where the precursor can take one of different routes. Insertion into the inner membrane of members of the mitochondrial carrier family and components of inner membrane translocons as Tim17p, Tim22p and Tim23p proteins is mediated by the TIM22 (translocase of the inner membrane) complex or carrier translocase (fig. 1B) [43, 44]. Precursors targeted to the matrix typically contain an N-terminal targeting signal that is decoded by a functionally distinct complex in the inner membrane, the TIM23 complex [43, 44]. Precursors that utilize the TIM22 translocon have targeting information within the mature polypeptide and usually lack an N-terminal targeting sequence [43, 44]. However, some MCs have N-terminal cleavable sequences, particularly in plants; in fact, one-third of *Arabidopsis* carriers contain N-terminal cleavable sequences [15], which are not essential for import [45]. Cleavable pre-sequences also appear in mammalian phosphate and citrate carriers [46] and in hydrogenosomal adenine translocator Hmp31 [11].

The TIM22 pathway contains components at the inner membrane as well as in the intermembrane space, the small Tim proteins. Yeast contain five small Tim proteins (Tim8p, Tim9p, Tim10p, Tim12p and Tim13p) [47]. These small Tims act as chaperone-like molecules to guide hydrophobic precursors across the aqueous intermembrane space, whereas the inner membrane complex mediates insertion into the inner membrane [48, 49]. The inner membrane substrates are guided toward the

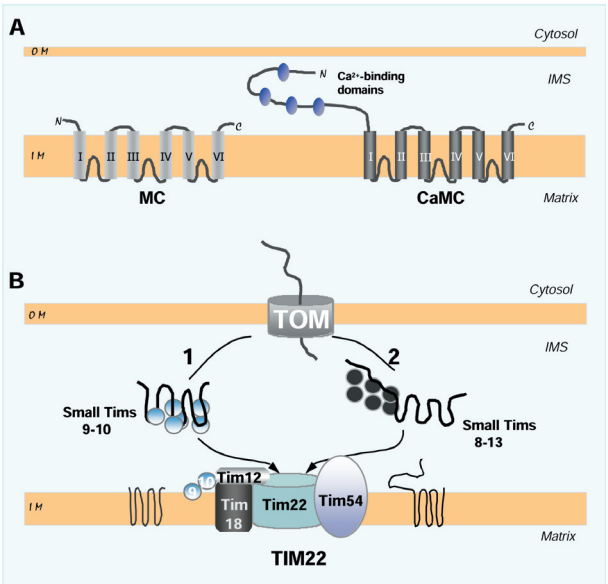

Figure 1. (A) Topology models of the two major subsets of MCs. Mitochondrial carriers (MCs) such as ADP/ATP translocase show a typical tripartite structure of six transmembrane α-helices that span the phospholipid bilayer of the mitochondrial inner membrane (IM) with the carboxy- and amino-ends facing the intermembrane space. Each repeat contains two hydrophobic regions connected by long hydrophilic loops orientated on the matrix side. Members of the calcium-binding mitochondrial carriers family (CaMC), such as aspartate/glutamate isoforms, have a large additional N-terminal domain in the intermembrane space (IMS) with calcium-binding domains. Ovals represent EF-hand Ca^{2+}-binding motifs. (B) Two major pathways for MCs transference across the intermembrane space.
In the outer membrane (OM) a single translocase, the TOM system, transports the mitochondrial proteins from the cytosol to the IMS. In the IM, the TIM22 translocon mediates the import of the inner membrane proteins such as those of the MCF and components of inner membrane translocons such as Tim22p and Tim23p proteins. Other proteins residing in the IMS, the small Tim proteins, escort inner membrane precursors across the IMS. The small Tims form different 70-kDa complexes, Tim9p-Tim10p and Tim8p-Tim13p, in the intermembrane space that bind, respectively, to typical hydrophobic carriers (pathway 1) and calcium-binding MCs (pathway 2). Pathways 1 and 2 transport precursors to the 300-kDa TIM22 complex at the IM that contains intermembrane space protein Tim12p, with a fraction of Tim9p and Tim10p and the IM components Tim18p, Tim22p and Tim54p. Tim22p forms the translocation pore, whereas Tim18p and Tim54p perform accessory functions. Tim8p-Tim13p binds to hydrophilic N-extensions, and Tim9p-Tim10p complex interacts with hydrophobic loops of MCs.

300-kDa insertion complex at the inner membrane that contains the essential proteins Tim12p, Tim22p, Tim54p and Tim18p, and a fraction of Tim9p and Tim10p (fig. 1B) [43, 44]. Tim22p is the major component of the complex forming a voltage-gated channel which mediates insertion into the inner membrane in the presence of a membrane potential [44]. The small Tim proteins, with a molecular mass around 10 kDa, assemble into 70-kDa complexes, Tim8p with Tim13p and Tim9p with Tim10p in a 3:3 ratio, which exhibit different substrate

specificities (fig. 1B) [47, 50]. The classical MCF proteins and import components Tim17p and Tim22p bind predominantly to the Tim9p-Tim10p complex [49, 51]. In contrast, the Tim8p-Tim13p complex binds to the hydrophilic amino-terminus of Tim23p protein, a component of the TIM23 translocon [48, 52, 53]. Recently, a set of MCF members were identified as partners of the Tim8p-Tim13p complex [41]. These are the human aspartate/glutamate carriers (AGCs), aralar1 and citrin, and their yeast homologue, Agc1p, whose long hydrophilic N-terminal domains interact with the Tim8p-Tim13p complex [41]. Pathway selection seems to be determined by the presence of the hydrophilic N-terminal extension; thus import of full-length aralar1 decreased by 80% in mitochondria that lack the Tim8p-Tim13p complex [41], and shortening of the aralar1 N-terminal domain results in preferential binding of aralar1 to the Tim9p-Tim10p complex [41]. Mutations in the locus for human Tim8p (*DDP1/TIMM8a locus*) cause Mohr-Tranebjaerg syndrome (MTS/DFN-1, deafness/dystonia syndrome) [50, 52]. In agreement with its role in AGC import, a lymphoblast cell line derived from an MTS patient shows decreased mitochondrial NADH levels due to defects in the malate-aspartate NADH shuttle (MAS) where AGC is the rate-limiting component [41]. The Tim8p-Tim13p complex probably also participates in SCaMC import as suggested by the differences in import efficiency detected for overexpressed full-length or amino-truncated versions of SCaMC-3 protein [42]. Regardless of the selection of import pathway complexes, mitochondrial targeting information for CaMCs is contained in their C-terminal half, as all amino-truncated variants are targeted into mitochondrial membranes [22, 28, 37, 42].

Screening the human genome for new MCF members

Molecular techniques presently available and the rapidly expanding genomic data from many organisms has greatly facilitated the search and characterization of new genes. Over the past few years, many proteins of unknown function with features of the MCF have emerged from sequencing of several genomes. Although MCF members are not very well conserved at the level of sequence identity (15–20%), they share a common topology with the presence of six transmembrane segments and the MCF signature that has opened up the possibility of identifying proteins containing these domains using *in silico* predictions. In the *S. cerevisiae* genome 35 members belonging to MCF have been described [12, 13]. Similar approaches in the *Arabidopsis* genome initially identified 45 putative MCs [15], and 58 in a subsequent analysis [16]. Likewise, a similar approach in the human genome suggests that at least 50 putative MCs (including isoforms) exist, although earlier only 23 were listed including the functionally characterized carriers SLC25A1 to SLC25A22 plus SLC25A27 [2].

Tables 1–3 show an exhaustive compilation of human mitochondrial carriers. A total of 49 human proteins with clear MC features have been detected. In addition to the MCs identified so far [2], recently identified MCs, SLC25A23 to SLC25A31 [28, 29, 54, 55], proteins reported to be MCF members but without assigned transport activities [56–58] and putative MCs detected in public human databases by *in silico* searches have been included. Human MCs have been classified according to previously established phylogenetic relations among *S. cerevisiae* MCs [12, 13]. El Moualij and co-workers used phylogenetic analysis to describe four main subsets of transporters: one including carriers involved in nucleotide transport as AACs and carriers for metabolites structurally related to nucleotides, a second group formed by carriers for phosphate and other cofactors, the third composed of carriers involved in the transport of citrate and structurally related substrates such as glutamate and oxodicarboxylate acids, and a final subdivision covering the carriers for dicarboxylic acids [12]. Only one modification has been made to this classification. Thus, as shown in table 2, the carriers for mitochondrial cofactors and tricarboxylic acids have been pooled in a single group. This is because some human carriers belonging to both subdivisions exhibit remarkable similarity, indicating a phylogenetic proximity greater than that of their yeast homologues. Table 3 includes the UCPs, which have no representatives in *S. cerevisiae* and show remarkable similarity to dicarboxylate carriers. In each table, the listing of MCs is based on their phylogenetic relationships. In addition, human MCs that lack yeast counterparts have been listed next to the closest human sequences indicated by BLAST analysis. Grouping carriers lacking a known function with their closest homologues may provide valuable insights into their possible function.

A comparison of this human subset of 49 MCs with the 34 members of yeast gene family (UGO1, initially described as an MC, has been excluded since it is located in the outer mitochondrial membranes, [59]) reveals 21 orthologous groups of MCs (tables 1–3). For each yeast/human orthologue group, representatives from available genomes have been added to provide additional information about how each functional group has evolved (see tables 1–3). Some predicted functional groups contain more than one highly related yeast or human paralogue, presumably generated by duplication events. The number of paralogues observed agrees with the role of gene duplication as a major force in genome evolution from the occasional wholesale duplication of entire genomes to the regular duplication of individual genes [60]. Duplicated genes are thought to be preserved through alternative mechanisms. They can be neofunctionalized, one copy

Table 1. Mitochondrial carriers related to ADP/ATP carriers

Yeast	Metabolite	Mammalian orthologues and/or related proteins	Remarkable counterparts in other species	References
YMR056c (AAC1)/ YBL030c (AAC2) YBR085w (AAC3)	ATP/ADP exchange	human SLC25A4 (NM_001151) at 4q35 SH; mouse (XM_134169) and rat (NM_053515), 95% identity	*C. elegans* (NP_501440, 70% identity, NP_491927, 66%); *D. melanogaster* CG16944 (NP_727450, 80%) and CG1683 (NP_788898, 73%); *T. nigroviridis* (CAG00577, 88%); *X. laevis* (AAH72091, 90%); *G. gallus* (CAG31047, 93%).	
		human SLC25A5 (NM_001152) at Xq24 SH; mouse (NM_007451) and rat (NM_057102), 98% identity	*C. elegans* (NP_501440, 71% of identity, NP_491927, 67%); *D. melanogaster* CG16944 (NP_727450, 81%) and CG1683 (NP_788898, 74%); *D. rerio* (NP_775345, 93%); *T. nigroviridis* (CAG00577, 93%); *G. gallus* (CAG31426, 93%).	Powell et al. [169]
		human SLC25A6 (92% identical to SLC25A5, NM_001636) at Xp22.32 and Yp11		
		human SLC25A31 (73% identical to SLC25A4, CAI05952) at 4q28.1 SH; mouse (XM_178386) and rat (XM_215549), 88–86% identity	*C. elegans* (NP_501440, 66% identity, NP_491927, 62%); *D. melanogaster* CG16944 (NP_727450, 73%) and CG1683 (NP_788898, 69%).	Dolce et al. [54]
YNL083w	ATP-Mg/Pi exchange*	human SLC25A24 (SCaMC-1, AJ619987) at 1p13.3 SH; mouse (NM_172685) and rat (XM_227597), 93–90% identity	*S. cereviasiae* Ynl083wp (NP_014316, 27%); *C. elegans* (NP_505970, 45%), (NP_510081, 43%) *D. melanogaster* CG32103 (NP_996067, 50%); *D. rerio* (NP_001004606, 66%); *X. tropicalis* (AAH84172, 72%); *G. gallus* (XP_422180, 79%).	Fiermonte et al. [29] del Arco and Satrústegui [28]
		human SLC25A25 (SCaMC-2, AJ619989) at 9q34.11 SH; mouse (NM_146118) and rat (NM_145677), 94–97% identity	*S. cereviasiae* Ynl083wp (NP_014316, 27%); *C. elegans* (NP_505970, 52%), (NP_510081, 48%); *D. melanogaster* CG32103 (NP_996067, 54%); *D. rerio* (NP_998422, 75%); *X. tropicalis* (AAH84177, 91%); *G. gallus* (XP_415513, 94%).	Chen [104]
		human SLC25A23 (SCaMC-3, AJ619988) at 19p13.3 SH; mouse (NM_025877) and rat (XM_217310), 93–93% identity	*S. cereviasiae* Ynl083wp (NP_014316, 25%); *C. elegans* (NP_505970, 49%), (NP_510081, 45%) *D. melanogaster* CG32103 (NP_996067, 51%); *D. rerio* (CAI20828, 64%); *X. laevis* (AAH56033, 64%); *G. gallus* (XP_415513, 66%).	
YPR011c	?	human MGC26694 (XM_209204) at 19p13.13 SH; mouse (NM_001007570) and rat (XM_224736), 92–91% identity	*S. cereviasiae* Ypr011cp (NP_015336, 33%, identity); *C. elegans* (CAE67090, 54%); *D. melanogaster* CG4241 (NP_996244, 57%); *T. nigroviridis* (CAG04865, 74%); *X. laevis* (AAH87392, 74%); *G. gallus* (XP_424684, 80%).	
YHR002w (LEU5)	coenzyme A accumulation	human SLC25A16 (NP_689920) at 10q21 (39% identical to SLC25A16) SH; mouse (NM_175194) and rat (XM_342129), 92–91% identity	*S. cereviasiae* Leu5p (NP_011865, 37%); *Anopheles gambiae* (EAA05161, 54%); *T. nigroviridis* (CAF98697, 76%); *G. gallus* (XP_421570, 82%).	Prohl et al. [31]
	?	LOC203427, a human SLC25A16-related protein (30% identity, NM_145305) at Xq24 SH; mouse (XM_110743) and rat (XM_233310), 86% identity	*D. rerio* (CAD68060, 59%); *G. gallus* (XP_420343, 70%).	
YGR096w	thiamine pyrophosphate/ dNDPs*	human SLC25A19 (CAC 27560) at 17q25.3 SH; mouse (AAH18167) and rat (AAH79002), 76% identity	*Giberella zeae* (EAA67856, 37% identity); *C. elegans* (NP_502337, 42%); *D. melanogaster* CG6608 (NP_731527, 37%); *D. rerio* (NP_991278, 62%); *G. gallus* (XP_420126, 67%).	Dolce et al. [96] Marobbio et al. [27]
YPR128c	peroxisomal ATP/AMP	Human SLC25A17 (NM_006358) at 22q13.2 SH; mouse (NM_011399) and rat (XM_216993), 95–94% identity	*S. cerevisiae* (YPR128c, 26% identity); *D. discoideum* (AAS38623, 35%); *D. melanogaster* CG32250 (NP_728982, 48%); *T. nigroviridis* (CAG11421, 68%); *X. tropicalis* (AAH74516, 78%); *G. gallus* (XP_424684, 89%).	Palmieri et al. [8] Van Roermund et al. [9] Visser et al. [10]

S. cerevisiae MCs are listed in separate rows according to the relatedness of protein sequences reported by el Moualij et al. [12]. *S. cerevisiae* functional paralogues are grouped. Duplicated carriers arising from whole-genome duplication in *S. cerevisiae* are considered unique carriers. For each *S. cerevisiae* carrier its human counterparts (reciprocal best hits) are indicated. Searches were performed using the BLAST algorithm. When more than one human homologue protein is detected, they are shown together. The available SLC25 names assigned by the Human Genome Organization (HUGO) nomenclature committee for the human MCF are used. For human carriers lacking SLC25 nomenclature, the accession number of predicted proteins and/or accession numbers for public databases are indicated. Mouse and rat orthologues for each human carrier are shown with their respective accession numbers and values of identity at amino acid levels with respect to human orthologues, as a percentage. Homologues from representative fungal genomes and non-mammalian animal groups are also indicated. Their degree of amino acid identity with the corresponding human homologue and accession numbers are also shown, in parentheses. In order to compress the list no plant homologues have been considered. Identity values were calculated by individual alignments by BLASTP. Predicted proteins showing near full-length similarity were chosen for this purpose. Metabolite(s) transported is indicated. * indicates that the transport function has only been shown for the human MC. Question marks are added to indicate those carriers with unknown functions. SH, homologues mapping at chromosomal synthenic positions; dNDPs, deoxynucleotide diphosphates.

Table 2. Mitochondrial carriers for mitochondrial cofactors, citrate and structure-related metabolites.

Yeast	Metabolite	Mammalian orthologues and related proteins	Remarkable counterparts in other species	References
YJR077c (MIR1)	phosphate	human SLC25A3 (NM_002635) at 12q23 SH; mouse (NM_133668) and rat (NM_139100), 94% identity. also pseudogenes are detected in human genome	*C. elegans* (NP_502087, 74% identity; NP_494870, 70%; NP_506148, 66%); *D. melanogaster* CG9090 (NP_611468, 75%), CG4994 (NP_729978, 74%); *T. nigroviridis* (CAF96756, 90%) *X. laevis* (NP_598429, 89%)	Phelps et al. [69]
YER053c	phosphate	not found	Yer053cp-counterparts only appear in hemiascomycetes fungi. *S. cerevisiae* Yjr077c (MIR, NP_012611, 40% identical).	Hamel et al. [72]
YJL133w (MRS3)/ YKR052c (MRS4)	iron accumulation**	human SLC25A28 (BC058937) at 10q23-q24 SH; mouse (NM_145156) and rat (XM_215249), 97% identity	*C. elegans* (NP_496447, 51% identity); *D. melanogaster* CG4963 (NP_651600, 52%); *T. nigroviridis* (CAF98715, 78%) *G. gallus* (XP_421702, 89%)	Wiesenberger et al. [140]
	?	human SLC25A28 isoform (NM_016612, 65% identity) at 8p21.2 SH; mouse (NM_026331) and rat (XM_224361), 91-93% identity	*C. elegans* (NP_496447, 52% identity); *D. melanogaster* CG4963 (NP_651600, 52%); *T. nigroviridis* (CAG01611, 71%) *G. gallus* (XP_417682, 77%)	Muhlenhoff et al. [142] Foury and Roganti [32] Li et al. [147]
YNL003c (PET8)	S-adenosyl-methionine	human SLC25A26 (AJ580932) at 3p14.1 SH; mouse (NM_026255) and rat (XM_342726), 88% identity	*C. elegans* (NP_501552, 48% identity); *D. melanogaster* CG4743 (NP_651415, 51%); *G. gallus* (XP_414419, 83%)	Marobbio et al. [25] Agrimi et al. [26]
YMR166c	?	not found	Ymr166cp-counterparts appear in Ascomycota fungi; *Kluyveromyces lactis* (XP_453501, 64% identity); *N. crassa* (EAA32670, 47%)	
YGR257c	Mn**	human CGI-69 (NM_016016) at 17q12 SH; mouse (NM_026542) and rat (XM_340914), 97% identity	*S. cerevisiae* Ygr257cp (NP_011773, 32%); *C. elegans* (NP_497836, 42% identity); *D. melanogaster* CG2616 (NP_6499731, 43%) and CG33075 (NP_788936, 40%); *T. nigroviridis* (CAG02222, 58%); *X. laevis* (AAH73249, 62%)	Yu et al. [150] Luk et al. [149]
	?	human CGI-69 isoform (51% identity, NP_061331) at 7q21.12 SH; mouse (NM_178766) and rat (XM_216078), 83-82% identity	*S. cerevisiae* Ygr257cp (NP_011773 , 34%); *C. elegans* (NP_497836, 48% identity); *D. melanogaster* CG2616 (NP_6499731, 42%) and CG33075 (NP_788936, 45%); *T. nigroviridis* (CAG11901, 63%); *X. laevis* (AAH76052, 63%)	
YDL119c	?	human FLJ20551 (NM_017875) at 3p21.31 SH; mouse (NM_144793) and rat (XM_217295), 85–83% identity	*S. cerevisiae* Ydl119cp (NP_010164, 31% identity); *A. nidulans* (XP_407800, 36%); *Apis mellifera* (XP_395257, 51%); *T. nigroviridis* (CAG11901, 63%); *X. laevis* (AAH77266, 68%)	
YDL198c (YHM1)	GTP/GDP	not found	Ydl198cp orthologues are found exclusively in Ascomycota fungi; *K. lactis* (XP_455686, 85% identity); *N. crassa* (EAA26888, 73%); *C. glabrata* (NP_448135, 88%)	Vozza et al. [24]
YJR095w (SFC1)	succinate/ fumarate	not found	Yjr095wp orthologues are found only in Ascomycota fungi and plants; *K. lactis* (XP_455225, 78% identity); *N. crassa* (EAA33522, 59%); *S. cerevisiae* Ybr291cp (NP_009850, 32%)	Palmieri et al. [19]

Yeast	Metabolite	Mammalian orthologues and related proteins	Remarkable counterparts in other species	References
YBR291c (CTP1)	citrate	human SLC25A1 (NM_005984) at 22q11.21 SH; mouse (NM_153150) and rat (NM_017307), 95–95% identity	*S. cerevisiae* Ybr291cp (NP_009850, 39% identity); *C. elegans* (NP_499187, 65%); *D. melanogaster* CG31305 (NP_731586, 66%); *T. nigroviridis* (CAG07840, 83%; CAG05322, 85%); *X. laevis* (AAH41303, 87%); *G. gallus* (XP_415059, 87%)	Kaplan et al. [170]
YFR045w	?	not found	Yfr045wp orthologues are found only in hemiascomycete fungi and plants; *Candida glabrata* (XP_445263, 40%); *K. lactis* (XP_451042, 40%); *S. cerevisiae* Ybr291cp (NP_009850, 31%)	
YMR241w (YHM2)	?	not found	Ymr241wp orthologues are found exclusively in Ascomycota fungi genomes; *K. lactis* (XP_455468, 82% identity); *N. crassa* (EAA27659, 71%); *C. glabrata* (NP_448544, 87%)	Cho et al. [66]
YPL134c (ODC1)/ YOR222w (ODC2)	oxo-dicarboxylates	human SLC25A21 (NM_030631) at 11q11.2 SH; mouse (NM_172577) and rat (NM_133614), 81-82% identity	*S. cerevisiae* ODC2 (NP_014865, 37% identity); *C. elegans* (NP_510638, 57%); *D. melanogaster* CG5254 (NP_569856, 57%), CG9582 (NP_60270, 41%); *T. nigroviridis* (CAG13165, 78%); *G. gallus* (XP_421247, 83%)	Fiermonte et al. [21]
YPR021c	aspartate/ glutamate	Human SLC25A12 (Aralar1, NM_003705) at 2q24 SH; mouse (NM_172536) and rat (XP_230015), 96–95% identity	*N. crassa* (XP_326734, 45% identity); *C. elegans* (NP_497274, 53%); *D. melanogaster* CG2139 (NP_733365, 58%); *D. rerio* (NP_997947, 74%); *T. nigroviridis* (CAG01991, 66%); *X. laevis* (AAH72270, 84%; AAH59349, 77%)	del Arco and Satrústegui [39] Kobayashi et al. [84]
	aspartate/ glutamate	human SLC25A13 (citrin, NM_014251) at 7q21.3, 78% identity with Aralar1 SH; mouse (NM_015829) and (XM_342640), 95% identity	*N. crassa* (XP_326734, 44% identity); *C. elegans* (NP_497274, 54%); *D. melanogaster* CG2139 (NP_733365, 58%); *D. rerio* (NP_997947, 80%); *T. nigroviridis* (CAG01991, 66%); *X. laevis* (AAH59349, 87%); *G. gallus* (XP_425987, 92%)	del Arco et al. [37] Cavero et al. [87]
	glutamate	human SLC25A22 (GC1, NP_078974) at 11p15.5 SH; mouse (NM_026647) and rat (XM_347185), 95–94 identity	*N. crassa* (XP_326734, 38% identity); *C. elegans* (CAE63270, 50%); *D. melanogaster* CG18347 (NP_650134, 58%), CG12201 (NP_731657, 53%); *D. rerio* (AAH75893, 69%; NP_998573, 72%); *G. gallus* (XP_427233, 81%)	Fiermonte et al. [23]
	glutamate	human SLC25A18 (GC2, NP_113669) at 22q11.2, 78% identity with GC1 SH; mouse (NM_207112, 87% identity), rat genome lacks with GC2	*N. crassa* (XP_326734, 38% of identity); *C. elegans* (CAE63270, 49%); *D. melanogaster* CG18347 (NP_650134, 53%), CG12201 (NP_731657, 48%); *D. rerio* (AAH75893, 65%; NP_998573, 67%); *G. gallus* (XP_427233, 66%).	
	?	human KIAA0446 (NM_014655; 28% and 29% identity with SLC25A22 and AGC25A12, respectively) at 1q22 SH; mouse (NM_178696) and rat (XM_345231), 96% identity	*N. crassa* (XP_326734, 28 of identity) *D. melanogaster* CG5805 (NP_651284, 39%); *D. rerio* (AAH85527, 72%); *X. laevis* (AAH76803, 87%); *G. gallus* (ESTs BX270822, CN220586, 86%)	
YOR130c (ORT1)	ornithine	human SLC25A15 (NM_014252) at 13q14 SH; mouse (NM_011017) and rat (XM_224969), 95% identity	*S. cerevisiae* Yor130cp (NP_014773, 29% identity) *C. elegans* (NP_501552, 48%); *D. melanogaster* CG4743 (NP_651415, 51%); *G. gallus* (XP_414419, 83%)	Camacho et al. [81]
	ornithine	Human SLC25A2 (intronless, NM_031947) at 5q21, 87% identity with SLC25A15 SH; mouse (XM_111757) and rat (XM_226032), 80–76% identity	No orthologues are detected from mammalian genomes.	Fiermonte et al. [75] Camacho et al. [74]

Yeast	Metabolite	Mammalian orthologues and related proteins	Remarkable counterparts in other species	References
YOR100c (CRC1)	carnitine/acyl-carnitine	human SLC25A20 (NM_000387) at 3p21.31 SH; mouse (NM_020520) and rat (NM_053965), 92–90% identity	*C. elegans* (NP_501223, 55% identity); *D. melanogaster* CG3057 (NP_995618, 52%), CG3476 (NP_609093, 47%); *T. nigroviridis* (CAG06860, 80%); *X. tropicalis* (NP_989099, 84%); *G. gallus* (XP_414400, 86%)	Huizing et al. [77]
YPR058w(YMC1)/ YBR104w (YMC2)	carnitine/ acyl-carnitine*	human carnitine/acylcarnitine like SLC25A29 (NM_152333) at 14q32.2 SH; mouse (NM_181328) and rat (XM_234550), 88–89%	*S. cerevisiae* Ypr058wp (NP_015383, 34% identity); *C. elegans* (NP_872134, 48%); *D. melanogaster* CG4995 (NP_723565, 47%); *T. nigroviridis* (CAG10455, 74%); *X. laevis* (AAH80378, 71%); *G. gallus* (XP_421366, 73%)	Sekoguchi et al. [55]
	?	three SLC25A29-related proteins about 40% identity with SLC25A29 human LOC153328 (NM_145282) at 5q31.1 SH; mouse (NM_177809) and rat (XM_341488), 84–76% identity	*D. rerio* (NP_001002367, 59%); *G. gallus* (XP_414625, 65%)	
	?	Human HDMCP (NM_207117) at 14q32.2 SH; mouse (XM_127129) and rat (AAT35561), 87–86% identity	*T. nigroviridis* (CAG01825, 46%); *G. gallus* (XP_421367, 59%)	
	?	Human hypothetical LOC283130 (NM_182556) at 11q13.1 SH; mouse (NM_134154) and rat (XM_219521), 85–87% identity	not found	Tan et al. [64]

As in table 1. ** indicates that transport functions are deduced only for yeast counterparts from indirect proof.

Table 3. Mitochondrial carriers related with dicarboxylate carrier.

Yeast	Metabolite	Mammalian Orthologues and related proteins	Remarkable counterparts in other species	References
YEL006w/YIL006w YIL134w (FLX1)	pyruvate* FAD*	human MFT (Mitochondrial Folate Transport, NM_030780) at 8q22.3 SH; mouse (NM_172402) and rat (XM_235359), 90% identity	*S. cerevisiae* Yil006wp (NP_012260, 35%), Yel006wp (NP_010910, 34%), Yil134wp (NP_012132, 31%); *C. elegans* (NP_495746, 53% identity); *D. melanogaster* CG8026 (NP_610468, 53%); *T. nigroviridis* (CAF98075, 74%); *G. gallus* (CAG32058, 77%)	Hildyard and Halestrap [137] Tzagoloff et al. [30] Titus and Moran [133]
YBR192w (RIM2)	?	Human HuBMSC_MCP (MGC4399, AF495714) at 1p36.22, also an expressed-intronless gene at 11p11.1, 99% identity SH; rat (XM_216577) and mouse (NM_027460), 91% identity	*S. cerevisiae* Ybr192wp (NP_009751, 35% identity); *C. elegans* (NP_496236, 48); *A. gambiae* (XP_310002, 53%); *T. nigroviridis* (CAG07516, 74%); *G. gallus* (XP_417600, 89%)	Wang et al. [58]
	?	FJL10618 (NM_018155, 60% identity with HuBMSC_MCP) at 3q23 SH; mouse (NM_138756) and rat (XM_236557), 94–95% of identity	*S. cerevisiae* Ybr192wp (NP_009751, 39% identity); *C. elegans* (NP_496236, 49%); *D. melanogaster* CG18317 (NP_608615, 49%); *D. rerio* (AAH76521, 86%); *T. nigroviridis* (CAG01980, 84%); *G. gallus* (XP_31696, 89%)	
	?	MTCH1 (or PSAP, NM_014341) at 6pter-p24.1, SH; mouse (NM_019880) and rat (XM_215358), 98% identity	*G. gallus* (XP_423245, 78%)	Xu et al. [56]
	?	MTCH2 (48% identity with MTCH1, NM_014342) at 11p11.2 SH; mouse (NM_019758) and rat (XM_215769), 93-91% identity various pseudogenes have also been detected	*D. melanogaster* CG6851 (NP_728482, 38%); *T. nigroviridis* (CAF96469, 68%); *X. laevis* (AAH54302; *G. gallus* (NP_990139, 79%)	Yerushalmi et al. [57]

Yeast	Metabolite	Mammalian Orthologues and related proteins	Remarkable counterparts in other species	References
YLR348c (DIC1)	dicarboxylates	human SLC25A10 (NM_012140) at 11q25.3 SH; mouse (NM_013770) and rat (NM_133418), 96% identity	*S. cerevisiae* Ylr348cp (NP_013452, 40% of identity); *N. crassa* (CAB91429); *D. melanogaster* CG8790 (NP_713793, 58%) and CG4323 (NP_732513, 51); *C. elegans* (NP_509133, 59%); *T. nigroviridis* (CAG03725, 80%); *X. laevis* (AAH70665, 80%)	Major et al. [171]
	oxoglutarate/ malate	human SLC25A11 (NM_003562) at 17p13.3 SH; mouse (NM_024211) and rat (NM_022398), 96% identity	*S. cerevisiae* Ylr348cp (NP_013452, 38% identity); *A. nidulans* (EAA65082); *D. melanogaster* CG1907 (NP_651703, 59%); CG18418 (NP_647924, 54%), CG7514 (NP_647924, 54%); *C. elegans* (NP_493694, 72%); *D. rerio* (AAH71521, 88%); *T. nigroviridis* (CAF90256, 84%)	
YKL120w (OAC1)	oxaloacetate**	human FLJ40217 (NM_201520) at 17p13.1 SH; mouse (NM_028048) and rat (XM_220594), 90% identity	*S. cerevisiae* Ykl120wp (NP_012802, 35%); *D. melanogaster* CG8323 (NP_610933, 46%), CG18327 (NP_610934, 47%), CG18324 (NP_725361, 48%); *T. nigroviridis* (CAG02891, 63%)	Palmieri et al. [20]
	?	human LOC284723 (50% identity with FLJ40217, NM_207348) at 1p36.13. SH; mouse (XM_357402) and rat (XM_233610), 88–89% identity	*S. cerevisiae* Ykl120wp (NP_012802, 35%); *D. melanogaster* (NP_610933, 45%; NP_610934, 43%; NP_725361, 43%); *T. nigroviridis* (CAG06857, 64%)	
	protons	human UCP1/SLC25A7 (NM_021833) at 4q28-q31 SH; mouse (NM_009463) and rat (NM_012682), 79% identity	*Cyprinus carpio* (AAS10175, 60%)	Aquila et al. [5]
	protons	human UCP2/SLC25A8 (NM_003355) at 11q13 SH; mouse (NM_011671) and rat (NM_019354), 96–94% identity	*C. elegans* (NP_493694, 33%, NP_505414, 29%); *D. melanogaster* CG6492 (NP_573246, 36%), CG7314 (NP_729738, 33%); *D. rerio* (NP_571251, 80%); *X. laevis* (AAH44682, 82%)	Fleury et al. [172]
	protons	human UCP3/SLC25A9 (NM_003355) at 11q13 SH; mouse (NM_009464) and rat (NM_013167), 85–86% identity	*C. elegans* (NP_505414, 33%, NP_493694, 32%,); *D. melanogaster* CG6492 (NP_573246, 33%), CG7314 (NP_729738, 32%); *D. rerio* (NP_955817, 72%); *X. laevis* (AAH44682, 72%, AAH86297, 72%); *G. Gallus* (XP_989438, 73%)	
	?	human UCP4/SLC25A27 (NM_004277) at 6p11.2 SH; mouse (NM_028711) and rat (NM_053500), 96–94% identity	*C. elegans* (NP_505414, 48%); *D. melanogaster* CG6492 (NP_573246, 57%), CG18340 (NP_505414, 48%); *D. rerio* (NP_956635, 69%); *X. laevis* (AAO26203, 74%)	Mao et al. [173]
	?	human SLC25A30 (XP_170736) at 13q14 SH; mouse (NM_026232) and rat (XM_341357), 92–93% identity	*C. elegans* (NP_505414, 36%); *D. melanogaster* CG7314 (NP_729738, 58%); *D. rerio* (NP_956458 78%); *X. laevis* (AAH72926, 88%); *G. Gallus* (XP_417040; 86%)	Haguenauer et al. [174]
	?	human UCP5/SLC25A14 (80% identity with SLC25A30, NM_003951) at Xq24. SH; mouse (NM_011398) and rat (NM_053501), 97–96% identity	*G. Gallus* (XP_420143, 89%)	Sanchis et al. [175]

As in table 1. * indicates that the transport functions are deduced for yeast counterparts from indirect proof and ** that trans *S. cerevisiae* orthologues.

evolving a new function and one retaining the original one, or subfunctionalized, where both gene copies are required to cover the multiple subfunctions once performed by the parental gene [61]. The emergence of novel MCs genes by duplication is a relatively frequent event, and examples of novel MCs which acquire new roles or perform part of the functions of the predicted parental genes are found. Duplication events explain the pairs of human paralogues in tables 1–3 (and references therein), and are also detected in the *S. cerevisiae* genome [62, 63].

Remarkably, a large number of mammalian MCs appear to arise from duplications of pre-existing carriers as functional paralogues in vertebrates. For 9 out of 21 yeast/human orthologue pairs, a second isoform is detected in vertebrate genomes. For other groups that lack representatives in *S. cerevisiae*, such as UCP4, the carriers for glutamate and 2-oxoglutarate/malate, or the homologues of human protein KIAA0446, several counterparts have been found in all animal genomes. In addition, other carriers are detected exclusively in vertebrate genomes, for example, homologues of human protein LOC203427 with similarity to Grave's protein or those for proteins HDMCP and LOC153328 related to carnitine/acylcarnitine carriers [55, 64].

Only three groups of yeast/mammalian orthologues remain functionally uncharacterized, *S. cerevisiae* carriers Ydl119cp, Ypr011c and RIM2 and their corresponding orthologues. Six *S. cerevisiae* carriers have no homologues other than in fungal or plant genomes. Two of them, Yhm2p (Ymr241wp) and Yhm1p (Ydl198cp), were initially involved in mitochondrial DNA (mtDNA) maintenance [65, 66], and for Yhm1p a role in GTP/GDP exchange and iron homeostasis has been proposed [24, 67]. Other *S. cerevisiae* carriers which lack clear animal counterparts are the succinate/fumarate (Yjr095wp) and phosphate (Yer053cp) carriers, as well as Ymr166cp and Yfr045wp, both of unknown function.

New MCs functionally redundant

Second phosphate carrier in *S. cerevisiae*

The mitochondrial phosphate carrier (PiC, SLC25A3) catalyses the transport of phosphate into the mitochondrial matrix, where Pi is essential for the oxidative phosphorylation of ADP to ATP. In mammals two PiC isoforms (A and B) are generated by alternative splicing from the same transcript [68]. Each isoform has a distinct physiological role as indicated by differences in tissue-specific distribution and the kinetic parameters [68]. In *S. cerevisiae* a single PiC, Mir1p/Yjr077cp, was purified from mitochondria and its gene identified [69]. Later, a closely related MC gene, *YER053c*, was identified in the genome of *S. cerevisiae*. The open reading frame (ORF) YER053c encodes a protein, Pic2p, 40% identical to Mir1p and mammalian PiCs. However, despite its sequence similarity, a tagged form of Pic2p was initially located in vacuoles [70], and the purified protein fails to mediate phosphate uptake activity in a liposome reconstitution experiment [71]. Nevertheless, a recent survey was indicated that Pic2p overexpression can compensate for the mitochondrial defect of a double mutant *Mir1*Δ*Pic2*Δ and restores phosphate transport activity in mitochondrial swelling experiments [72]. Pic2p has also been found to be associated with mitochondrial membranes [72].

Searches in whole-genome sequences of other yeasts, such as *Neurospora crassa* or *Aspergillus nidulans*, or in different metazoan genomes do not reveal any PiC-related sequences in addition to that of the PiC orthologues. The genome of *Yarrowia lipolytica,* a hemiascomycete yeast like *S. cerevisiae,* that is highly redundant with an abundance of duplicated genes [73], codes for two proteins related to Pic2p (accession number XP_501487 and XP_505403). It is possible that these redundant PiCs arose from gene duplication in hemiascomycetes. Recent sequencing and complete genome annotation of *Ashbya gossypii* and *Kluyveromyces waltii,* two hemiascomycetes with small genomes, has provided proof for the occurrence of whole-genome duplication (WGD) in the *S. cerevisiae* lineage after it split from their common ancestor [62, 63]. After genome duplication some genes (about 10%) have been maintained in both locations while others are lost or diversified [62, 63]. The pairs of duplicated MC genes in the *S. cerevisiae* lineage are *YBL030c* (*AAC2*)/*YBR085w* (*AAC3*), *YIL006w*/*YEL006w*, *YJI133w* (*MRS3*)/*YKR052c* (*MRS4*), *YPL134c* (*ODC1*)/*YOR222w* (*ODC2*) and *YPR058w* (*YMC1*)/*YBR104w* (*YMC2*) [62, 63], but not Mir1p or Pic2p**.** These duplicated carriers show about 60% identity with each other, while Mir1p and Pic2p, which seem to be derived from a more ancient individual gene duplication, have lower identity, about 40%. It is thought that second variants or paralogues are maintained because they provide an advantage to the organism under particular conditions. Pic2p expression increases at high temperature, suggesting that Pic2p could play a role under specific stress conditions [72].

Novel carriers for carnitine/acylcarnitine and ornithine/citrulline exchange

Recently, additional mitochondrial carriers for ornithine/citrulline and carnitine/acylcarnitine exchange have been fully characterized [55, 74, 75]. The carnitine-acylcarnitine carrier (CAC, SLC25A20) shuttles long-chain acylcarnitine esters in exchange for free carnitine across the inner mitochondrial membrane [76]. This transport is an essential step in mitochondrial long-chain fatty acid β-oxidation [76]. A second mammalian carnitine-acylcarnitine, named CACL for carnitine-acylcarnitine translocase-like (also SLC25A29), shows only a moderate de-

gree of identity with CAC, 37%, as well as a completely unrelated genomic exon/intron organization. Despite its marked dissimilarities, however, CACL has palmitoylcarnitine transporting activity in mitochondria [55]. The CACL transcript is found in tissues such as heart and liver, where CAC is also expressed at a high level [77], and its expression in liver is induced by partial hepatectomy and fasting [55]. Likewise, as occurs for CAC, heterologous expression of mouse CACL in yeast defective for *CRC1* (CAC) and *CIT2* (glyoxisomal citrate synthase) genes relieves the growth impairment of this double mutant on oleate medium, an impairment caused by its inability to oxidize fatty acids [55, 78]. Despite their similarities, it appears that these CACs are not entirely redundant at a functional level. In humans, CACL expression could not carry out CAC functions in fatty acid metabolism. Thus, patients with a CAC deficiency exhibit cardiac symptoms and abnormal liver functions [77].

The closest CACL-related yeast proteins are the uncharacterized YMC1 and YMC2 (each 35% identical to CACL) and CRC1, the yeast CAC orthologue (30% identity with CACL), suggesting that more carriers involved in carnitine (or derivates) transport could exist in yeast. It is possible that these related carriers arose from a common ancestor as a result of an ancient duplication event in the yeast lineage. In addition, vertebrate genomes code for three proteins highly related to CACL. The corresponding human counterparts, LOC153328, LOC283130 and HDMCP (HCC downregulated MC protein) [64], show about 38–41% identity with human CACL sequences and 40–48% identity among one another. Other vertebrate genomes such as pufferfish (*Tetraodon nigroviridis*) and chicken have LOC153328 and HDMCP counterparts, but not for LOC283130, this protein presenting homologues only in mammalian genomes (table 2). Interestingly, conservation of the protein sequence is slightly lower than that detected for CACL orthologues (table 2), suggesting an accelerated rate of amino acid change in one of the duplicate gene copies following gene duplication [61, 79]. The site of higher divergence among these CACL-related proteins is the hydrophilic loop between TM3 and TM4 domains. HDMCP has an extraordinarily long TM3-TM4 loop similar in length to that of the glutamate carrier [23, 64]. Interestingly, this structural feature appears only in the mammalian HDMCPs, whereas *T. nigroviridis* and chicken counterparts do not have this amino acid stretch. HDMCP, identified as a transcript whose expression is downregulated in hepatocellular carcinomes (HCCs), maps immediately upstream to the CACL gene, and its expression is restricted to liver [64]. Overexpression of HDMCP in cell lines leads to the loss of mitochondrial membrane potential accompanied by reduction of intracellular levels of ATP, but the identity of the metabolite transported remains unknown [64]. In conclusion, the existence of CAC, CACL and additional CACL-related carriers opens up the possibility that new mitochondrial carriers for carnitine, its derivatives or structurally related molecules may exist in vertebrates.

The ornithine/citrulline carrier (ORC), purified initially from rat liver mitochondria [80], catalyses the electroneutral exchange of cytosolic ornithine for matrix citrulline plus a H^+. Its main function is to connect the cytosolic and intramitochondrial reactions of the urea cycle. The human ORC1 gene, SLC25A15, is defective in the hyperornithinemia-hyperammonemia-homocitrullinuria (HHH) syndrome (triple H syndrome, MIM 238970) [81]. The phenotype of HHH patients is generally milder than those associated with defects in any urea cycle enzyme, suggesting that other carriers could compensate for the defective ornithine transporter [81]. This was confirmed following characterization of a second human carrier for ornithine/citrulline exchange (ORC2, SLC25A2) [74, 75]. Unlike the CACL carrier, human ORC2 shows high identity, 87%, with ORC1 protein although it displays an entirely different gene structure, since ORC2 lack intronic sequences [74, 75]. The transport properties of the human isoforms (ORC1 and ORC2) have been analyzed [75]. Both transport L-isomers of ornithine, lysine, arginine and citrulline by exchange or unidirectional mechanisms. ORC2 has a broader specificity than ORC1, and also transports L- and D-histidine, L-homoarginine and D-isomers of ornithine. ORC1 is the isoform predominantly expressed. Moreover, the Km values of ORC2 for ornithine and citrulline are higher than that of ORC1, and hence ORC2 cannot completely replace ORC1 functions in the urea cycle in HHH patients [75]. ORC2 orthologues are found only in mammalian databases (see table 2) and, as judged from the genomic sequences available, have a common genomic structure. They are intronless genes that probably originated by retrotransposition of a spliced messenger RNA (mRNA) derived from the intron-containing paralogous (retrogenes) [60]. As occurs in CACL and CACL-related proteins, mammalian ORC2 orthologues have higher ratios of amino acid substitutions than among the corresponding ORC1 paralogues (table 2) [79].

MCs for glutamate

The existence of two transporters for glutamate, the unidirectional aspartate/glutamate exchanger and the glutamate/hydroxyl carrier, were inferred several decades ago from studies performed in rat liver and brain mitochondria revised in [1]. Their main biochemical properties have been studied in intact mitochondria and in reconstituted proteoliposomes [1, 82, 83], but the identity of responsible proteins has long remained unknown. The AGC catalyses a 1:1 electrogenic exchange of aspartate for glutamate plus a H^+ playing an important role in the malate/aspartate NADH shuttle, urea synthesis

and gluconeogenesis from lactate [1]. On the other hand, mitochondrial glutamate influx through the glutamate carrier is required to provide substrate for glutamate dehydrogenase [1].

The glutamate transporters have been identified at the molecular level. Initially, two related carriers belonging to the recently described subfamily of CaMCs aralar1 (SLC25A12) and citrin (SLC25A13) [37, 39, 84] were found to be human AGC paralogues [22]. Next, the human AGC sequences were used to find candidates for mitochondrial glutamate carriers. This resulted in the identification of two human carriers showing 33% identity with the C-terminal domains of AGCs that were functionally characterized as glutamate carrier (GC) paralogues: GC1 (SLC25A22) and GC2 (SLC25A15) [23].

The transport properties and functional roles of the AGCs aralar1 and citrin have been analysed [22, 37, 39, 40, 84–89]. Their large molecular mass, about 70 kDa, matches that of partially purified AGC preparations from bovine heart mitochondria [37, 39, 82]. The transport properties of both carriers reconstituted into proteoliposomes fully match those of the native AGC regarding substrate specificity, transport affinities, inhibitor sensitivity and voltage dependence [22]. In addition, AGC overexpression in different human cell lines increases the activity of MAS [22, 40, 89]. Transport activity is stimulated by Ca^{2+} on the external side of the inner mitochondrial membrane where the Ca^{2+}-binding domains of these proteins are localized, indicating that Ca^{2+} activation of AGC occurs independent of Ca^{2+} entry into mitochondria by calcium uniporter [22]. The MAS is known to be activated by Ca^{2+} and Ca^{2+}-mobilizing hormones in liver [90, 91] and heart [92], at least at low workloads [93], but the mechanism responsible for the activation was thought to be calcium entry in mitochondria followed by Ca^{2+} activation of α-KGDH. The finding that Ca^{2+} activates the AGCs from the external face of the mitochondria [22] indicates that Ca^{2+}-induced MAS activation requires cytosolic calcium signals but not calcium entry in mitochondria and activation of α-KGDH. In fact, Ca^{2+}-induced MAS activation in neurons (where aralar1 is the only AGC isoform present) [86] is specifically activated by small calcium signals [B. Pardo and J. Satrústegui, unpublished results].

The distribution of human AGC paralogues is isoform specific; thus, while aralar1 is preferably expressed in excitable tissues such as brain, skeletal muscle, heart and insulin-secreting beta cells [85, 86, 89], human citrin is the major AGC isoform in liver [37, 84, 85]. The lack of overlap in AGC isoform expression in many adult tissues explains the phenotypes associated with mutations in AGC genes. Mutations in citrin are responsible for adult-onset type II citrullinemia (CTLN2 [MIM 603471]), characterized by a liver-specific decrease in argininosuccinate synthetase activity that causes hyperammonemia and citrullinemia [84], while the human aralar1 gene has been associated by linkage analysis with autism [94]. Citrin-deficient mice show a marked decrease in asparatate efflux and MAS activity in liver mitochondria but fail to show CTLN2-like symptoms [88].

The second transport activity involved in mitochondrial transport of glutamate is that performed by the GC that provides the substrate for matrix glutamate dehydrogenase [1]. It has also been proposed that GC may operate in the reverse direction to limit the intramitochondrial accumulation of glutamate [1]. Because glutamate is cotransported with a proton by the GC, the transport is influenced by ΔpH, and therefore its entry is favoured in energized mitochondria [1, 23]. Despite their close phylogenetic relationship, GC isoforms are quite different from AGCs. The GC paralogues differ in their kinetic parameters; CG1 has a very high Km value for glutamate, 5.18 mM, whereas the Km value of GC2 is lower, 0.26 mM [23]. Both human GC isoforms are ubiquitous, but GC1 is expressed in higher amounts than GC2 in all tissues [23]. Recently, mutations in GC1/SLC25A22 have been associated with an autosomal recessive form of neonatal myoclonic epilepsy [95]. A missense mutation in SLC25A22 affecting a highly conserved amino acid in GCs cosegregates with the disease. In cultured skin fibroblasts from patients glutamate oxidation appears strongly affected [95]. During human development, SLC25A22 is specifically expressed in the brain within territories proposed to contribute to the genesis and control of myoclonic epilepsy [95].

The *S. cerevisiae* AGC orthologue, Agc1p, has been also reported [87]. It is a protein of 902 amino acids encoded by the ORF YPR021c, with a long amino extension lacking calcium-binding domains and a C-terminal region with high similarity, 54%, with human AGCs [87]. It is noteworthy that no clear yeast GC orthologue has been identified. Thus *S. cerevisiae* Agc1p and orthologues in other fungi are the closest relatives of the mammalian GCs. Interestingly, unlike its human orthologues, yeast Agc1p catalyses both aspartate-glutamate exchange and substrate uniport activities. Agc1p in *S. cerevisiae* functions as a uniporter to supply the mitochondria with glutamate for nitrogen metabolism and ornithine synthesis, and in addition it participates as an AGC in a MAS critical for growth on acetate and fatty acid as carbon sources [87]. It is thus possible that all glutamate transporters arise from a common ancestor that in yeast has evolved into Agc1p, which possesses both exchange and uniport activities. In mammals it could give rise to AGCs (strict aspartate-glutamate exchanger) and the related GCs (ΔpH-dependent glutamate uniporter).

AGC counterparts with similarity throughout the whole protein to human AGCs are detected in ascomycetes fungi not belonging to the Hemiascomycota subdivision, such as *Neurospora crassa* and *Aspergillus nidulans*. These AGCs maintain the bipartite structure, a long amino

domain with calcium-binding domains plus a C-terminal half homologue to MCs, in all the groups studied (table 2). Two isoforms, counterparts for each human AGC isoform, are found in verterbrates. GC homologues are also found in most organisms from nematodes to mammals, with more than one representative in insects, *T. nigroviridis* and *Caenorhabditis elegans* (table 2). However, authentic orthologues for human GC2/SLC25A15 are exclusively detected in mammals (table 2).

New MCs involved in transport of nucleotides or structurally related substrates

One of main clusters of MCs found through phylogenetic analysis is that formed by the adenine nucleotide carriers (ANC) and homologous sequences [12, 13, 15, 96, 97]. Identification of ANC-related sequences in the *C. elegans* genome was followed by searches for human counterparts that led to the finding of a family of at least seven ANC-related proteins (table 1) [96]. Although the functions of some members of the cluster are still unknown, the identity of the metabolites transported by most ANC-related carriers has been determined. In all cases they are nucleotides or structurally related molecules such as coenzyme A or thiamine pyrophosphate [8, 10, 27–29, 31, 96]. Therefore, the ANC-related carriers of yet unknown function, such as yeast Ypr011cp and human protein LOC203427 (table 1), could transport similar substrates.

ATP-Mg/Pi carrier

The ATP-Mg/Pi exchanger stands out among the mitochondrial transport activities involving nucleotides whose molecular identification has remained elusive. This transporter was characterized functionally in intact liver mitochondria in the 1980s [98, 99, see 100 for review], but identification at the molecular level has been achieved only recently [28, 29]. This carrier performs the net transport of adenine nucleotides across the inner mitochondrial membrane because it catalyses a reversible electroneutral counterexchange of ATP-Mg for Pi [101]. The ATP-Mg/Pi carrier, therefore, differs from classical ANCs, which mediate a membrane potential-dependent, strictly equimolar exchange of cytosolic ADP for matrix ATP and therefore cannot account for changes in the matrix adenine nucleotide content [102]. Both adenine nucleotide carriers are further distinguishable by their different inhibitor sensitivities, the ATP-Mg/Pi exchanger being insensitive to classical translocase inhibitors such as CART or bongkrekic acid [98]. Furthermore, ATP-Mg/Pi exchange is activated by extramitochondrial micromolar Ca^{2+} concentrations [103].

The proteins corresponding to the ATP-Mg/Pi carriers comply with the features expected for these carriers; they are ANC-related carriers that bear calcium-binding domains [28, 29, 104]. Three conserved paralogues, about 70–80% identical, of about 500 amino acids have been characterized in humans, SCaMC-1/APC1, SCaMC-2/APC3 and SCaMC-3/APC2, (for ATP-Mg/Pi carriers) [28, 29]. These isoforms have an N-terminal extension bearing four EF-hand calcium-binding motifs with high similarity to calmodulin facing the extramitochondrial space (fig. 1A) [28, 29]. The transport properties and kinetic characteristics of recombinant SCaMC-1/APC1 and SCaMC-3/APC2 proteins reconstituted in proteoliposomes have confirmed that they are indeed distinct isoforms of the ATP-Mg/Pi carrier [29]. An exclusive feature of SCaMC genes is the presence in SCaMC-1 and SCaMC-2 isoforms of alternative first exons which generate a total of six amino-termini variants [28, 29]. All variants are found to be able to be imported into mitochondrial membranes [28, 29, 38], and their amino-terminal extension is unnecessary for import [28, 42]. The large number of isoforms and spliced variants might provide different calcium sensibilities to the transport activity of these carriers.

ATP-Mg/Pi carriers are widely conserved, with orthologues in most eucaryotic organisms [28]. In yeast genomes a single related protein is detected, whereas other species exhibit two (*C. elegans*, or chicken) or three isoforms (*Danio rerio*, *Xenopus* or *A. thaliana*) with high similarity among each other [28]. Except for the *Schizosaccharomyces pombe* orthologue, all proteins display EF-hand motifs at equivalent positions [28]. It is possible that these isoforms arose by a gene fusion process between a primitive adenine nucleotide translocase gene and calmodulin-like sequences. The role of ATP-Mg/Pi carriers as a pathway for the adenine nucleotide transport alternative to classical ANCs has been confirmed in yeast [104]. Ynl083wp/Sal1p, the yeast ATP-Mg/Pi orthologue, is required for growth on glucose of yeast mutants that lack Aac2p [104, 105]. Interestingly, mutations in EF-hands that abolish calcium binding in Sal1p also prevent glucose growth in yeast lacking Aac2p, suggesting that calcium-binding is functionally important [104]. Indeed, a CART-resistant ATP transport in yeast mitochondria absent in mutants lacking Sal1p is calcium sensitive, with an $S_{0.5}$ of about 30 μM [S. Cavero and J. Satrústegui, unpublished observations]. The function of the ATP-Mg/Pi exchanger has been proposed to be essential for modulation of the matrix adenine nucleotide concentration in many physiopathological situations, for example, in the recovery of mitochondrial adenine nucleotide content after hypoxia or ischemia [100]. It is also responsible for uptake and accumulation of adenine nucleotides in rat liver mitochondria immediately after birth [99], a process required for postnatal mitochondrial maturation [106].

A new organelle where new ANCs have been isolated is the hydrogenosome, the hydrogen-producing organelle found in some microbial eucaryotes that lack mitochondria [11, 107, 108]. Hydrogenosomes lack the entire electron transport chain, and ATP is generated by substrate level phosphorylation and exported to the cytosol (reviewed in [109]). Two classes of ANCs according to their sensitivity to classical AAC inhibitors and phylogenetic relations have been identified in hydrogenosomes [11, 107, 108]. Hydrogenosomes of anaerobic ciliates and chytrid fungi posses genuine mitochondrial-type ANCs, whereas those of the parabasalian flagellates *Tricomonas vaginalis* and *Tricomonas gallinae* do not cluster with veritable ADP/ATP carriers [12, 107, 108]. Hydrogenosomal ANCs of anaerobic fungi *Neocallimastix frontalis* and *Neocallimastix patriciarum* have properties similar to mitochrondrial counterparts in primary structure, mode of transport and sensitivity toward inhibitors [107, 108], and phylogenetic analysis reveals their relationship with mitochondrial AAC from aerobic fungi. Specific inhibitors of the mitochondrial AAC, bongkrekic acid and CART, interfere with hydrogen production from malate by *Neocallimastix* hydrogenosomes [107]. On the other hand, the hydrogenosomal ANC of *T. vaginalis,* the protein Hmp31, and its homologue of *T. gallinae,* are clearly different from that of *Neocallimastix* and mitochondrial translocases [11, 97, 107, 108]. *T. gallinae* ANC-like Hmp31 transports ADP and ATP with high efficiency, but exhibits resistance against bongkrekic acid [97]. Phylogenetic analysis also indicates that *Tricomonas* Hmp31 does not belong to *Neocallimastix ANCs* or the ANC family and appears more related to ATP-Mg/Pi carrier [97].

Whether hydrogenosomes have co-evolved vertically with mitochondria from a common proto-mitochondrial endosymbiont ancestor or have been created several times along evolution remains controversial [110, 111]. Hydrogenosome-containing eucaryotes do not form a coherent phylogenetic group [109]. Tricomonads belong to the phylum Parabasalia constituted by a group of ancient eucaryotes [112], therefore *Tricomonas* Hmp31, which differs significantly from classical mitochondrial ANC, might represent an ancient atypical ANC. Moreover, its similarity with the ATP-Mg/Pi carrier opens up the possibility that these two carriers share a common origin.

Human deoxynucleotide carrier

The human deoxynucleotide carrier (DNC, SLC25A19), identified initially as a human MC with relevant homology to ANCs [96], has been functionally characterized by transport assays with purified bacterially overexpressed proteins reconstituted into proteoliposomes [96]. The protein transports all deoxynucleoside diphosphates (dNDPs) and with less efficiency deoxynucleoside triphosphates (dNTPs) in exchange for ADP or ATP [96]. Therefore, its functional role could be to supply precursors for mitochondrial DNA synthesis. Likewise, this carrier also provides a potential route for uptake of toxic antiviral nucleosides into the mitochondria since it is able to transport dideoxynucleotides (ddNTPs) [96]. The human SLC25A19 gene has been associated with the Amish microcephaly disoder (MCPHA [MIM 607196]) [113]. One mutation in the SLC25A19 gene affecting an amino acid that is highly conserved in homologous proteins segregates with the disease in affected individuals. Reconstitution assays show that the mutant protein is unable to transport dATP [113]. MCPHA disorder is characterized by severe congenital microcephaly and premature death [114], suggesting that mitochondrial deoxynucleotide uptake may be essential for the prenatal brain growth [113].

Orthologues for the human SLC25A19 carrier are observed in all organisms, from vertebrates to yeast (see table 1). Notably, these carriers show a slightly lower similarity among each other than that found between orthologues from other MCs. Surprisingly, the closest yeast carrier (28% identity), and expected functional counterpart, Ygr096cp, has been involved in mitochondrial thiamine pyrophosphate uptake [27]. The transport properties of the reconstituted protein have been determined following an experimental approach similar to that used for SLC25A19. In agreement with its transport capabilities, cells lacking Ygr096cp have reduced levels of thiamine pyrophosphate (ThPP) in mitochondria, and show decreased activities of mitochondrial ThPP-requiring enzymes such as acetolactate synthase or α-ketoglutarate dehydrogenase [27].

The YGR096c and human SLC25A19 genes are the first pair of mammalian-yeast MC orthologues with apparently diverging functions. However, a recent report questions the role of SLC25A19 in mitochondrial uptake of dNTPs and ddNTPs [115]. Lam and co-workers found high (mM) Km values for dNTPs in uptake assays with the product of human SLC25A19 reconstituted in proteoliposomes, higher than physiological dNTPs concentrations [115]. Furthermore, SLC25A19 overexpression did not increase dTTP uptake in mitochondria and did not sensitize cells to mtDNA depletion caused by dideoxynucleotide analogues [115]. In addition, downregulation of SLC25A19 expression by small interfering RNA (siRNA) was is also unable to change the action of dideoxynucleotide analogues on mtDNA depletion and the rate of dTTP uptake into isolated mitochondria [115]. On the other hand, MCPHA patients show elevated levels of α-ketoglutarate in urine [113, 114]. It has been proposed that these increased α-ketoglutarate levels might be the result of a defect in the ThPP-dependent α-ketoglutarate dehydrogenase complex [114]. Therefore, a possible role of the human carrier coded by SLC25A19 in mitochon-

drial ThPP uptake would also agree with the biochemical features of MCPHA patients. However, whether human SLC25A19 has ThPP-transport ability remains unproven [96].

A different deoxynucleotide transport activity, a mitochondrial deoxycytidine triphosphate (dCTP) transporter has been characterized in human lymphocytic cells [116], but its molecular identity remains unknown. On the other hand, at least another transport system, the equilibrative nucleoside transporter, ENT, a non-MCF member [117], controls mitochondrial deoxynucloetide content and mtDNA synthesis besides the mitochondrial dNTP transporter [118]. Moreover, mutations in the muscle isoform of the adenine nucleotide carrier, ANT1, have been associated with mtDNA deletions and altered levels of dATP inside mitochondria [119]. Finally, there is evidence that dNTPs could arise inside mitochondria [120] and that a mitochondrial ribonucleotide reductase could exist [120]. In sum, the role of nucleoside and deoxynucleotide transport systems in mitochondria remains to be clarified.

Peroxisomal ATP carrier

The peroxisomal membrane has long been considered permeable to small solutes [121]. However, new data indicate that yeast peroxisomes are impermeable to small solutes and cofactors such as NAD(H), NADP(H), acetyl-CoA or even to protons [122, 123], suggesting the existence of specific shuttle systems across the peroxisomal membranes [124]. A peroxisomal protein belonging to MCF, Pmp47 from *Candida Boidinii*, was found to be necessary for medium-chain fatty acid utilization [125, 126]. Later, its *S. cerevisiae* counterpart, Ypr128cp, as well as its human homologue, Pmp34p, were involved in adenine nucleotide transport [8–10]. Disruption of the *YPR128c* gene in yeast results in impaired growth on laurate, a medium-chain fatty acid (MCFA), as a single-carbon source [126], a process that is dependent on intraperoxisomal ATP [124, 127], whereas normal growth was observed with long-chain fatty acids [127]. Ypr128cp has been overexpressed, purified and reconstituted into phospholipid vesicles, and it appears to represent a novel type of adenine nucleotide carrier transporting ATP, ADP and AMP with high efficiency [8]. In addition, Ypr128cp efficiently transports the adenine deoxynucleotides and is not inhibited by CART or bongkrekic acid [8]. Because activation of medium-chain fatty acids yields AMP and no peroxisomal adenylate kinase has been identified, it has been proposed that a major function of Ypr128cp is to exchange AMP for ATP across the peroxisomal membrane [8]. Ypr128cp catalyses unidirectional transport in addition to exchange of substrates. In both modes, nucleotide exchange is H^+-compensated and electroneutral [128]. Therefore, Ypr128cp is involved in the formation of a ΔpH across the peroxisomal membrane [128]. Homologues to Ypr128cp are found in all groups investigated (table 1). Its human orthologue, PMP34 or SLC25A17, has been also characterized [10]. Heterologous SLC25A17 expression in *YPR128c*Δ yeast rescues the defect in MCFA oxidation, and transport assays with reconstituted protein in proteoliposomes also indicate that human Pmp34p transports adenine nucleotides [10]. In humans, the peroxisomal β-oxidation system is required for chain shortening of certain branched-chain fatty acids and very long chain fatty acids (VLCFAs), and therefore it needs ATP in the peroxisomal matrix for their activation [127].

GTP/GDP exchanger

A yeast carrier, encoded by the ORF YDL198c (previously known as Yhm1p) and not strictly belonging to the ADP/ATP translocase cluster [12], has been identified and functionally characterized as a mitochondrial GTP/GDP transporter [24]. YDL198c was initially identified as a multicopy suppressor of the inability of the *abf2* mutant to growth at 37 °C on glycerol [65]. Abf2p is a mitochondrial histone-like protein essential for mtDNA maintenance in cells grown on glucose medium [66], and this finding linked YDL198c function with mtDNA stability [65]. Another yeast MCF member, that encoded by *YMR241w*, has likewise been isolated as a multicopy suppressor of the adf2 mutant and associated with mtDNA maintenance [66].

The identity of the metabolite transported by Ydl198cp was determined in transport assays after reconstitution of the protein in proteoliposomes [24]. Ydl198cp transports GTP and GDP by a counter-exchange mechanism. Unlike electrogenic ATP/ADP translocase, GTP/GDP exchange is compensated by H^+ cotransport so that the net transport is electroneutral and driven by the ΔpH component of the proton motive force [24]. Its physiological role is probably to exchange cytosolic GTP for intramitochondrial GDP; and mitochondrial matrix GTP is required for important processes such as nucleic acid or protein synthesis [24]. Cells lacking this carrier have reduced GTP and increased GDP levels in mitochondria. Furthermore, these mutant cells undergo the complete loss of mitochondrial DNA and hence are unable to grow on nonfermentable carbon sources [24]. In addition, Ydl198cp-deficient yeast exhibit abnormally high iron mitochondrial uptake, causing iron depletion in the cytosol and misregulation of the high-affinity iron uptake systems [67]. It is not clear how defects in mitochondrial GTP levels lead to increased mitochondrial iron accumulation or mtDNA instability.

Ydl198cp does not exhibit significant homology with any other mitochondrial carrier characterized so far in animals or plants. In principle, this result is unexpected because Ydl198cp orthologues found in other Ascomy-

cota fungi exhibit very high levels of identity, ranging from 88% in *Candida glabrata* to 74% in *N. crassa* (table 2). These values are similar to those detected for very conserved MCs such as the fungal ATP/ADP translocase orthologues. An identical phylogenetic distribution and conservation is found for Ymr241wp (Yhm2p), the second mitochondrial carrier related to mtDNA stability for which the transported metabolite(s) remains unidentified (table 2) [66]. Its high degree of conservation in fungi suggests that Ydl198cp performs an essential function in yeast which is unnecessary for other organisms. In vertebrates and many other organisms, the presence of the GTP/GDP carrier may not-strictly be necessary, since most organisms have a mitochondrial GTP-producing succinyl-CoA ligase, whereas this enzyme is linked to ATP production in *S. cerevisiae* [24]. Alternatively, other members of MCF could carry out the transport of GTP or GDP. Thus, isolated heart mitochondria have been reported to transport guanine nucleotides [129]. Two distinct transport mechanisms, sensitive and insensitive to atractyloside, have been identified in heart mitochondria [130]. The first, probably carried out by the ADP/ATP translocase, is totally inhibited at physiological concentrations of ATP, ruling out a role in guanine transport *in vivo*. Atractyloside-insensitive guanine nucleotide uptake may be carried out by the ATPMg/Pi carrier, accounting for the net transport of guanine nucleotides across the inner membrane [130].

Carriers for mitochondrial cofactors and related molecules

S-Adenosylmethionine carrier

Mitochondria from yeast and mammals do not have the machinery necessary for the biosynthesis of S-adenosylmethionine (AdoMet), which is present exclusively in the cytosol. AdoMet needs to be imported in mitochondria, where it serves as the methyl donor for DNA, RNA and protein methylation, for sterol methylation and as an essential cofactor in the last step of biotin biosynthesis in yeast [26 and references therein]. The first evidence suggesting the existence of a carrier-mediated system responsible for AdoMet uptake was obtained in rat liver mitochondria [131]. Then, genes coding for mitochondrial carriers capable of exchanging cytosolic AdoMet for mitochondrial S-adenosylhomocysteine (AdoHcy) were been identified in yeast and humans [25, 26].

An *S. cerevisiae* strain lacking the mitochondrial carrier for AdoMet, Ynl003cp, *YNL003c*Δ, also known as PET8 by its petite ρ^+ phenotype on respiratory substrates [132], exhibits auxotrophy for biotin that is not restored by the biotin precursor dethiobiotin. Recombinant Ynl003cp reconstituted in proteoliposomes catalyses an active AdoMet exchange, but not biotin or dethiobiotin transport [25]. As dethiobiotin is converted to biotin by an intramitochondrial AdoMet-requiring biotin synthetase Bio2p, biotin auxotophy in dethiobiotin-containing medium must be caused by insufficient supply of AdoMet to the mitochondrial matrix [25]. Indeed, biotin auxotophy in dethiobiotin-containing medium is rescued by expressing the cytosolic S-adenosylmethionine synthetase 1 inside mitochondria, in agreement with the transport specificity of Ynl003cp [25]. It catalyses the uniport uptake of AdoMet for mitochondrial methylation reactions, and probably the exchange between cytosolic AdoMet and intramitochondrial AdoHcy generated from AdoMet in methylation reactions.

Orthologues to the yeast mitochondrial S-adenosylmethionine carrier have been identified in other eucaryotes, including humans. The human orthologue, SLC25A26, has been fully characterized [26]. Despite their high degree of conservation, 45% identity at the amino acid level, the biochemical properties of reconstituted human AdoMet carrier differ from those of the yeast orthologue. The human protein catalyses AdoMet-AdoHcy exchange, but almost no AdoMet uniport: it has a higher affinity for AdoMet and stronger sensitivity to inhibitors of the glutamate carrier than the yeast protein [26]. In phylogenetic analysis the human AdoMet carrier is positioned close to the cluster made up of transporters for amino acids like the isoforms of the glutamate and the aspartate/glutamate carriers [26], suggesting that the structural determinants for transport specificity by this carrier are likely derived from methionine.

A mitochondrial carrier slightly related to Ynl003cp and Agc1p, Ymr166cp, which shows 28% identity with both proteins, is present in *S. cerevisae*. It has no homologues in fungal or plant genomes, and in vertebrates, the closest sequences are those of AdoMet carrier.

MC involved in folate uptake

Candidates for mitochondrial folate transporters (MFTs) have been identified through assays of functional complementation [133, 134]. In mammalian cells, folate metabolism is distributed between cytosolic and mitochondrial compartments. Mitochondrial folates represent about 35% of the total cellular pool and are required as cofactors for the mitochondrial glycine cleavage system, the synthesis of glycine from serine and for the synthesis of the formylmethionine initiator of mitochondrial protein synthesis [133–135]. The identification of a candidate transporter for folates has taken advantage of *glyB* cells, a Chinese hamster ovary (CHO) subline, which is auxotrophic for glycine and does not accumulate folate cofactors in mitochondria [136]. A first approach, by means of retroviral transduction with human complementary DNAs (cDNA) into *glyB* cells, has allowed, isolation of a transduced cell line that is no longer auxotrophic

for glycine [133]. A human cDNA encoding a 35-kDa protein homologous to MC complements the auxotrophy of *glyB* cells and reinstates folate transport into the mitochondria [133]. Subsequent analysis has confirmed the mitochondrial location of human MFT (hMFT), and that endogenous *glyB* folate transporter harbours an inactivating mutation that changes a conservative glycine residue, located at α-helice TM4, for glutamate [134].

Homologous proteins showing high degree of identity with hamster MFT are found in nearly all eucaryotic groups (table 3). In *S. cerevisiae,* however, a mitochondrial carrier for folates remains unidentified. The closest related transporters in yeast are those encoded by duplicated ORFs Yel006wp and Yil006wp, which show, respectively, 34 and 35% identity at the amino acid level with hMFT. But present evidence indicates that it is unlikely that these proteins are folate carriers. Introduction of both genes into *glyB* cells does not sustain growth in glycine-lacking medium [133], and additionally, Yil006wp has been involved in mitochondrial pyruvate transport [137]. Human MFT is also closely related, 31% identity, with the yeast flx1p, the carrier involved in flavin nucleotide transport into mitochondria [30]. *Flx1* mutants have a lower concentration of mitochondrial FAD and diminished FAD flux across mitochondrial membrane vesicles than wild-type strains [30]. Likewise, flx1p deletion results in the specific decrease of activities of the mitochondrial FAD-binding enzymes lipoamide and succinate dehydrogenase [137]. Thus, Flx1p is also unlikely to be a yeast counterpart of mammalian MFT. However, in spite of this, BLAST searches using *S. cerevisiae* Yel006wp/Yil006wp and flx1p or their *A. nidulans* orthologues as a query reveal that no carriers other than MFT-related proteins are found in *C. elegans*, *Drosophila melanogaster* and vertebrate genomes. Thus, whether Yel006wp/Yil006wp or flx1p could be functional orthologue(s) of MFT in *S. cerevisiae* is still an open question.

MCs involved in mitochondrial accumulation of inorganic compounds

Some members of MCF have been involved in mitochondrial metal ions homeostasis participating either directly or indirectly in mitochondrial import of sulfur, iron and manganese. A structurally unrelated protein, Mrs2p, belonging to the bacterial CORA family of magnesium transporters, has been involved in mitochondrial magnesium homeostasis [139].

Although mitochondria contain a wealth of iron-containing proteins, the existence of a specific carrier for iron has remained elusive. Recently, two yeast carriers, Mrs3p and Mrs4p, originally isolated as multicopy suppressors of intron II splicing defects [140, 141], were shown to be involved in iron transport [32, 142]. *MRS4* is co-induced with several iron-uptake genes in genome-wide transcription profiles [143]. In addition, two important synthetic pathways requiring mitochondrial iron import, heme synthesis and iron-sulfur cluster synthesis appear to be influenced by cellular levels of *MRS4* and closely related *MRS3* [142]. Deletions of these genes also affect vacuolar iron transport [144]. The double deletion strain Δ*MRS3*Δ*MRS4* has a growth defect in low-iron media and decreased heme and iron-sulfur cluster synthesis. In contrast, mitochondrial heme formation and *de novo* Fe-S cluster synthesis is significantly increased upon overproduction of these carriers [142]. Additional evidence has been obtained from a yeast strain lacking Yfh1p, the yeast frataxin orthologue [32]. Loss of Yfh1p leads to defects in iron-sulfur cluster synthesis and excessive mitochondrial iron accumulation [145]. ΔYfh1 cells are protected from mitochondrial iron toxicity when MRS3 and MRS4 are deleted [32]. Conversely, *MRS4* overexpression in a ΔYfh1 strain elicits an increased iron content and loss of mtDNA [32]. However, iron uptake is not completely abrogated by the double *MRS3*/*MRS4* deletion, leading to the suggestion that both encode mitochondrial iron transporters whose function becomes apparent only under low iron conditions, and that these carriers do not represent the sole system for mitochondrial iron acquisition [142]. An important unresolved aspect is the form in which iron is imported, free or chelated. In this respect, the only conclusive evidence is that iron must be reduced prior to being incorporated into the mitochondrial matrix to be a substrate for ferrochelatase [146].

In humans, there are two Mrs3p/Mrs4p orthologues with 65% identity among each other, hMRS3/4 (SLC25A28) [147] and human *Mscp* [148], which are about 40% identical to the yeast counterparts. Human SLC25A28 has been shown to be the functional orthologue since it can rescue the thermosensitive growth defect observed in the double Δ*MRS3*Δ*MRS4* strain [147]. Mouse *Mscp* is highly expressed in the spleen, although its expression decreases during splenic lymphocyte maturation [148]. Both human isoforms display spliced variants lacking TM1-TM3 domains which do not lose the capacity to be imported into mitochondrial structures [147]. All vertebrate genomes studied have conserved counterparts for the two human genes (see table 2), but nematodes, insects and ascomycetes fungal genomes have a single one.

Although the transporter(s) responsible for mitochondrial manganese uptake is still unknown, a member of MC appears to be involved in the activation of mitochondrial manganese-containing superoxide dismutase 2 (SOD2) in yeast [149]. A genetic approach designed to search out new factors participating in the posttranslational activation of SOD2 identified the mitochondrial carrier Ygr257cp (designated *MTM1* for manganese trafficking factor for mitochondrial SOD2). Inactivation of yeast *MTM1* leads to the loss of SOD2 activity, but

mitochondrial levels of manganese are normal [149]. In these cells, SOD2 activity is restored only when treated with high supplements of manganese, but not with other heavy metals. This suggest that Mtm1p could function by facilitating the insertion of the essential manganese cofactor, but does not exclude that Mtm1p might transport another metabolites required for SOD2 assembly [149]. Among human MCs, two putative orthologues of *S. cerevisiae MTM1* have been detected, the previously reported CGI-69 (32% identity) [150] and the expressed protein NP_061331, which has 51% identity at the amino acid level with CGI-69 (table 2). Although, its function is yet unknown, human CGI-69 is widely expressed, showing highest levels in testis. Its expression in brown adipose tissue is upregulated by cold [150]. Vertebrates have counterparts for the two human carriers, and both are equally conserved (table 2).

The role of dicarboxylate carrier (DIC) in sulfur ion transport was known long ago [151, 152]. DIC catalyses the exchange of certain mitochondrial dicarboxylates (e.g. malate and succinate) with inorganic phosphate or cytosolic inorganic sulfur-containing compounds. It facilitates the entry of thiosulfate, sulfite and sulfate into mitochondria, taking part in the mitochondrial metabolism of sulfur-compounds (reviewed in [153]). In *S. cerevisiae* uptake of sulfate and thiosulfate by mitochondria can be also performed by means the oxaloacetate/sulfate carrier (OAC) encoded by *YKL120w* (table 3) [20]. It transports oxaloacetate, sulfate, thiosulfate and malonate into the yeast mitochondrial matrix along with protons or in exchange for hydroxyl ions [20]. One of its main functions is to carry oxaloacetate produced by yeast cytoplasmic pyruvate carboxylase into the mitochondrial matrix for replenishing the intermediates of the Krebs cycle [20]. Mammals do not require oxalacetate uptake in mitochondria because pyruvate carboxylase is localized in the mitochondrial matrix [20]. However, BLAST searches in human and mouse genomic databases reveal the existence of proteins highly related to OAC, with two proteins 35% identical to yeast OAC sequences, FLJ40217 and LOC284723, in humans (table 3). Expression data obtained from expressed sequence tag (EST) analysis indicate that both genes are ubiquitously expressed. As expected for a functionally relevant MC, homologous proteins are detected in all eucaryotic genomes analysed (see table 3). In *D. melanogaster*, three OAC-related genes are found clustered together in a tandem array. Although their characterization is still pending, the high similarity between FLJ40217 and LOC284723 and yeast OAC strongly suggests that these proteins are functional orthologues. However, since under normal physiological conditions animal mitochondria do not transport oxaloacetate [14], these animal counterparts could preferentially transport sulfur-compounds or oxaloacetate-related metabolites.

Uncharacterized MCs

In spite of the enormous advances of the last years, a number of MCF members remain uncharacterized. On the one hand, there are MCF proteins fully characterized at the molecular level but whose transport specificities are not yet known. A notorious subset of MCs identified at the molecular level lacking a known function is that comprising proteins related to the mammalian folate carrier. On the other hand, mitochondrial carriers for relevant metabolites such as pyruvate, glutamine, choline and even NAD^+, whose transport activities have been extensively investigated in isolated intact mitochondria, await identification.

Two pairs of MCs showing a relationship to mammalian MFT, but of unknown substrate specificity, have been reported. Each is composed of two highly related proteins that probably represent functional paralogues. One is that formed by MTCH2 (also designated as Mimp) [57] and MTCH1 [56], which show about 24% identity with hMFT and lack counterparts in the yeast genome (table 3). MTCH2 was initially detected by differential display as an upregulated gene in NIH-3T3 cells transfected with growth factors [57]. Its overexpression in intact cells decreases the mitochondrial membrane potential in a dose-dependent manner [57]. Its isoform MTCH1 or PSAP (presenilin-associated protein) interacts with the C-terminus of presenilin-1, and its overexpression causes apoptotic death accompanied by cytochrome c release and caspase-3 activation [56]. MTCH1 and MTCH2 have non-overlapping expression patterns; MTCH2 is the predominant form expressed in liver, kidney, heart, skeletal muscle and testis [57] whereas MTCH1 is found in brain [56].

The second pair of MCs is composed of HuBMSC-MCP (human bone marrow stromal cell-derived mitochondrial carrier protein) [58] and its closely related protein FJL10618. Although these proteins are related with the human folate carrier (28% identity), they represent independent carriers with specific orthologues from yeast to mammals (see table 3). Their yeast counterpart (35–39% identity) is RIM2 (Ybr192wp), an MC reported as essential for mtDNA maintenace and viability under respiratory growth conditions [58, 141]. An expressed intron-less gene, 99% identical to HuBSMC-MCP at the nucleotide level, is detected exclusively in the human genome. HuBMSC-MCP expression is detected in various cell lines and human tissues [58], and a number of human ESTs for FJL10618 sequences have been detected. Homologues for FJL10618 are found in vertebrate genomes (table 3). Overexpression of HuBSMC-MCP enhances endocytotic capacity in dendritic cells suggesting that it might provide energy for the endocytotic process [58]. These proteins have been proposed as candidates for the mammalian mitochondrial pyruvate carrier [137, 154].

Although no definitive proof for the occurrence of a specific mitochondrial NAD^+ translocator has been provided, plant mitochondria actively accumulate NAD^+ [155, 156]. NAD^+ uptake is concentration dependent, exhibits Michaelis-Menton kinetics and is specifically inhibited by an azido derivate of NAD^+ [156]. In animal cells, rapid influx of NAD^+ through mitochondrial membranes has also been reported [157]. Recently, an NAD^+/ADP exchanger from an obligate intracellular symbiont bacteria has been shown to be involved in the import of NAD^+ molecules through cytoplasmic membranes when synthesized heterologously in *E. coli* [158]. Significantly, this novel carrier shows homology with functionally characterized ATP/ADP translocases and nucleotide transporters from other bacteria [159]. In a similar scenario an ANC-related gene may have evolved into a plant mitochondrial NAD^+/ADP exchanger.

Likewise, the choline and glutamine carrier genes are probably among the MC sequences without assigned function. Choline is transported into the mitochondria, where it is oxidized to betaine by an NAD^+-linked enzyme [160]. Its structure resembles that of L-carnitine, both with a trimethyl C-terminal end. It is possible, therefore, that carnitine/acylcarnitine carriers or a related carrier could carry out choline uptake. In fact, L-carnitine has a competitive effect on the mitochondrial uptake of a derivate of choline, ddCDP-choline [161], suggesting that both substrates might be transported by structurally related carriers. Mitochondrial glutamine transport has been extensively studied (reviewed in [162, 163]) in intact mitochondria [164], submitochondrial particles [162] and after partial purification from kidney mitochondria [163]. It has been reported that purified glutamine carrier catalyses both the unidirectional transport of glutamine and glutamine/glutamine exchange with similar first-order rate constants [163]. In addition, the carrier mediates the transport of L-asparagine and with lower efficiency than that of L-aspartate and L-glutamate [163]. Its substrate specificity suggests that the glutamine carrier is structurally homologous to the glutamate and aspartate/glutamate carriers. From phylogenetic analysis, a possible candidate is human KIAA0446 protein, which has well-conserved orthologues in all organisms, including insects (table 2).

In addition to these unresolved aspects of the MCF, the scenario of mitochondrial metabolite transport is complicated by the existence of mitochondrial proteins capable of transporting metabolites and not belonging to MCF. One of these is a new class of tricarboxylate carrier (TCC) which has five putative transmembrane domains without the characteristic tripartite structure. This 37–38-kDa protein transports citrate, shows homology with sideroflexins [165] and is highly expressed in the central nervous system [165].

A further source of complexity and diversity may arise from the report that a number of MCs could transport more that one molecule, the specific metabolite and another relatively unrelated molecule. For example, a role for the dicarboxylate and 2-oxoglutarate carriers in glutathione (GSH) transport has been suggested by use of selective substrates and inhibitors [166, 167]. GSH, which is required for normal mitochondrial function, is not synthesized within mitochondria, but is taken up from the cytosol [168]. Functional expression of the 2-oxoglutarate carrier in *X. laevis* oocytes conferred GSH transport activity that was suppressed by a specific inhibitor of the carrier [167].

Concluding remarks

Proteins from the MCF have highly conserved structural features that have allowed the identification of a substantial number of novel carriers shortly after the first eucaryotic genomes were completed. The establishment of phylogenetic relations between mitochondrial carriers emerged as a helpful tool for identifying function. Members of MCF may be classified in subsets that share relevant structural characteristics and, interestingly, whose substrates also maintain structural relationships. Development of an efficient transport assay in proteoliposomes containing reconstituted carriers, obtained by overexpression in yeast or bacterial systems, constitutes a great advance. These assays, performed mainly by F. Palmieri and J. E. Walker groups, have enabled identification of the transported substrate by a large number of MCs. However, the carriers for very relevant metabolites, such as pyruvate or glutamine, are still unidentified, and the molecule transported for a number of carrier proteins is also unknown. Notable clues to the physiological implications of MCF members have been provided by yeast and mice deficient for specific MC genes. The relevance of metabolic functions performed by these carriers is becoming evident from the growing number of human diseases associated with them.

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