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Discoveries, metabolic roles and diseases of mitochondrial carriers: A review☆

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ABSTRACT

Mitochondrial carriers (MCs) are a superfamily of nuclear-encoded proteins that are mostly localized in the inner mitochondrial membrane and transport numerous metabolites, nucleotides, cofactors and inorganic anions. Their unique sequence features, i.e., a tripartite structure, six transmembrane **α**-helices and a three-fold repeated signature motif, allow MCs to be easily recognized. This review describes how the functions of MCs from *Saccharomyces cerevisiae*, *Homo sapiens* and *Arabidopsis thaliana* (listed in the first table) were discovered after the genome sequence of *S. cerevisiae* was determined in 1996. In the genomic era, more than 50 previously unknown MCs from these organisms have been identified and characterized biochemically using a method consisting of gene expression, purification of the recombinant proteins, their reconstitution into liposomes and transport assays (EPRA). Information derived from studies with intact mitochondria, genetic and metabolic evidence, sequence similarity, phylogenetic analysis and complementation of knockout phenotypes have guided the choice of substrates that were tested in the transport assays. In addition, the diseases associated to defects of human MCs have been briefly reviewed. This article is part of a Special Issue entitled: Mitochondrial Channels edited by Pierre Sonveaux, Pierre Maechler and Jean-Claude Martinou.

1. Introduction

Mitochondrial carriers (MCs) are a superfamily of transporters named the MCF, or in mammals SLC25 (solute carrer family 25), which corresponds to the 2.A.29 family according to Milton Saier (see http://www.tcdb.org). All MCF members have a tripartite structure consisting of three tandemly repeated homologous domains about 100 amino acids in length. Each repeat contains the conserved signature motif PX[D/E]XX[K/R]X[K/R]X_{20-30}[D/E]GXXXX[W/Y/F][K/R]G (PROSITE PS50920, PFAM PF00153 and IPR00193) and two hydrophobic transmembrane segments connected by a long hydrophilic matrix loop [1]. Furthermore, the N- and C-termini are located on the cytosolic side of the inner mitochondrial membrane [2,3]. Unlike other MCs, the aspartate–glutamate and ATP-Mg/phosphate carriers have additional long N-terminal regulatory domains containing EF-hand Ca^{2+}-binding motifs. For a review dealing with the 3D structure of MCs the reader is referred to [4]. Because of enzyme compartmentalization, MCs provide a link between metabolic reactions occurring inside and outside the mitochondria by catalyzing the translocation of numerous solutes across the membrane. For this reason they are essential in many biochemical processes such as the citric acid cycle, oxidative phosphorylation, the transfer of reducing equivalents of NADH and NADPH, gluconeogenesis, amino acid and fatty acid metabolism as well as in cofactor organelle import, mitochondrial replication, transcription and translation, Ca^{2+} cell signaling, and insulin secretion.

Despite their structural similarity, MCs are functionally diverse because they transport a large variety of solutes that differ in size and nature, from H^+ to NAD^+ and coenzyme A (CoA). Based on the substrates transported they can be classified as carriers for amino acids, carboxylates, ketoacids, nucleotides, dinucleotides, coenzymes and other substrates. More appropriately, in view of their substrate specificity MCs have been divided into subfamilies (e.g. the ADP/ATP carrier subfamily), which are also characterized by distinctive structural features [5,6]. Until now at least 25 subfamilies have been recognized and characterized for substrate specificity, mode of transport and kinetic properties. The majority of transported substrates carry negative charges, although some carry positive charges or are zwitterions. Most MCs catalyze a 1:1 substrate exchange (or antiport), a few substrate uniport, or an H^+-compensated anion symport, or a combination of these transport modes. Based on the electrical nature of the transport reactions they mediate, MCs can be electrophoretic (electrogenic), when a net electrical charge is translocated across the membrane, or electroneutral. Therefore, as their driving force MCs utilize the concentration gradient of the

Abbreviations: AAC, ADP/ATP carrier; CoA, coenzyme A; EPRA method, expression-purification-reconstitution-assay method; MC, mitochondrial carrier; MCF, mitochondrial carrier family; Pi, phosphate.

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transported solutes and, in most cases, either the electrical or the chemical component, or both, of the proton motive force generated across the mitochondrial membrane by the H^+ pumps of the respiratory chain.

MCs are widespread in all eukaryotes although some subfamilies are specific for a particular taxonomic kingdom. A few are exceptionally present in pathogenic viruses and bacteria perhaps as a result of horizontal gene transfer from their eukaryotic host. Furthermore, contrary to meanstream thinking, some MCs are localized in non-mitochondrial membranes, such as those of peroxisomes and chloroplasts. With very few exceptions, MCF members are present in minute amounts in organelle membranes, about 20 pmol/mg mitochondrial protein or less. In addition, the expression levels of MCs vary to a great extent in different organs; some are ubiquitous while others are tissue-specific, the latter being especially common in the case of different isoforms of the same carrier. Lastly, all MCs are nuclear encoded and are thought to contain internal targeting sequences that are recognized by the mitochondrial protein import machinery. A few MCs possess a cleavable presequence, which is not required but may help targeting in various ways [7,8].

This review focuses on the molecular identification, main transport properties and metabolic functions of the MCs discovered since 1996 from *Saccharomyces cerevisiae*, *Homo sapiens* and *Arabidopsis thaliana*, which are listed in Table 1. As a premise, a brief history of the discovery and extension of the MCF members (Section 2) and a description of the experimental approaches used for identifying their functions (Section 3) are provided. The discoveries of the *S. cerevisiae* MCs and human MCs identified from their yeast orthologs are treated in Section 4. The remaining characterized human MCs are reviewed in Section 5. In each section the newly discovered carriers are reported following the chronological order in which they have been identified (see also Table 1). Furthermore, a summary of the Arabidopsis MCs identified so far can be found in Section 6. In addition, the diseases known to be associated with alterations of the human MC genes are also dealt with (see Section 7).

2. Brief history of the discovery and extension of the MCF

In pioneering studies the presence of various carrier systems for the transport of metabolites in mitochondria was assessed by direct methods of transport, i.e. measuring the distribution of radioactive compounds between the intra- and extra-mitochondrial space, or by indirect methods such as light scattering recording of mitochondria exposed to iso-osmotic solutions of ammonium salts, or absorption and fluorimetric change recording of the redox state of intramitochondrial NAD^+ or $NADP^+$ [9–13]. In 1975, the ADP/ATP carrier (AAC) was purified from bovine heart mitochondria in a native state in complex with carboxyatractyloside. In the 20 years that followed, 10 other MCs were isolated from mitochondria to homogeneity in a reconstitutively active state using a general scheme involving solubilization of mitochondria with nonionic detergents and chromatography on hydroxyapatite and celite [1,14–16]. This laborious task ultimately proved the existence of mitochondrial transporters for metabolites in the mitochondrial membrane and demonstrated that they are independent, functional, and structural identities consisting of single polypeptides of about 30–32 kDa. Of these purified carrier proteins, six were sequenced by direct amino acid analysis or by molecular biology techniques based on minimal protein sequence information. They are the AAC, the uncoupling protein, and the carrier for phosphate (Pi), oxoglutarate, citrate and carnitine-acylcarnitine [17–22]. The analysis

Table 1
Mitochondrial carriers (MCs) discovered since 1996.

Section	Mitochondrial carrier	Yeast carrier	Human carrier
4	**MCs discovered in *Saccharomyces cerevisiae* and human MCs identified beginning with the yeast orthologs**		
4.1	*Identification of S. cerevisiae MCs based on information derived from studies with intact mitochondria*		
4.1.1	Dicarboxylate carriers	*YLR348c* (Dic1p)	*SLC25A10* (DIC)
4.2	*Identification of S. cerevisiae MCs using genetic and metabolic information*		
4.2.1	Ornithine carriers	*YOR130c* (Ort1p)	*SLC25A15/2* (ORC1–2)
4.2.2	Succinate–fumarate carrier	*YJR095w* (Sfc1p)	
4.2.3	Peroxisomal adenine nucleotide carrier	*YPR128c* (Ant1p)	
4.2.4	Thiamine pyrophosphate carriers	*YGR096w* (Tpc1p)	*SLC25A19* (TPC)
4.2.5	*S*-adenosylmethionine carriers	*YNL003c* (Sam5p)	*SLC25A26* (SAMC)
4.2.6	GTP/GDP carrier	*YDL198c* (Ggc1p)	
4.2.7	Pyrimidine nucleotide carriers	*YBR192w* (Pyt1p)	*SLC25A33/36* (PNC1–2)
4.3	*Identification of S. cerevisiae MCs using sequence similarity*		
4.3.1	Carnitine carrier	*YOR100c* (Crc1p)	
4.3.2	Oxaloacetate-sulfate carrier	*YKL120w* (Oac1p)	
4.3.3	Aspartate–glutamate carrier	*YPR021c* (Agc1p)	
4.4	*Identification of S. cerevisiae MCs using phylogenetic analysis*		
4.4.1	Oxodicarboxylate carriers	*YPL134c*, *YOR222w* (Odc1–2p)	*SLC25A21* (ODC)
4.4.2	NAD^+ carriers	*YIL006w*, *YEL006w* (Ndt1–2p)	
4.4.3	Citrate-oxoglutarate carrier	*YMR241w* (*Coc1p*)	
4.4.4	Adenosine 5′-phosphosulfate carrier	*YPR011c* (Apsc1p)	
4.5	*Other identified S. cerevisiae MCs*		
5	**Other identified human MCs**		
5.1	*Human MCs discovered based on transport activities revealed from studies with intact mitochondria*		
5.1.1	Aspartate–glutamate carriers		*SLC25A12/13* (AGC1–2)
5.1.2	Glutamate carriers		*SLC25A22/18* (GC1–2)
5.1.3	ATP-Mg/phosphate carriers		*SLC25A24/23/25/41* (APC1–4)
5.2	*Human MCs discovered using phylogenetic analysis*		
5.2.1	ADP/ATP carrier isoform 4		*SLC25A31* (AAC4)
5.2.2	Carrier for CoA and 3′-phosphoadenosine 5′-phosphate (PAP)		*SLC25A42* (CoAPC)
5.2.3	Peroxisomal carrier for CoA, FAD and NAD^+		*SLC25A17* (CFNC)
5.2.4	Four-carbon metabolite/phosphate carrier		*SLC25A8* (UCP2)
5.2.5	Carrier for basic amino acids		*SLC25A29* (hBAC)
6	**MCs from *Arabidopsis thaliana***		

of their primary structure led to the recognition of three homologous domains, the three-fold repetition of the signature motif and to the important conclusion that all belong to the same protein family called the MCF. A further relevant finding for future research in the field was the successful expression of the bovine oxoglutarate carrier in *Escherichia coli*, its solubilization, and functional reconstitution into liposomes in 1993 [23]. This was the first time that a eukaryotic membrane protein had been produced in bacteria and renatured. Meanwhile, with the advance of genomic DNA sequencing projects, many proteins of unknown function bearing the characteristic MCF sequence features emerged from various organisms. These proteins were annotated in databases as putative or hypothetical MCs, or simply as unknown proteins. At present, a database search for MCs reveals 4780 SLC25-related members in metazoa, 3119 in green plants, 10,076 in fungi and 1001 in Protista (EBI Accession Code IPR002067). Generally, in all organisms MC genes are distributed almost uniformly in all chromosomes. After completing the genomic sequence of *S. cerevisiae* in 1996, a systematic investigation of the molecular identification and transport proteins of putative MCs was undertaken. A few years later, this investigation was extended to the MCs of *H. sapiens* and subsequently to those of *A. thaliana*, aided by the completion of whole genome sequencing of these two organisms. In the genomic era, in a relatively short time and without prior protein purification, more than 50 new MCs have been identified at the molecular level and characterized in terms of substrate specificity, inhibitor sensitivity, transport modes and kinetic parameters by direct transport assays [6,24–27]. In many instances, the tissue distribution and role of MCs in cell metabolism and specific cell function, such as insulin secretion [28], were also disclosed.

3. Experimental approaches for identifying the function of MCs

Discovering the function of novel members of the MCF requires identification of the substrate(s) they transport. Until now, the most successful strategy employed to identify the substrate specificity of MCs of unknown function consists of gene expression, purification of the recombinant protein and its reconstitution into liposomes followed by direct transport assays, herein named the "expression–purification–reconstitution-assay" (EPRA) method [23]. In particular, this experimental approach involves searching for MC genes in sequence databases, cloning them into appropriate vectors, expressing the gene products in *E. coli* and/or *S. cerevisiae*, purifying and incorporating the recombinant proteins into liposomes and characterizing the function of the reconstituted proteins using transport assays. In reconstituted liposomes, protein-mediated transport is measured by following the flux of radioactive substrates from the outside to the inside of proteoliposomes, or vice versa, as uniport or as exchange in the presence of an appropriate substrate inside and outside the proteoliposomes. In most of these studies, the degree of similarity to known MCs, use of knock-out, knowledge of cell metabolism, genetic information, phylogenetic clustering, complementation of phenotypes and subcellular localization have provided useful clues for testing candidate substrates in reconstituted liposomes. However, with none of these methods can functional identification and transport functions of a carrier protein be identified with certainty. In a few instances expression of MCs has been achieved in baculovirus-infected insect cells [29], a cell-free wheat germ extract system [30], and *Lactococcus lactis* [31]. In the first two hosts MCs were expressed in mitochondria, while in the latter in the plasma membrane. Furthermore, in the latter case the proteoliposomes used to measure transport were prepared by freezing and thawing of Lactococcus plasma membranes and pure liposomes.

The above-mentioned "gene-to-function" procedure appears simple and straightforward, but it can fail for a number of reasons. Many MCs proved to be toxic to *E. coli* BL21(DE3). Therefore, new *E. coli* strains such as C41(DE3) and C0214(DE3), capable of tolerating expression of a given MC (e.g. the bovine Pi carrier [32]), had to be selected by spontaneous mutation of the original host strain or a derived strain. Nowadays, numerous *E. coli* strains are commercially available for over-expression of proteins. Furthermore, MCs are expressed in the cytosol of bacteria as inclusion bodies. It is therefore crucial to use a suitable detergent to solubilize the carrier in a form whereby it can be refolded and achieve a high degree of purity of the expressed transporters. Until now *N*-dodecanoylsarcosine (sarkosyl), selected from among many other detergents tested [23], has proven to be satisfactory in almost all cases. Only exceptionally did it require substitution with lauric acid [33]. Normally, recombinant MCs can be purified to apparent homogeneity after the purification of inclusion bodies and a series of washing steps with nonionic detergents like Triton X-114. Using affinity chromatography on a Ni-NTA-agarose column of MCs tagged with a tail of 6–8 histidines was sometimes useful as a final purification step. In addition, it was observed that the purity of MCs overexpressed using the C41(DE3) and C0214(DE3) strains is usually much greater than that obtained using the BL21(DE3) strain [34]. Refolding occurs during the incorporation of recombinant proteins into liposomes; therefore, this process must be gradual. When using, for example, the cyclic-detergent-removal procedure, mixed micelles containing a nonionic detergent, the recombinant carrier solubilized in sarkosyl, and phospholipids are slowly and repeatedly passed through the same column filled with a hydrophobic resin such as Amberlite [35]. Often cardiolipin and an SH-reducing compound must be added to the reconstitution mixture and sometimes from the beginning, that is during solubilization of the expressed protein.

4. MCs discovered in *S. cerevisiae* and human MCs identified beginning with the yeast orthologs

Immediately after the genome of *S. cerevisiae* was entirely sequenced it was screened for MCF members based on the sequence features of this protein family. This genome was found to contain 35 genes encoding MCs [36–38]. At that time, only the three isoforms of the AAC (Aac1–3p) and the carriers for Pi (Mir1p) and citrate (Ctp1p) had been found in *S. cerevisiae* after their cloning in mammals [39–43]. To date, nearly all the transport functions of the remaining 30 *S. cerevisiae* MCs have been discovered as summarized

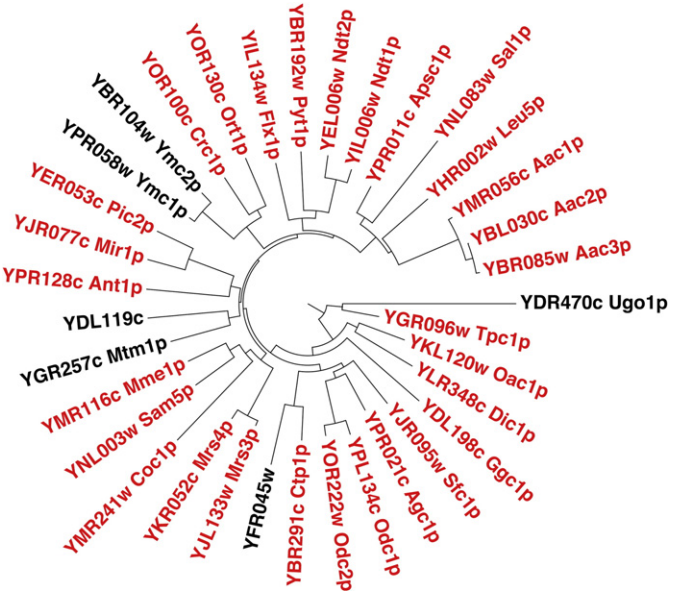

Fig. 1. Phylogenetic tree of *Saccharomyces cerevisiae* MCs. The tree of the 35 MC sequences from *S. cerevisiae* was constructed using PhyML v3.1 in seaview4 [163] from a Muscle multiple-sequence alignment and drawn in FigTree v1.4.2. The names of MCs and their acronyms are found on the terminal nodes with those biochemically characterized colored in red.

Fig. 2. Phylogenetic tree of human MCs. The tree of the 53 MC sequences from *Homo sapiens* was built using PhyML v3.1 in seaview4 [163] from a Muscle multiple-sequence alignment and drawn in FigTree v1.4.2. The names of MCs and their acronyms are found on the terminal nodes with those biochemically characterized colored in red.

below (Fig. 1). Aside from the identified yeast MCs, the human MCs disclosed starting from the yeast sequences are also dealt with in this section (these and all the other human MCs are shown in Fig. 2).

4.1. Identification of S. cerevisiae MCs based on information derived from studies with intact mitochondria

4.1.1. The dicarboxylate carriers Dic1p (YLR348c) and DIC (SLC25A10)

In the search for the function of the *YLR348c* gene product, the EPRA method was performed using the substrates of transport activities assessed in studies with intact mitochondria. Results showed that the YLR348c protein, now named Dic1p, transports dicarboxylates (malate, succinate or malonate), Pi, sulfate and thiosulfate by a strict counter-exchange mechanism [36], as previously described for the DIC activity in isolated mitochondria and the DIC purified from rat liver mitochondria [44–47]. In contrast, this carrier does not transport oxoglutarate at an appreciable rate (although YLR348c is the closest relative of the bovine and human oxoglutarate carriers among the 35 *S. cerevisiae* MCs). To gain insight into the physiological role of Dic1p a yeast *DIC1* null mutant was generated [48]. This mutant failed to grow on ethanol or acetate as the sole carbon source. Growth was restored by adding low concentrations of oxaloacetate, aspartate, glutamate and citrate, but not of leucine and lysine, which are unable to generate intramitochondrial Krebs cycle intermediates [48]. Notably, in the case of the yeast mutant lacking the gene for Sfc1p (see Section 4.2.2), the lack of growth on acetate or ethanol was not restored by the addition of compounds that generate intramitochondrial Krebs cycle intermediates [48]. These findings indicate that the primary function of Dic1p is to provide Krebs cycle intermediates in the matrix of mitochondria. In wild-type *S. cerevisiae* growing on acetate or ethanol, Dic1p catalyzes the import of succinate into mitochondria in exchange for internal P_i. Given that P_i is recycled back into mitochondria by the Pi carrier, the combined activity of Dic1p and the Pi carrier leads to a net uptake of succinate. The conversion of succinate to fumarate and oxaloacetate within mitochondria allows the oxidation of acetyl-CoA produced from acetate or ethanol and triggers Sfc1p activity (Fig. 3).

Later, the sequence of the yeast Dic1p was used to search for the human dicarboxylate carrier (encoded by *SLC25A10* and called SLC25A10 or DIC). Screening *Caenorhabditis elegans* for homologs of the biochemically characterized yeast Dic1p resulted in the identification of K11G12.5, which shares 37% sequence identity with Dic1p and was confirmed by the EPRA method to be a dicarboxylate carrier based on its transport properties. Subsequently, two partially overlapping murine ESTs (AA199557, AA041737) were found to be 47% identical to K11G12.5 on protein sequence level. Both ESTs were used to identify full-length homologs in murine, rat, and human cDNAs sharing about 89% sequence identity. Using the EPRA method the recombinant rat protein was shown to be a dicarboxylate carrier by characterizing its transport properties [49]; the carrier transports malate, succinate, malonate, Pi, sulfite, sulfate and thiosulfate by an electroneutral antiport mechanism. Its half-saturation constants (Km) for malate and Pi are about 0.5 and 1.5 mM, respectively, as in rat liver mitochondria [44] and liposomes reconstituted with recombinant dicarboxylate carrier from *S. cerevisiae* [36], *C. elegans* and rat [49]. The human DIC is expressed in liver, kidney, heart, brain, lung, pancreas and adipose tissue [50–52]. It fulfills several important metabolic functions under different physiological conditions and in different tissues. It is involved in the anaplerotic supply of intermediates for the Krebs cycle or participates by exporting malate in exchange for Pi in gluconeogenesis occurring mainly in liver and to a lesser extent in kidney (Fig. 4). DIC also plays a role in the urea cycle by importing malate generated from fumarate produced by argininosuccinate lyase. It is involved in fatty acid synthesis by exporting malate in exchange for external Pi in the citrate-pyruvate shuttle leading to net export of citrate from mitochondria and the production of NADPH in the cytosol [24]. The fact that DIC expression is upregulated in a variety of tumors and its knockdown induces a less malignant phenotype (whose growth is more sensitive to oxidative stress and more dependent on glutamine) supports the hypothesis that DIC is involved in the transfer of reducing equivalents of NADPH [53]. Furthermore, DIC plays a role in sulfur metabolism because i) sulfite is formed within mitochondria during the degradation of cysteinsulfinate and from thiosulfate by the action of thiosulfate sulfurtransferase (rhodanase) and thiosulfate reductase, ii) small amounts of thiosulfate are produced in mitochondria from H_2S, and iii) sulfite is oxidized outside or inside mitochondria [45,46,54]. This implies that cysteinsulfinate, thiosulfate and H_2S must enter the mitochondrial matrix and that sulfite and/or sulfate must exit the organelles.

4.2. Identification of S. cerevisiae MCs using genetic and metabolic information

4.2.1. The ornithine carriers Ort1p (YOR130c) and ORC1–2 (SLC25A15/A2)

Before the discovery of the ornithine carrier (encoded by *YOR130c*, also called *ARG11* and after its identification *ORT1*), it had been reported that mutations in the *ARG11* gene of *S. cerevisiae* caused poor growth rates in the absence of arginine and that mitochondrial import of glutamate and export of ornithine were required to accomplish arginine biosynthesis in the cytosol. Therefore, the protein encoded by *ARG11* could be a glutamate or an ornithine carrier. The transport properties of the reconstituted *ORT1* product disclosed with the EPRA method showed that this protein is an ornithine carrier since it catalyzes ornithine transport in exchange for protons, arginine or lysine but not histidine or any other amino acid [55]. On the basis of these findings, the main physiological function of the yeast ornithine carrier is to export mitochondrial ornithine to the cytosol, where it is converted to arginine, in exchange for protons at the expense of the proton motive force (Fig. 3). At high cytosolic concentrations of arginine or lysine, the ornithine carrier can

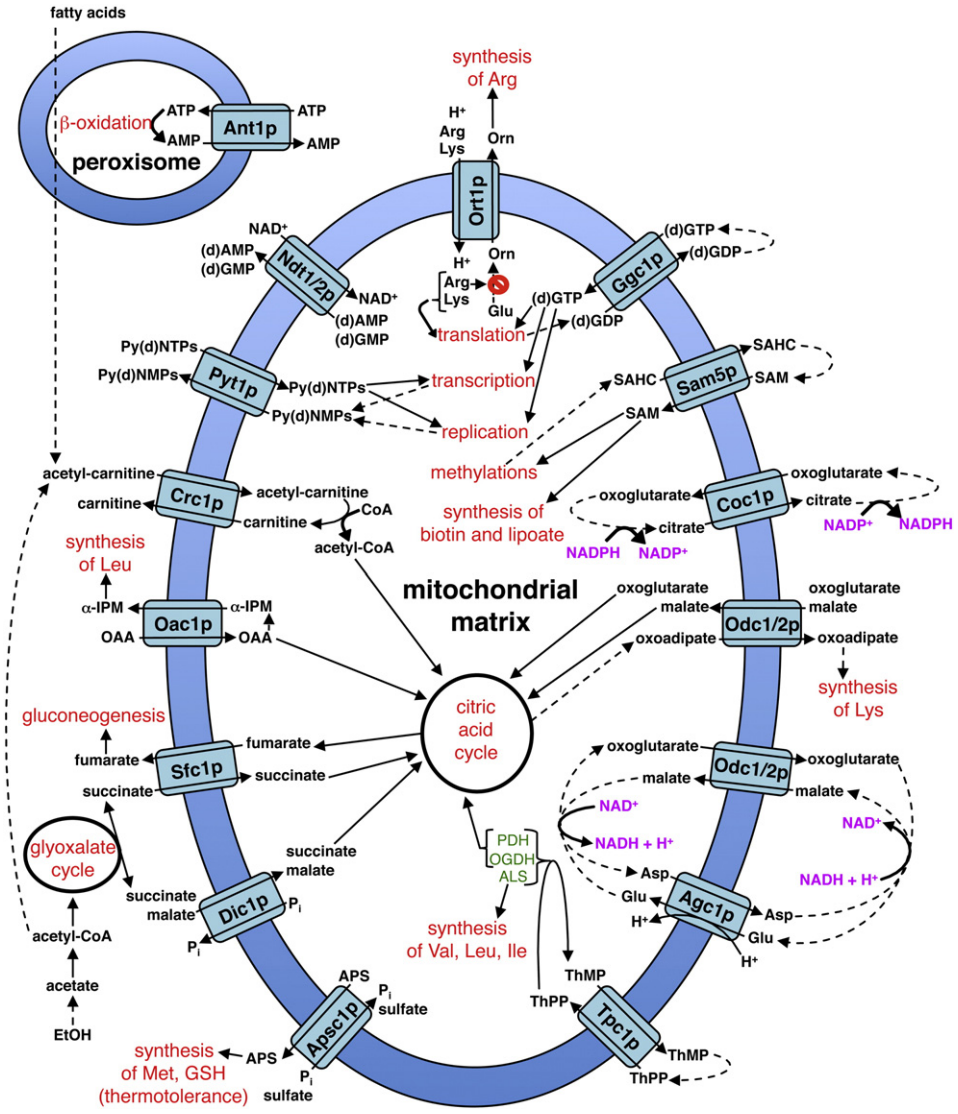

Fig 3. Roles of *Saccharomyces cerevisiae* MCs. The MCs are indicated by their acronyms, where Coc1p is Yhm2p and Apsc1p is YPR011cp. Processes are indicated in red whereas enzymes are abbreviated in green: ALS, acetolactate synthase; OGDH, 2-oxoglutarate dehydrogenase; PDH, pyruvate dehydrogenase. Abbreviations of compounds: α-IPM, α-isopropylmalate; APS, adenosine 5′-phosphosulfate; EtOH, ethanol; GSH, glutathione; OAA, oxaloacetate; Orn, ornithine; Py(d)NMPs, pyrimidine (deoxy-)nucleoside monophosphates; Py(d)NTPs, pyrimidine (deoxy-)nucleoside triphosphates; SAM, S-adenosylmethionine; SAHC, S-adenosylhomocysteine; ThMP, thiamine monophosphate; ThPP, thiamine pyrophosphate. Arrows with dashed black lines indicate several steps.

catalyze the entry of arginine or lysine into mitochondria in exchange for ornithine. In the mitochondrial matrix arginine and lysine are consumed in protein synthesis, and arginine inhibits the first two enzymes of its biosynthetic pathway in a feedback mechanism (Fig. 3).

The human ornithine carrier is a typical example of a MC identified starting from the *S. cerevisiae* ortholog. In man there are two isoforms of the ornithine carrier, called SLC25A15 (or ORC1) and SLC25A2 (or ORC2), which are 32 and 27% identical to Ort1p, respectively, and 87% identical to each other. The two human ornithine carrier isoforms were characterized by the EPRA method for substrate specificity, inhibitor sensitivity, kinetic parameters and level of expression in several tissues [56]. ORC1 transports the L-forms of ornithine, citrulline, arginine and lysine, and ORC2 transports the same substrates as ORC1 as well as their D-forms, histidine and homoarginine. Recently, asymmetric dimethylarginine (ADMA) was found to be transported exclusively by ORC2 [57]. Both isoforms are expressed in all tissues examined, but ORC1 at higher levels especially in liver, pancreas, lung and testis. Like the yeast ornithine carrier, ORC1 and ORC2 transport ornithine in exchange for a proton although at a low rate. This reaction is important in several tissues for the oxidation of ornithine inside mitochondria as well as for the synthesis of polyamines in the cytosol (Fig. 4). Unlike the yeast carrier, the two human isoforms also transport citrulline. The reaction between cytosolic ornithine and intramitochondrial citrulline, which is electroneutral because the positive charge of ornithine is compensated by the movement of a proton together with citrulline [58], is fundamental in liver for the functioning of the urea cycle. The ornithine carrier, also named ornithine-citrulline carrier at the time it was purified from rat liver mitochondria [59], is indeed an essential component of the urea cycle to link the reactions of urea synthesis from ammonia occurring in the cytosol to those occurring in the mitochondria (Fig. 4). The importance of the ornithine/citrulline + H^+ exchange is underlined by a disease named HHH (hyperornithinemia–hyperammonemia–homocitrullinuria) syndrome (OMIM 212138), which is caused by mutations in the *SLC25A15* gene encoding ORC1 [26,27,56,60]. HHH syndrome is a disorder of the urea cycle mainly characterized by the metabolic alterations indicated by its name, increased production of polyamines and increased excretion of orotic acid and uracil. Common clinical symptoms are episodes of confusion, lethargy, neurological features

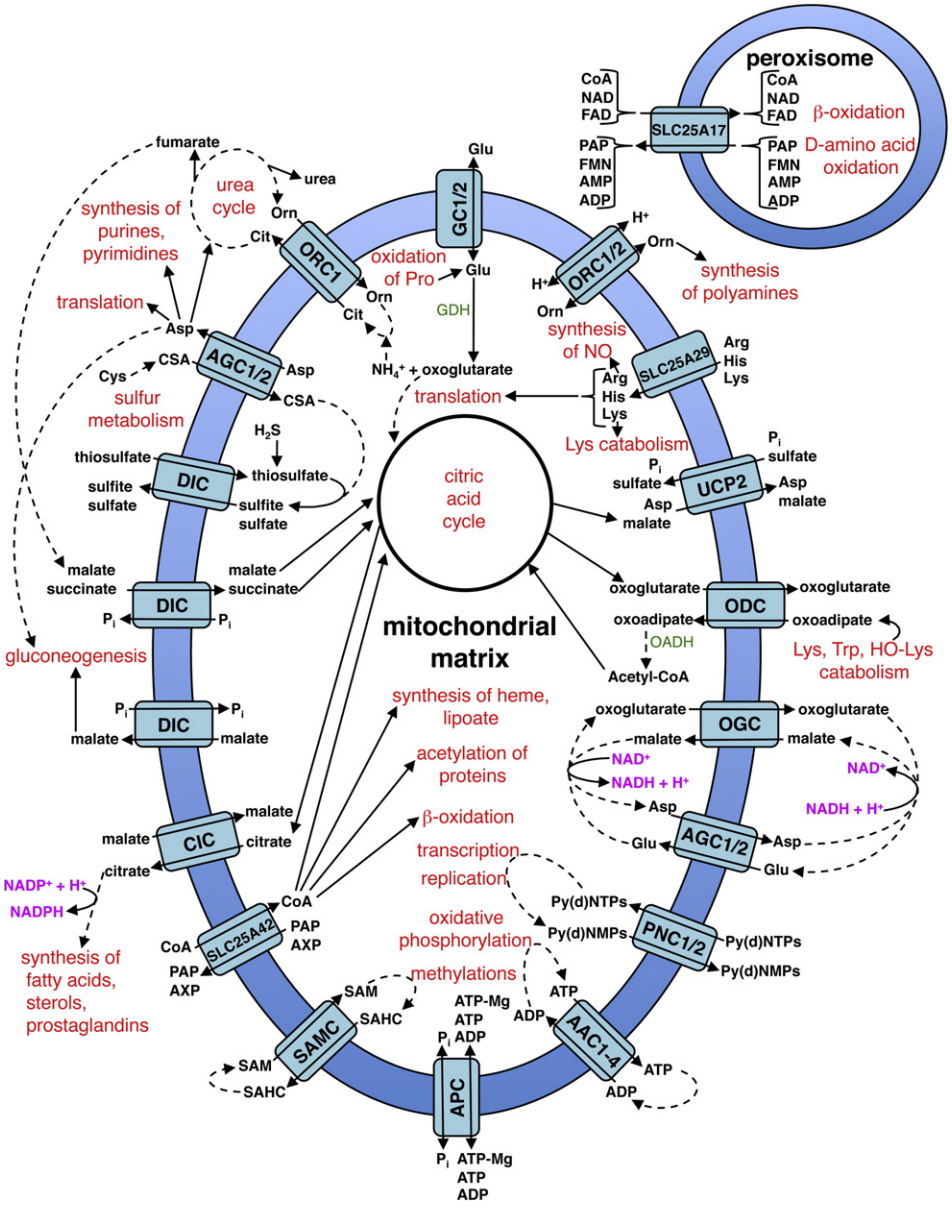

Fig 4. Roles of human MCs. Processes are indicated in red whereas enzymes are abbreviated in green: GDH, glutamate dehydrogenase; OADH, 2-oxoadipate dehydrogenase. Abbreviations of compounds: AXP, adenine nucleotides; CSA, cysteinesulfinate; HO-Lys, hydroxylysine; PAP, adenosine 3′,5′-diphosphate; SAM, S-adenosylmethionine; SAHC, S-adenosylhomocysteine. Arrows with dashed lines indicate several steps.

such as mental retardation, learning difficulties and seizures, and coma or fulminant liver failure due to hyperammonemia.

4.2.2. The succinate–fumarate carrier Sfc1p (YJR095w)

The "genetic–metabolic approach" was also successfully exploited for the molecular identification of the succinate-fumarate carrier encoded by *YJR095w* or *ACR1*. In fact, at the start of this identification process it was already known that i) the *ACR1* null mutant was unable to grow on ethanol or acetate, and that ii) in *S. cerevisiae* ethanol or acetate metabolism succinate was produced in the cytosol by the glyoxylate pathway. As succinate dehydrogenase is accessible to succinate only from the mitochondrial matrix [61], it was thought that cytosolic succinate had to be imported into mitochondria and thus the protein encoded by *ACR1* could be a succinate transporter. Indeed, by means of the EPRA method it was found that recombinant and reconstituted Sfc1p transports succinate and fumarate by a strict counter-exchange mechanism [62]. Sfc1p also transports oxoglutarate and oxaloacetate to a lower extent (Km values about 2 mM). From a physiological point of view this carrier is considered essential for gluconeogenesis from acetate and ethanol (Fig. 3). This view is substantiated by the finding that *SFC1* is co-regulated with five key enzymes of the glyoxylate and gluconeogenic pathways.

4.2.3. The peroxisomal adenine nucleotide carrier Ant1p (YPR128c)

To gain insight about possible substrates for Ant1p (encoded by YPR128c or *ANT1*), the *S. cerevisiae* deletion strain for *ANT1* was produced and characterized [63]. Also, the subcellular localization of Ant1p was investigated. The results of these studies showed that Ant1p is required for growth on fatty acids and is localized to peroxisomes, in whose matrix fatty acid β-oxidation takes place in *S. cerevisiae*. In addition, *ANT1* expression is upregulated in the presence of fatty acids. In light of these observations, it was thought that Ant1p could be responsible for one of the following transport reactions: import of fatty acids, ATP, or cofactors (NAD^+ and FAD), that are required for β-oxidation, into peroxisomes or export of AMP that is formed in this process. The transport properties of reconstituted Ant1p disclosed

using the EPRA method showed that this protein transports ATP, ADP and AMP with high efficiency and the corresponding deoxynucleotides, to a lower extent, in a carboxyatractyloside- and bongkrekic acid-insensitive reaction [63]. Therefore, the physiological role of Ant1p is to transport cytosolic ATP into the peroxisomal lumen in exchange for AMP produced in the activation of fatty acids (Fig. 3). Notably, Ant1p was the first peroxisomal protein proven to be a transporter and the first member of the MCF found to be located in a membrane different from the mitochondrial inner membrane.

4.2.4. The thiamine pyrophosphate carriers Tpc1p (YGR096w) and TPC (SLC25A19)

Similarly as for Ant1p, clues for the molecular identification of Tpc1p, encoded by *YGR096w* or *TPC1*, came from the characterization of the phenotype of the *TPC1* knockout strain. Interestingly, *TPC1*Δ cells required thiamine or branched-chain amino acids for growth on fermentable carbon sources [64]. This finding led to the idea that Tpc1p could be involved in supplying the cofactor thiamine pyrophosphate (ThPP) to the first enzyme (acetolactate synthase, ALS) of the branched-chain amino acid synthesis pathway that in *S. cerevisiae* is located in mitochondria. The EPRA method demonstrated that recombinant Tpc1p reconstituted in liposomes catalyzes antiport and ΔpH-stimulated uniport transfer of thiamine pyrophosphate and thiamine monophosphate [64]. It also transports (deoxy)nucleotides, though to a lower extent, with the following order of efficiency: NMP > NDP > NTP, but not nucleosides, purines or pyrimidines. The physiological function of Tpc1p was suggested to be mitochondrial import of thiamine pyrophosphate for ALS and other matrix enzymes that use this cofactor (pyruvate dehydrogenase and oxoglutarate dehydrogenase) and export of thiamine monophosphate that is formed by mitochondrial thiamine pyrophosphatase and can be recycled in the cytosol (Fig. 3).

The closest human Tpc1p-homolog SLC25A19 (TPC) had previously been discovered as a deoxynucleotide carrier (DNC) [65] based on the fact that it clustered with AAC. As demonstrated by the EPRA method this carrier efficiently transports deoxynucleoside diphosphates (dNDPs) and ADP. Significant activity was also found with GDP, CDP, UDP and dideoxynucleoside triphosphates, whereas much lower activity was found with NTPs, dNTPs, dNMPs and Pi. No activity was observed with NMPs, (deoxy)nucleosides, purines and pyrimidines. Later, based on the above-mentioned sequence similarity with Tpc1p, thiamine pyrophosphate and thiamine monophosphate were also found to be transported efficiently by DNC [66], suggesting that the carrier's main function was similar to that of yeast Tpc1p. A mutation in the *SLC25A19* gene, found to be markedly deleterious in studies with reconstituted liposomes, causes congenital Amish microcephaly (OMIM 607196), which is characterized by severe congenital microcephaly, no orientation to light and sound, no motor development, increased urine levels of 2-oxoglutarate, lactic acidosis and premature death [66,67]. The accumulation of 2-oxoglutarate is probably due to impaired function of α-ketoglutarate dehydrogenase, while lactic acidosis is the result of reduced pyruvate dehydrogenase activity; both are intramitochondrial enzymes that require the cofactor ThPP. Another presumably less deleterious TPC mutation causes neuropathy with bilateral striatal necrosis (OMIM 613710), which manifests with recurrent episodes of flaccid paralysis and encephalopathy associated with bilateral striatal necrosis and chronic progressive polyneuropathy [68]. At variance with congenital Amish microcephaly, patients exhibit normal head circumference, development, cognitive skills and absence of 2-oxoglutarate in urine.

4.2.5. The S-adenosylmethionine carriers Sam5p (YNL003c) and SAMC (SLC25A26)

Like Tpc1p, the molecular identification of the *YNL003c* protein product, now called Sam5p, began with the characterization of the *SAM5* knockout phenotype. *SAM5*Δ cells were found unable to grow on fermentable sugars in the absence of the biotin precursor dethiobiotin [69]. This observation suggested that Sam5p could be involved in dethiobiotin import into mitochondria, where it is converted to biotin by matrix biotin synthetase (Bio2p), in the export of biotin or in the mitochondrial import of *S*-adenosylmethionine (SAM), which is a cofactor for Bio2p. Direct transport measurements carried out with the EPRA method showed that Sam5p catalyzes uniport and antiport of SAM and *S*-adenosylhomocysteine (SAHC), whereas no dethiobiotin or biotin transport was detected [69]. Based on these observations it was deduced that the physiological role of Sam5p is to import SAM, which is synthesized in the cytoplasm, into mitochondria by uniport (for the synthesis of biotin and lipoate) or in exchange for matrix SAHC; SAHC is produced in the methylation reactions occurring in mitochondria and is hydrolyzed exclusively in the cytosol (Fig. 3). Strong support for the physiological role of Sam5p came from the finding that the *SAM5*Δ phenotype can be suppressed by expression of an engineered mitochondrial matrix-targeted version of SAM synthetase (Sam1p) [69].

Starting from the yeast ortholog, the human mitochondrial SAM carrier (SAMC encoded by *SLC25A26*) was identified by its transport properties. Using the EPRA method, SAMC was proven to transport SAM and SAHC by an exchange mechanism [70]. Unlike Sam5p, SAMC is a more obligatory aniporter and exhibits a higher transport affinity for SAM (Km of 23 μM compared to 75 μM for Sam5p). The inhibition constant of SAHC for SAMC is 10.5 μM. The physiological function of SAMC is to catalyze the import of SAM, the universal donor of methyl groups, into mitochondria in exchange for matrix SAHC, which is produced, as in *S. cerevisiae*, in the intramitochondrial methylation reactions and is hydrolyzed in the cytosol (Fig. 4). Recently, a genetic disease (OMIM 616794) caused by mutations in the *SLC25A26* gene of three families disclosed in Belgium, Japan and Sweden has been described [71]. *SLC25A26* deficiency reduces the ability of mitochondria to methylate numerous targets, with deleterious consequences on mitochondrial RNA stability, translation, protein modification, and biosynthesis of coenzyme Q and lipoic acid. Common clinical symptoms of the disease are respiratory insufficiency and lactic acidosis; severity and other manifestations vary from progressive muscle weakness to neonatal mortality.

4.2.6. The GTP/GDP carrier Ggc1p (YDL198c)

Ggc1p (encoded by *YDL198c*, now named *GGC*) was discovered in *S. cerevisiae* based on metabolic considerations and does not seem to be present in either mammals or plants. After several blinded tests GTP was thought to be a possible substrate for the YDL198c protein because its production inside *S. cerevisiae* mitochondria is unlikely, due to the formation of ATP instead of GTP by succinyl-CoA ligase and the absence of a nucleoside diphosphate kinase in the matrix. The EPRA method demonstrated that Ggc1p translocates GTP, GDP, dGTP, dGDP, the structurally related ITP and IDP and, to a lesser extent, guanosine 5′-tetraphosphate and the (deoxy)nucleoside di- and triphosphates of U and T [72]. Ggc1p catalyzes counter-exchange of the transported substrates and is unaffected by the AAC inhibitors carboxyatractyloside and bongkrekic acid. The Km of Ggc1p for GTP and GDP (about 1 μM and 5 μM, respectively) are about 1 order of magnitude lower than for dGTP and dGDP and about 2 orders of magnitude lower than for ITP and IDP. Furthermore, the GTP/GDP exchange is not electrophoretic (as the ADP/ATP exchange catalyzed by AAC), but proton-compensated by the movement of protons in the same direction as GTP. The primary function of Ggc1p is most likely to mediate the influx of GTP (dGTP) into mitochondria in exchange for internal GDP, nucleotides involved in essential processes such as intramitochondrial nucleic acid and protein synthesis (Fig. 3). Consistently, the *GGC1* nul mutant did not grow on non-fermentable carbon sources and exhibited mitochondria with reduced levels of GTP and increased levels of GDP [72].

4.2.7. The pyrimidine nucleotide carriers Pyt1p (YBR192w) and PNC1–2 (SLC25A33/A36)

Prior to the identification of the pyrimidine nucleotide carrier encoded by *YBR192w* (alias *RIM2* and *PYT1*), the only information available was that deletion of *RIM2* caused loss of mtDNA and lack of growth on non-fermentable carbon sources. On these grounds, it was reckoned that Rim2p could be a transporter for pyrimidine nucleotides. At that time there were many considerations in favor of the existence of such a transporter: 1) several transporters for purine nucleotides were known [39–41,63,72,73]; 2) pyrimidine (deoxy)nucleoside triphosphates are required for mtDNA and mtRNA synthesis; and 3) (deoxy)pyrimidine nucleoside diphosphates in *S. cerevisiae* are synthesized outside the mitochondria where nucleoside diphosphate kinase and ribonucleotide reductases are also located. As assessed with the EPRA method, in liposomes Rim2p, or Pyt1p, transports pyrimidine (deoxy)nucleoside mono-, di- and triphosphates by an obligatory counter-exchange mechanism [74]. The Km values for TTP, UTP and CTP are 207, 404 and 435 μM, respectively. The main function of Pyt1p is probably to transport pyrimidine (deoxy)nucleoside triphosphates into the mitochondria, where they are incorporated into mtDNA and mtRNA, in exchange for pyrimidine (deoxy)nucleoside monophosphates, which are produced intramitochondrially in the breakdown of mtDNA and mtRNA (Fig. 3).

The closest human relatives of yeast Pyt1p are SLC25A33 (PNC1) and SLC25A36 (PNC2), which have 60% identical protein sequences. Biochemical characterization of the recombinant proteins performed using the EPRA method demonstrated that PNC1 preferentially transports UTP, UDP, TTP, TDP and dUTP, whereas PNC2 transports the (deoxy)nucleoside di- and triphosphates of the bases C, U, I and G [33]. At variance with PNC1, PNC2 also transports nucleoside monophosphates. The Km values of PNC1 for UTP, TTP and CTP are in the 0.08–0.18 mM range, and those of PNC2 for CTP and UTP are 0.21 and 1.19 mM, respectively. PNC1 catalyzes only strict antiport, whereas PNC2 catalyzes both anti- and uniport. Therefore, PNC1 and PNC2 are likely to be involved in supplying (deoxy)nucleotides for mitochondrial replication, DNA repair and transcription (Fig. 4). Studies on PNC1 in human cells have shown that its overexpression reduced reactive oxygen species (ROS) and increased cell size, while its silencing diminished mtDNA, oxidative phosphorylation, cell size and mitochondrial UTP levels, but increased ROS [75,76]. Furthermore, the expression of PNC1 is induced by insulin-like growth factor via the mTor signaling pathway. Its expression levels are higher in cancer cells [75,76], which suggests that the function of PNC1 is associated with rapidly growing cells.

4.3. Identification of S. cerevisiae MCs using sequence similarity

In some instances, comparing the sequences of known mammalian carriers with the proteins encoded in the *S. cerevisiae* genome has helped to predict the function of MC yeast sequences.

4.3.1. The carnitine carrier Crc1p (YOR100c)

The yeast YOR100cp (Crc1p after identification), which among the *S. cerevisiae* MCs is the closest relative of the rat and human carnitine carrier [22,77] (31% and 32% identity, respectively), catalyzes the transport of carnitine and acylcarnitines, as proven by the EPRA method [78]. At variance with the mammalian orthologs [79], Crc1p transports medium- and long-chain acylcarnitines to a much lower extent than acetylcarnitine and propionylcarnitine. Both orthologs, on the other hand, catalyze fast antiport and slow uniport of substrates. The β-oxidation of fatty acids in *S. cerevisiae* is restricted to peroxisomes where they are oxidized to acetyl-CoA, which is converted to acetylcarnitine that is further metabolized in mitochondria. Therefore, the main biochemical role of the yeast carnitine carrier is to import acetylcarnitine, and not medium- and long-chain acylcarnitines, into mitochondria in exchange for free carnitine (Fig. 3). This conclusion is consistent with the dual localization in mitochondria and peroxisomes of the major fungal acetylcarnitine transferase, whose expression is under transcriptional control by an oleate responsive element also found in the *CRC1* promoter. The yeast Crc1p is also essential in the utilization of ethanol and acetate in yeast (Fig. 3). In fact, the acetyl moiety produced as acetyl-CoA in the cytosol (that is not metabolized by the glyoxylate pathway) is transferred to carnitine by an outer mitochondrial membrane-associated acetylcarnitine transferase (encoded by a gene whose expression is induced by ethanol and acetate but not oleate) and transported into the mitochondrial matrix by Crc1p.

4.3.2. The oxaloacetate-sulfate carrier Oac1p (YKL120w)

In a novel attempt to identify the yeast counterpart of the mammalian oxoglutarate carrier, its second closest yeast homolog, YKL120wp, encoded by the gene currently termed *OAC1*, was investigated. Using the EPRA method, the recombinant YKL120wp was found to transport oxaloacetate, sulfate and malonate efficiently by exchange or unidirectional symport together with protons, but not oxoglutarate [80]. These results suggest that the main function of Oac1p is the import of oxaloacetate in mitochondria (Fig. 3), since pyruvate carboxylase is located in the cytoplasm of yeast and Oac1p is expressed at high levels in synthetic medium compared to rich medium [80]. However, the *OAC1*Δ strain was able to grow equally on several fermentable and non-fermentable carbon sources, which could be explained by cytosolic oxaloacetate being transformed into malate that is imported into mitochondria by Dic1p. In support of this hypothesis, the *OAC1*Δ*DIC1*Δ strain did not grow on non-fermentable carbon sources, suggesting that these MCs have an anaplerotic role for the Krebs cycle [80]. Furthermore, Oac1p has been shown to transport α-isopropylmalate, which is produced in the mitochondrial matrix and used in the cytosol for leucine biosynthesis [81]. Consistent with this transport function of Oac1p, the *OAC1*Δ strain requires leucine supplement for optimal growth on fermentable carbon sources [81].

4.3.3. The aspartate–glutamate carrier Agc1p (YPR021c)

Another transporter discovered by the "similarity sequence approach" is the yeast aspartate–glutamate carrier Agc1p [82], as the two human isoforms of this carrier, AGC1 (SLC25A12) and AGC2 (SLC25A13), had previously been identified and characterized biochemically [83] (see Section 5.1.1). The C-terminal domain of Agc1p, which resembles a typical MC, is about 43% identical to its human counterparts, whereas its N-terminal domain does not contain Ca^{2+}-binding EF-hand motifs in contrast to what was known for the human AGCs. As shown by the EPRA method, Agc1p catalyzes the electrophoretic exchange of aspartate$^-$ for glutamate and proton and, unlike the human isoforms, catalyzes uniport of aspartate and glutamate [82]. From a physiological standpoint, given that the *AGC1*Δ strain is able to grow in minimal synthetic medium supplemented with non-fermentable carbon sources with the exception of acetate and oleic acid, it is thought that Agc1p is required in combination with the oxodicarboxylate carrier (Section 4.4.1) for accomplishing the malate–aspartate shuttle in yeast cells growing on acetate or fatty acids (Fig. 3). Unlike the human AGCs, Agc1p does not seem to be required for aspartate export to the cytosol, in agreement with the presence of pyruvate carboxylase in the *S. cerevisiae* cytosol, and the fact that Agc1p is not required for gluconeogenesis.

4.4. Identification of S. cerevisiae MCs using phylogenetic analysis

Once the substrate specificity of one or more proteins belonging to the same phylogenetic tree cluster is disclosed, this information can be somewhat suggestive of the function of other cluster members.

4.4.1. The oxodicarboxylate carriers Odc1–2p (YPL134c and YOR222w) and ODC (SLC25A21)

Odc1p and Odc2p are 61% identical in sequence and cluster on a phylogenetic tree together with the previously identified Sfc1p and citrate carrier Ctp1p (Fig. 1). The EPRA method demonstrated that

Odc1p and Odc2p transport C5-C7 oxodicarboxylates with the highest specificity for 2-oxoadipate and 2-oxoglutarate by a counter-exchange mechanism; they also transport the corresponding dicarboxylates and malate to a lesser extent by the same mode of transport [84]. Odc1p expression is repressed by glucose and Odc2p is constitutively expressed, suggesting that Odc1p has a particular role under respiratory conditions, whereas Odc2p plays a role under anaerobic conditions and in the presence of glucose. In yeast, lysine is synthesized via the 2-aminoadipate pathway in which the precursor 2-oxoadipate is produced in the mitochondrial matrix and the intermediate 2-aminoadipate is converted into lysine in the cytoplasm. The transport characteristics of Odc1p and Odc2p suggest that they export 2-oxoadipate from the mitochondrial matrix in exchange for 2-oxoglutarate or malate (Fig. 3). Another important physiological role of the Odc1–2p isoforms in yeast could be the mitochondrial export of oxoglutarate in exchange with malate for the transfer of reducing equivalents by the malate/aspartate shuttle (Fig. 3) and cytosolic nitrogen assimilation.

In the search for human homologs of yeast Odc1p and Odc2p before human gene sequencing was completed, ESTs of *C. elegans* and *Drosophila melanogaster* were first screened, which resulted in finding one protein product in each organism with 36–38% identity with the yeast proteins. These two ESTs were used to find a partial human cDNA, which was extended producing a protein sharing 32 and 33% sequence identity with Odc1p and Odc2p, respectively. This protein was proved by the EPRA method to be the human oxodicarboxylate carrier (encoded by *SLC25A21* and called SLC25A21, or ODC) [85]. Indeed, the recombinant and reconstituted ODC transports 2-oxoadipate and 2-oxoglutarate with Km values of 0.11 and 0.22 mM, respectively, by a counter-exchange mechanism. It also transports 2-aminoadipate, citrate and a few other compounds with an affinity of 1 or 2 orders of magnitude lower than that for 2-oxoadipate. At variance with the yeast Odc1p and Odc2p, the human ODC does not transport malate. The main function of the ubiquitously expressed ODC is probably to catalyze the exchange between cytosolic 2-oxoadipate and intramitochondrial 2-oxoglutarate (Fig. 4). In the mitochondrial matrix 2-oxoadipate, which is formed in the catabolism of lysine, tryptophan and hydroxylysine, is oxidized by oxoadipate dehydrogenase. Under certain conditions ODC might import 2-aminoadipate, because the transaminases interconverting 2-aminoadipate and 2-oxoadipate are present both in the cytosol and in the mitochondrial matrix. The knock-down of an alternative splice variant of murine ODC (ODC-AS), that has 29 residues replacing the 22 N-terminal residues of the originally identified ODC, reduces lipid accumulation in white and brown adipocytes [86]. This finding was interpreted to indicate that ODC-AS may be involved in cytoplasmic triglyceride biosynthesis. Interestingly, the expression of ODC-AS is specifically down-regulated in brown adipocytes upon exposure to cold during lipid consumption to yield heat. Finally, it has been suggested that defective ODC could cause 2-oxoadipate acidemia, which is characterized by the accumulation and excretion of large amounts of 2-oxoadipate, 2-aminoadipate and 2-hydroxyadipate in urine [85]. However, molecular defect(s) in the *SLC25A21* gene have not yet been identified.

4.4.2. The NAD^+ carriers Ndt1p and Ndt2p (YIL006w and YEL006w)

The protein sequences of the yeast MCs Ndt1p and Ndt2p, which are 55% identical on sequence level, contain a characteristic variant of the second signature motif repeat in which the normally negatively charged residue is substituted with a tryptophan (PIWVVK) [87]. This sequence feature is found in a group of MC subfamilies clustering in a phylogenetic tree, which includes the yeast members Flx1p and Pyt1p that were known to transport FAD and pyrimidine nucleotides, respectively (Fig. 1). Therefore, the clustering of Ndt1p and Ndt2p suggested that their substrates could be nucleotide related. Indeed, the EPRA method demonstrated that the bacterially expressed Ndt1p transports NAD^+ as well as (d)AMP and (d)GMP at lower rates, but not NADH, $NADP^+$ and NADPH [88]. Concerning the mode of transport, Ndt1p catalyzes fast antiport and slow uniport of NAD^+. The affinities of Ndt1p for NAD^+, AMP and GMP are about 0.35, 0.98 and 0.95 mM, respectively. Ndt2p was expressed in *E. coli* but could not be renatured and/or reconstituted functionally. Given i) the high sequence identity between Ndt1p and Ndt2p, ii) that reduced levels of mitochondrial NAD^+ and NADH were found in the *NDT1*Δ and *NDT2*Δ single mutants and even more so in the double deletion strain, iii) that the activities of the NAD^+-dependent mitochondrial enzymes pyruvate dehydrogenase and acetaldehyde dehydrogenase were reduced in the *NDT1*Δ and *NDT2*Δ single mutants and even more in the double mutant, and iv) that the activities of these enzymes in mitochondrial extracts of the mutant strains were increased to the level of the wild-type extract by adding NAD^+ [88], it was inferred that the function of both Ndt1p and Ndt2p is to catalyze the uptake of NAD^+ into mitochondria by uniport or in exchange with internal (d)AMP and (d)GMP (Fig. 3). Notably, no activity of NAD^+ synthetase or NMN adenylyltransferase was found in *S. cerevisiae* mitochondria, indicating that NAD^+ is not synthesized in yeast mitochondria and has to be imported from the cytoplasm [88]. Recently, it has been proposed that a NAD^+ carrier, similar to that of yeast and plants, is absent in mammalian mitochondria which contain an NMN adenylyltransferase [89].

4.4.3. The citrate-oxoglutarate carrier (YMR241w or COC1)

The closest homolog of Yhm2p (encoded by *YMR241w* and named Coc1p) is Dic1p with 24% identical sequence. However, the amino acids of the similarly located putative binding site of Yhm2p are nearly identical to those of the previously identified rat (CTP) and yeast (Ctp1p) citrate carriers [90,91]. This observation led to the hypothesis that citrate could be a substrate of Yhm2p [92]. Indeed, as assessed by the EPRA method the reconstituted Yhm2p was found to translocate citrate, oxoglutarate and, to a lesser extent, oxaloacetate, succinate and fumarate by a counter-exchange mechanism. The Km of Yhm2p for citrate and oxoglutarate were about 0.2 and 1.2 mM, respectively. Unlike the citrate carriers, Yhm2p does not transport malate and isocitrate and is not inhibited by 1,2,3-benzenetricarboxylate. *YHM2*Δ cells exhibited an increase in the NADPH/$NADP^+$ ratio in their mitochondria and a decrease in the NADPH/$NADP^+$ and GSH/GSSG ratios in the cytosol as compared to wild-type cells. Furthermore, *YHM2*Δ cells and more so *YHM2*Δ/*ZWF1*Δ cells exposed to H_2O_2 responded to the oxidative insult less efficiently than did wild-type cells [92]. These results suggest that in *S. cerevisiae* Yhm2p, or Coc1p, as a single membrane component, can catalyze the citrate-oxoglutarate NADPH shuttle from the mitochondrial matrix to the cytosol (Fig. 3). Evidence is still lacking to suggest whether this shuttle can operate in the reverse direction under different conditions. Notably, the mitochondrial citrate (isocitrate)-oxoglutarate NADPH shuttle that has been proposed to exist in mammals would involve two membrane components, i.e. the citrate/malate and oxoglutarate/malate carriers.

4.4.4. The adenosine 5′-phosphosulfate carrier (YPR011c)

Phylogenetic analyses showed that YPR011cp (or APS carrier encoded by *YPR011c*) is found in a cluster that includes MCs transporting CoA (i.e., human SLC25A42, yeast Leu5p, Arabidopsis AT1G14560 and AT4G26180) and adenine nucleotides ([6] and Fig. 1). Furthermore, MCs for CoA are the closest homologs of YPR011cp in humans and Arabidopsis with 29–34% sequence identity [93]. However, YPR011cp was unable to complement a *LEU5*Δ strain suggesting that it does not transport CoA. Instead, transport measurements carried out with the EPRA method demonstrated that reconstituted YPR011cp transports Pi, sulfate, adenosine 5′-phosphosulfate (APS) and 3′-phospho-adenosine 5′-phosphosulfate (PAPS) almost exclusively by an exchange mechanism [93]. APS and PAPS are structurally very similar to adenosine 3′,5′-diphosphate (PAP), which is a substrate for the human CoA carrier SLC25A42, but is not transported by YPR011cp. The inhibition constants (Ki)

of APS and PAPS for the YPR011cp-mediated [^{35}S]sulfate/sulfate exchange are 38 and 96 μM, respectively, compared to 343 μM for sulfate and 420 μM for Pi. It is thought that APS is translocated across the mitochondrial membrane via YPR011cp in exchange for Pi or sulfate (Fig. 3), which can recycle back via other MCs. Moreover, evidence has been provided that the APS carrier (or Apsc1p) is involved in yeast thermotolerance and in the synthesis of methionine and glutathione at elevated temperatures [93].

4.5. Other identified S. cerevisiae MCs

The yeast MC gene *YMR166c* (currently named *MME1*, mitochondrial magnesium exporter 1) was initially isolated as a suppressor of the *MRS2*Δ phenotype (Mrs2p being a mitochondrial CorA-like Mg^{2+} transporter). Subsequent deletion of *MME1* led to an increase in mitochondrial Mg^{2+} levels, and the EPRA method showed the ability of Mme1p to transport Mg^{2+} [94]. Five other MCs from *S. cerevisiae* have been discovered based on methods other than transport assays with isolated recombinant proteins reconstituted into liposomes. Measurements of FAD export from submitochondrial vesicles of wild-type, mutated and deleted Flx1p (encoded by *YIL134w*) strains suggested that Flx1p is responsible for the transport of FAD [95]. Measurements in mitochondria of a *LEU5*Δ strain of potential Leu5p (encoded by *YHR002w*) substrates linked to mitochondrially located leucine biosynthesis, such as CoA and α-isopropylmalate, showed that Leu5p is required for the import of CoA, or a precursor of CoA, into mitochondria [96]. Transport experiments performed with mitochondria from wild-type, *SAL1*Δ and *AAC1–3*Δ yeast strains demonstrated that Sal1p (encoded by *YNL083w*) catalyzes a Ca^{2+}-stimulated transport of ADP, ATP, ATP-Mg and Pi [97] similarly to the human ATP-Mg/Pi carrier (APC) homologs previously discovered [73] (see Section 5.1.3). Through the characterization of strains engineered by yeast genetics Mrs3p and Mrs4p (encoded by *YJL133w* and *YKR052c*, respectively) were found to have an important role in mitochondrial iron accumulation [98,99]. Finally, based on 40% sequence identity between the first identified yeast Pi carrier Mir1p (encoded by *YJR077c*) and Pic2p (encoded by *YER053c*), complementation and mitochondrial swelling studies performed with *MIR1*Δ suggested that Pic2p is also a Pi carrier [100].

5. Other identified human MCs

The genome of *H. sapiens* encodes 53 MCs. In addition to the 6 disclosed before 1996 (see Section 2) and those starting from the yeast orthologs (see Section 4), 13 other human MCs have been identified using information originating from studies with intact mitochondria and from phylogenetic analyses. Fig. 2 depicts a phylogenetic tree of all MCs from *H. sapiens*; those identified and characterized until now in terms of substrate specificity and other characteristics are shown in red.

5.1. Human MCs discovered based on transport activities revealed from studies with intact mitochondria

5.1.1. The aspartate–glutamate carriers (SLC25A12 or AGC1 and SLC25A13 or AGC2)

One of the MCs identified in man and then in yeast (Section 4.3.3) is the mitochondrial aspartate–glutamate carrier (abbreviated as AGC). Its ability to exchange aspartate for glutamate (or cysteinesulfinate) had been described in mitochondria and in liposomes reconstituted with the carrier purified from mitochondrial extracts [12,101,102]. However, the protein(s) responsible for this exchange activity had not been identified at the molecular level. The AGC molecular identification project began when Dr. T. Saheki and Dr. J. Satrustegui asked our group to collaborate in finding the function of citrin (SLC25A13, AGC2) and aralar (SLC25A12, AGC1), respectively. Both proteins were very interesting because they bind Ca^{2+}, are located in mitochondria and have a peculiar structure consisting of two domains: the C-terminal domain, that contains all the characteristic sequence features of the MCF, and the N-terminal domain that contains four EF-hand Ca^{2+}-binding motifs and protrudes outside the inner mitochondrial membrane. Furthermore, AGC2 was present in all the tissues examined, whereas AGC1 was expressed only in brain, heart and skeletal muscle.

By means of the EPRA method, it was found that AGC1 and AGC2, as well as their C-terminal domains, transport L-aspartate, L-glutamate and L-cysteinesulfinate by strict counter-exchange, but not many other compounds including the D-isoforms of aspartate and glutamate, L-aminoadipate, L-asparagine, L-glutamine and L-homocysteinesulfinate [83]. Furthermore, all four recombinant proteins exhibited a higher affinity for aspartate (Km of about 50 μM) than for glutamate (Km of about 200 μM). In addition, in reconstituted liposomes AGC1–2 and their C-terminal domains displayed increased $^{14}C\text{-}Asp_{out}/Glu_{in}$ and decreased $^{14}C\text{-}Glu_{out}/Asp_{in}$ exchange rates in the presence of a membrane potential ($\Delta\Psi$) of about 100 mV positive inside. By contrast, no effect was observed when homo-exchanges, i.e. Asp/Asp or Glu/Glu, were measured under the same conditions. Given that the C-terminal domains of AGC1 and AGC2 account for all the transport properties and activity (in terms of turnover number) of the entire proteins, they constitute the catalytic portion of these carriers while the N-terminal domains containing the Ca^{2+}-binding sites comprise their regulatory portion [83]. This conclusion was confirmed by the finding that in Chinese hamster ovary (CHO) cells overexpressing AGC1 or AGC2 the agonist-induced synthesis of ATP in mitochondria (triggered by an agonist-induced calcium signal and monitored using a mitochondrially targeted luciferase) was much higher than in control cells, whereas in CHO cells overexpressing the C-terminal domains of either AGC1 or AGC2 it was virtually identical to that observed in control cells [103]. Therefore, the absence of the Ca^{2+}-binding regulatory domains of AGCs abolishes the activation of mitochondrial metabolism, demonstrating that Ca^{2+} is the mediator of this effect and AGCs are a sensor of cytosolic Ca^{2+}. These results clearly point to an unexpected novel mechanism of Ca^{2+} signaling through the regulation of a carrier activity. Recently, the crystal structures of the N-terminal domain of AGC1 and AGC2 have been solved and a mechanism explaining how Ca^{2+} activates AGC activity has been proposed [104].

Along with the oxoglutarate carrier (OGC), AGCs are essential components of the malate–aspartate shuttle, which transfers the reducing equivalent of NADH from the cytosol to the mitochondrial matrix (Fig. 4). AGCs are also important for exporting aspartate to the cytosol, which is necessary for the urea cycle, translation, gluconeogenesis from lactate, and purine and pyrimidine biosynthesis in cells such as hepatocytes, whose extracellular aspartate uptake is limited. Furthermore, cysteinesulfinate, an intermediate in cysteine degradation to sulfate, most likely relies on AGCs for import into the mitochondrial matrix (Fig. 4). The relevance of AGC1 and AGC2 in cell metabolism is demonstrated by the existence of genetic diseases associated with these transporters. Mutations in the AGC1 gene (*SLC25A12*), which markedly reduce transport activity of the encoded carrier, cause AGC1 deficiency (OMIM 612949) [105,106]. The disease symptoms are congenital hypotonia, developmental delay, and infantile epilepsy in conjunction with severe hypomyelination and reduced N-acetyl-aspartate in the brain. It is reckoned that lack of functional AGC1, which is the predominant AGC isoform in neurons, diminishes cytosolic aspartate required for myelin biosynthesis via N-acetyl-aspartate, while the reducing equivalent transfer of NADH is preserved by the glycerol-3-phosphate shuttle [26,27]. Notably, AGC1 knockout mice display growth retardation, impaired central nervous system myelination and reduced brain levels of N-acetyl-aspartate [107]. In addition, AGC1 has also been associated with autism [108]. Mutations in the gene encoding AGC2 (*SLC25A13*) cause two age-dependent diseases: neonatal intrahepatic cholestasis by citrin deficiency (NICCD, OMIM 605814) and adult-onset type-II citrullinemia (CTLN2, OMIM 215700), which are mainly due to lack of aspartate in the

cytosol and impaired cytosolic NADH reoxidation in the liver (because AGC1 is absent in this organ). NICCD manifests with some of the following symptoms: transient intrahepatic cholestasis, failure to thrive, hepatomegaly, citrullinemia, ketotic hypoglycemia, hypoproteinemia, elevated threonine-to-serine ratio and increased levels of Met, Thr, Tyr, Phe, Lys, Arg and galactose [109]. NICCD is usually benign and the symptoms disappear after 1 or a few years of age. However, in adulthood the patients may develop type-II citrullinemia. CTLN2 is characterized by dislike for sugars, preference for fat and proteins, alcohol intolerance, citrullinemia, hypoproteinemia, hyperammonemia, which leads to encephalopathy and neuropsychiatric symptoms, and in some patients hyperlipidemia and fatty liver [110]. For other aspects concerning AGCs the reader is referred to [111].

5.1.2. The glutamate carriers (SLC25A22 or GC1 and SLC25A18 or GC2)

Previous studies with intact isolated mitochondria have shown that glutamate can also be transported by a carrier other than AGC [12]. However, again the protein(s) responsible for this activity had not been identified or isolated from mitochondria. In this case the sequences of the two AGC isoforms were used to screen eukaryotic databases to find candidates for this putative glutamate carrier. Two human ESTs encoded proteins that were 63% identical to each other and 33% identical to the C-terminal domains of the two AGC isoforms [112]. These two proteins were localized to mitochondria (Lasorsa F. M. and Palmieri F. unpublished data), and the transport properties of recombinant GC1 and GC2, established by the EPRA method, showed that they are isoforms of a mitochondrial glutamate/H^+ symporter [112]. Indeed, both isoforms transport only L-glutamate and not structurally related compounds such as D-glutamate, aspartate, glutamine, asparagine, or any other compound tested; they transport L-glutamate by an electroneutral H^+-compensated mechanism, and are inhibited by tannic acid, bromocresol purple and SH reagents. However, the two GC isoforms differ in their kinetic parameters, since isoform 1 has a much higher Vmax and Km for glutamate than isoform 2. They also differ in tissue distribution because GC1 is expressed in higher amounts than GC2 in all tissues (except brain) and is particularly abundant in liver and pancreas. From a physiological perspective, GCs play important roles in amino acid degradation, nitrogen metabolism and the urea cycle. Given that the GC-mediated glutamate transport across the mitochondrial membrane is dependent on ΔpH, the entry of glutamate via GCs into the mitochondrial matrix, where glutamate dehydrogenase (GDH) is located, is favored in energized mitochondria. It has been calculated that the rate of glutamate transport into mitochondria would be high enough to feed the urea cycle, even if glutamate were the only ammonium source for mitochondrial synthesis of carbamoyl phosphate. However, under certain conditions GCs may also export glutamate from mitochondria, for example, when ammonia rather than glutamate is the major nitrogen source for urea synthesis or when glutamate is produced inside the matrix from proline oxidation (Fig. 4). In this respect, it is worth mentioning that GC1 knockdown in insulin-secreting β-cells and isolated rat islets markedly inhibits the secretory response to high glucose as a result of reduced transfer of glutamate from mitochondria to cytoplasm [113]. Considering the organ expression levels and kinetic constants of the two GC isoforms, GC2 probably fulfills the basic cellular demand for mitochondrial glutamate transport whereas GC1 is required in specific tissues for more active transport, for example in association with urea cycle or protein-rich diets. Different mutations in the *SLC25A22* gene encoding GC1 cause neonatal epileptic encephalopathy with suppression bursts (NEESBs, OMIM 609304) [114,115] and a different form of early onset epilepsy, known as migrating partial seizures in infancy (MPSI) [116]. These two diseases have several clinical symptoms in common but differ in seizure type and electroencephalogram pattern. Both NEESBs- and MPSI-causing mutations abolished glutamate transport in reconstituted liposomes.

5.1.3. The ATP-Mg/phosphate carriers (SLC25A24/A23/A25/A41 or APC1–4)

Knowledge of a third transport activity shown in intact mitochondria and consisting of a Ca^{2+}-activated exchange of ATP-Mg for Pi has been very useful for the molecular identification of the ATP-Mg/Pi carrier (APC). Indeed, by using the sequence of the human AAC (AAC1 encoded by *SLC25A4*) three human ESTs encoding peptides containing Ca^{2+}-binding motifs in their sequences were found. The full-length cDNAs encoded three proteins that had an overall structure similar to that of the two isoforms of AGC, i.e. characterized by the presence of a long N-terminal domain containing EF-hand Ca^{2+}-binding motifs. The three APCs encoded by *SLC25A24* (APC1), *SLC25A23* (APC2), and *SLC25A25* (APC3) and sharing 66–75% sequence identity were expressed in bacteria and identified by the EPRA method. In fact, recombinant APC1 and the C-terminal domains of both APC1 and APC2 transport P_i, ATP-Mg, ATP, ADP and AMP by a strict counter-exchange mechanism [73]. Deoxyadenine nucleotides, 3′-AMP, 3′-5′-ADP, pyrophosphate as well as the G, C, T and U nucleotides are also transported by the same mechanism but to a much lesser extent. The Km of APC1 and APC2 for external ATP-Mg, ATP and ADP range between 0.2 and 0.5 mM and are lower than those for external AMP (0.9 mM) and Pi (1.6 mM). Furthermore, for both APC1 and APC2 the rate of all the exchanges tested were unaffected by valinomycin in the presence of a K^+ gradient, indicating that APC proteins catalyze an electroneutral exchange of substrates. In contrast, in the presence of the K^+/H^+ exchanger nigericin and of an inwardly directed K^+ gradient (conditions that create a pH difference across the liposomal membranes, basic inside the vesicles), the uptake of [^{14}C]ATP in exchange for internal ATP-Mg or Pi was stimulated, whereas the uptake of [^{14}C]ATP-Mg in exchange for internal ATP was decreased. Remarkably, all the homo-exchanges tested and the ATP-Mg/P_i exchange were not affected. Therefore, the ATP/ATP-Mg and the ATP/P_i hetero-exchanges are driven by ΔpH, indicating that the charge imbalance of the exchanging substrates is compensated by the movement of protons [73]. Interestingly, APCs differ from AAC because they have a much broader substrate specificity, catalyze an electroneutral H^+-compensated counter-exchange, are poorly inhibited by the potent inhibitors of AAC, carboxyatractyloside and bonkgrekic acid, and possess three EF-hand Ca^{2+}-binding sites. Due to the high sequence identity of the three APC isoforms it was assumed that APC3 also had transport characteristics similar to APC1 and APC2, although it could not be renatured/reconstituted in a functional form for biochemical characterization despite successful recombinant expression in *E. coli*. In further studies, SLC25A41 was identified as a fourth human APC isoform, which unlike the previously discovered APC1–3, is calcium-independent [117]. Recently, the crystal structure of the N-terminal intermembrane space domain of APC1 has been determined and mechanisms for the Ca^{2+} activation of these proteins have been suggested [118,119].

The metabolic function of APCs is to catalyze net transport of adenine nucleotides across the mitochondrial inner membrane by using P_i as counter-substrate (Fig. 4), which can be subsequently transported back to its original compartment by PiC (SLC25A3). Therefore, APCs are involved in regulating the mitochondrial concentration of adenine nucleotides, which affects the activities of adenine nucleotide-dependent enzymes engaged in mitochondrial matrix processes, such as gluconeogenesis from lactate and the urea cycle as well as mitochondrial protein import, replication, transcription and translation. Interestingly, knockdown of APC1, which is upregulated in a variety of tumors, increases the mitochondrial matrix concentration of free Ca^{2+}, which was interpreted to suggest a reduced ion sequestration by the decreased level of matrix adenine nucleotides [120] (but see [121]). For other aspects concerning APCs the reader is referred to [111].

5.2. Human MCs discovered using phylogenetic analysis

5.2.1. The ADP/ATP carrier isoform 4 (SLC25A31 or AAC4)

By screening human databases with the human AAC1 (SLC25A4) sequence a MC of unknown function was found. This protein clusters with the three known isoforms of AAC in a phylogenetic tree of human MCs

(Fig. 2), is 66–68% identical to human AAC1–3 (which are 88–92% identical among themselves), and contains the characteristic RRRMMM motif of the AAC subfamily. By means of the EPRA method, reconstituted AAC4 was proven to be a novel isoform of AAC [122]. Indeed, like AAC1–3 AAC4 i) transports ADP, ATP, dADP and dATP but none of the other compounds tested including many other (deoxy)nucleotides, ii) functions by an obligatory counter-exchange, iii) is highly sensitive to carboxyatractyloside and bonkgrekic acid, and iv) the physiologically relevant ADP_{out}/ATP_{in} exchange (Fig. 4) is dependent on the $\Delta\Psi$ applied across the liposomal membrane, i.e. is electrophoretic, whereas the homo-exchanges were not affected by $\Delta\Psi$. At variance with AAC1–3, AAC4 exhibits a much lower affinity for its substrates, Km being 72 μM for ADP and 120 μM for ATP. Another peculiarity of AAC4 is its nearly exclusive expression in testis [122]. Notably, AAC4-deficient male mice displayed reduced testicular size, germ cell meiosis arrest and infertility demonstrating a crucial role for this carrier in spermatogenesis [123].

5.2.2. The carrier for CoA and 3′-phosphoadenosine 5′-phosphate (SLC25A42 or CoAPC)

Prior to the molecular identification of the MC for CoA and adenosine 3′,5′-diphosphate (PAP) (encoded by *SLC25A42*), this carrier was known to be highly expressed in virtually all human tissues [124]. Furthermore, it clustered together with yeast Leu5p [96] and a protein of unknown function, the human SLC25A16, in a phylogenetic tree of *H. sapiens* and *S. cerevisiae* MCs. Transport measurements carried out with the EPRA method provided evidence that SLC25A42 transports CoA, PAP, (deoxy)adenine nucleotides and dephospho-CoA [125]. Among the transported substrates, this carrier displays the highest affinity for PAP (about 27 μM) while the affinity for CoA (about 100 μM) is equal to or slightly higher than the free CoA concentration in the cytosol. The main physiological function of the strict antiporter SLC25A42, or CoAPC, is the import of CoA (which is synthesized outside mitochondria) into the mitochondrial matrix in exchange for internal PAP (generated from CoA) or other substrates (Fig. 4). CoA is a cofactor of a long list of matrix enzymes involved in the β-oxidation of fatty acids, citric acid cycle, heme biosynthesis, type II fatty acid synthesis (lipoate production), *N*-acetylglutamate (a regulator of the urea cycle) synthesis, branched-chain amino acid catabolism as well as acetylation of proteins. Recently, a missense mutation in the *SLC25A42* gene has been found to cause mitochondrial myopathy leading to lactic acidosis, muscle disorganization and weakness [126]. This disease highlights the important physiological role of CoAPC.

5.2.3. The peroxisomal carrier for CoA, FAD and NAD^+ (SLC25A17 or CFNC)

SLC25A17 is localized in peroxisomes [127]. A clue about which substrate(s) could be transported by SLC25A17 came from its clustering with the carriers for FAD/folate, pyrimidine nucleotides, NAD^+, the peroxisomal Ant1p and its relatives in Arabidopsis [6,33,63,74,88,95,128–131]. By involving a wide range of substrates the EPRA method showed that recombinant SLC25A17 transports FAD, FMN, CoA, acetyl-CoA, NAD^+, AMP, ADP and PAP, but not $NADP^+$, NMN, ATP or many other compounds [132]. SLC25A17, or CFNC, catalyzes almost exclusively a counter-exchange of substrates. The affinities of CoA, FAD and NAD^+ for SLC25A17 are lower or not much higher than the cytosolic-free concentrations of these cofactors. The physiological role of SLC25A17 is to catalyze the import of CoA, FAD and NAD^+ into peroxisomes, where they are essential for the activity of various enzymes (Fig. 4). The most likely candidates to exchange with external CoA, FAD and NAD^+ are PAP (generated from CoA), FMN (generated from FAD), and AMP and ADP (generated from ATP) in the peroxisomal lumen.

5.2.4. The four-carbon metabolite/phosphate carrier (SLC25A8 or UCP2)

SLC25A8 clusters together with UCP1, UCP3–6 as well as with the dicarboxylate carrier (DIC) and oxoglutarate carrier (OGC) (Fig. 2). Based on its sequence homology with UCP1, SLC25A8 was usually considered to be an uncoupling protein. However, of the six human UCPs, only UCP1 has been demonstrated to carry protons and act as an uncoupling protein in mitochondria [133,134]. The EPRA method demonstrated that recombinant UCP2 transports aspartate, malate, malonate, oxaloacetate, $Pi + H^+$ and sulfate $+ H^+$ [135]. The proposed biochemical role of UCP2 is to export four-carbon metabolites from mitochondria in exchange for cytosolic Pi (or sulfate) and H^+ (Fig. 4), a reaction that would be stimulated in respiring cells by both the electrical and chemical components of the proton motive force. Consistently, UCP2-knockdown in HepG2 cells enhances mitochondrial oxidation of glucose and diminishes glutaminolysis [135]. For an account of the vast literature on UCP2 the reader is referred to [136].

5.2.5. The carrier for basic amino acids (SLC25A29 or hBAC)

SLC25A29 clusters together with the carnitine-acylcarnitine carrier (CAC), ORC1, ORC2 and three unknown MCs (Fig. 2). SLC25A29 was thought to be a CAC-like transporter or ornithine transporter isoform 3 (ORNT3) [137,138]. Recently, biochemical characterization of recombinant SLC25A29 performed using the EPRA method has demonstrated that this carrier transports arginine, lysine, homoarginine, methylarginine and, to a much lesser extent, ornithine and histidine, but not carnitine, acylcarnitines or many other tested compounds [139]. The transport affinities (Km) of SLC25A29 for arginine and lysine are about 0.42 and 0.71 mM, respectively. The Ki values of ornithine and histidine for the SLC25A29-mediated arginine/arginine homo-exchange are about 1.6 and 2.7 mM, respectively. Therefore, this carrier is a transporter for basic amino acids with a preference for arginine and lysine. Given its ability to catalyze substantial uniport (besides a counter-exchange transport as do all MCs), a primary physiological role of SLC25A29, or hBAC, is to import arginine, lysine and histidine into the mitochondrial matrix for protein synthesis and amino acid degradation (Fig. 4). In addition, in mitochondria arginine is a substrate of arginase II and NO synthase, and lysine is a substrate for the first enzyme of the saccharopine pathway, which is the major route for lysine degradation in upper eukaryotes.

6. MCs from *A. thaliana*

A significant advance in characterizing members of the MCF has also been made in *A. thaliana* [6], the plant model organism whose genome was the first to be completely sequenced in the plant kingdom. The number of MCs in the genome of plants (dicots, monocots and algae) is similar to or higher than the number found in yeast and mammals; the genome of *A. thaliana* encodes 58 MCs (Fig. 5). These carriers have generally been identified at the molecular level starting from previously discovered MCs in *S. cerevisiae* and/or *H. sapiens*. Several Arabidopsis MCs were disclosed and biochemically characterized by expression in *E. coli* as inclusion bodies and by purification, renaturation/reconstitution and transport assays in liposomes (the EPRA method). This group of MCs comprises: AT5G19760, the di- and tri-carboxylate carrier DTC [140]; AT2G33820 and AT1G79900, the basic amino acid carriers AtBAC1 and AtBAC2 [141,142]; AT4G39460 and AT1G34065, the *S*-adenosylmethionine carriers SAMC1/SAMT1 and SAMC2 [143,144]; AT2G22500, AT4G24570 and AT5G09470, the dicarboxylate carriers DIC1, DIC2 and DIC3 [145]; AT4G01100, the adenine nucleotide transporter ADNT1 [146]; AT2G47490 and AT1G25380, the NAD^+ transporters AtNDT1 and AtNDT2 [130]; AT2G39970, the NAD^+, NADH, CoA and adenosine 3′,5′-phosphate carrier PXN [30,147]; AT5G01500, the 3′-phosphoadenosine 5′-phosphosulfate (PAPS) and 3′-phosphoadenosine 5′-phosphate (PAP) transporter PAPST/AtTAAC [148]; and AT5G61810, AT5G51050 and AT5G07320, the ATP-Mg/Pi carriers AtAPC1, AtAPC2 and AtAPC3, respectively [149]. These MCs are localized to mitochondria with the exception of NDT1 and PAPST, which are found in chloroplasts, PXN in peroxisomes, and SAMC1 in both mitochondria and chloroplasts. DTC, which is the closest Arabidopsis homolog of bovine oxoglutarate carrier (OGC) with 40%

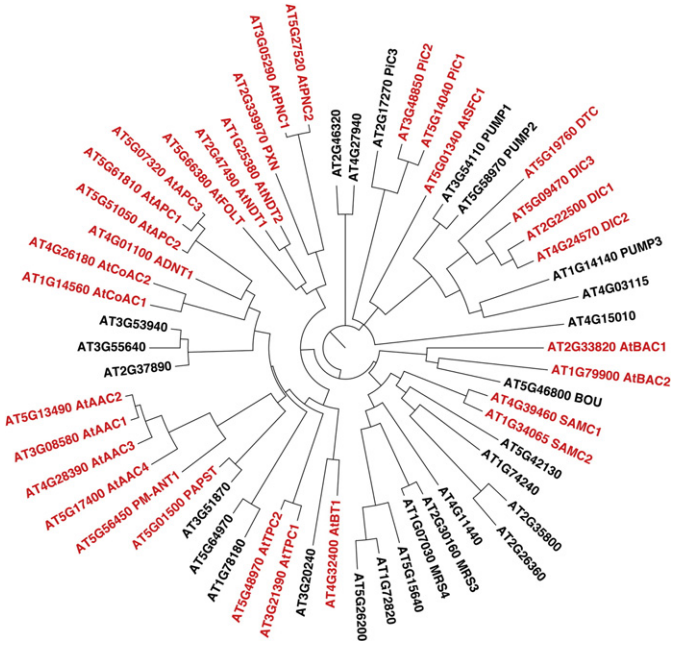

Fig. 5. Phylogenetic tree of *Arabidopsis thaliana* MCs. The tree of the 58 MC sequences from *A. thaliana* was made by using PhyML v3.1 in seaview4 [163] from a Muscle multiple-sequence alignment and drawn in FigTree v1.4.2. The names of MCs and their acronyms are found on the terminal nodes with the biochemically characterized ones colored in red.

sequence identity, was shown to transport oxoglutarate, malate, succinate, maleate, oxaloacetate and malonate, like OGC, but also tricarboxylates such as citrate, isocitrate, *cis*-aconitate and *trans*-aconitate by obligatory electroneutral exchanges [140]. AtBAC1 and AtBAC2, the closest Arabidopsis homologs of yeast Ort1p (with 29% sequence identity), transport arginine, lysine, histidine and ornithine; AtBAC2 also transports citrulline [141,142]. Furthermore, both AtBAC1 and AtBAC2 are able to complement an *ORT1* null mutant strain. Although Arabidopsis DIC1–3 are the closest homologs of human DIC (with 34–38% sequence identity), they were originally classified as uncoupling proteins and named PUMP3–5; however, they were later shown to transport malate, oxaloacetate, succinate, Pi, sulfate, thiosulfate and sulfite [145], which is the typical substrate repertoire of the mitochondrial dicarboxylate carriers from other species. At the time of its identification, ADNT1 did not share any significant sequence similarity with biochemically characterized MCs (including AACs) and therefore many compounds were screened to identify its substrates [146]. Among the substrates tested ADNT1 was found to catalyze transport of (deoxy)adenine nucleotides and adenosine 5′-sulfophosphate in a carboxyatractyloside- and bongkrekic acid-insensitive counter-exchange reaction. AtNDT1 and AtNDT2, which are 26–35% identical to the two yeast NAD^+ carriers, transport NAD^+, ADP and AMP as well as other nucleotides to a much lower extent and can restore NAD^+ transport activity in *NDT1*Δ and *NDT2*Δ yeast strains [130]. The closest Arabidopsis relative of the human peroxisomal CoA, FAD and NAD^+ transporter SLC25A17 is PXN, which was shown to transport NAD^+, NADH, ADP and AMP [30] as well as CoA, acetyl-CoA and adenosine 3′,5′-phosphate [147]. The C-terminal domains of AtAPC1–3 have 43–48% identical protein sequence with those of human APC1–3. AtAPC1–3 transport mainly Pi, AMP, ADP, ATP and adenosine 5′-phosphosulfate (APS); AtAPC2 and AtAPC3 also transport sulfate and thiosulfate [149].

Another group of Arabidopsis MCs was discovered and characterized by expression in the plasma membrane of *E. coli* and transport experiments performed with whole cells. This method is limited to the substrates that lack endogenous transporters in host cells. All of them transport ATP and ADP: AT3G08580, AT5G13490 and AT4G28390, the mitochondrial ADP/ATP carriers AtAAC1, AtAAC2 and AtAAC3 [150]; AT5G17400, the ER-located AAC AtAAC4 [151]; AT3G05290 and AT5G27520, the peroxisomal nucleotide carriers AtPNC1 and AtPNC2 [131,152]; AT4G32400, the dually localized plastid and mitochondrial Brittle1-homolog AtBT1 [153,154]; and AT5G56450, the plasma membrane adenine nucleotide transporter PM-ANT1 [155].

The function of a third group of Arabidopsis MCs was suggested based on their complementation of *S. cerevisiae* deletion strains lacking the corresponding yeast homologs: AT5G01340, the succinate/fumarate carrier AtSFC1 complements the growth defect of the *SFC1*Δ yeast strain on ethanol [156]; AT5G14040 and AT3G48850, the Pi carriers PIC1 and PIC2 complement the double *MIR1*Δ*PIC2*Δ yeast strain phenotype and mitochondrial Pi-induced swelling [100]; AT5G66380, the folate carrier AtFOLT complements the growth defect and the folate concentration in mitochondria of auxotrophic CHO cells lacking the human folate carrier [129]; AT3G21390 and AT5G48970 complement the thiamine auxotrophy of the *TPC1*Δ yeast strain grown on fermentable carbon sources [157]; and AT1G14560 and AT4G26180 complement the growth defect of the *LEU5*Δ yeast strain grown on non-fermentable carbon sources [158]. These results indicate that this group of Arabidopsis carriers shares the essential transport function with their yeast counterpart, but their complete substrate specificities and kinetic properties may vary.

7. MCs and related diseases

In the last two decades the rapid advance in the molecular identification and characterization of MCs aided the discovery of a remarkable number of MC-associated diseases. Recently the advent of exon sequencing has further accelerated the process of identifying gene mutations of MCs, including MCs of unknown function. The 13 MC-related diseases (17 phenotypes) known to date are listed in Table 2 following the numbering of the defective carrier they have been associated with. These disorders are rare errors of metabolism caused by mutations of the genes encoding MCs. Some disorders are characterized by defective oxidative phosphorylation while others by specific metabolic dysfunctions, depending on the physiological role of the affected carrier in intermediary metabolism. Their symptomatology is therefore multifarious and dependent on the affected metabolism and its significance in specific tissues. With the exception of autosomal dominant progressive external ophthalmoplegia (PEO), all the other MC-related disorders are inherited in an autosomal recessive manner.

Deficiencies of MCs for aspartate/glutamate (AGC1 and AGC2), ornithine (ORC1), thiamine pyrophosphate (TPC), glutamate (GC1), *S*-adenosylmethionine (SAMC) and CoA plus PAP (SLC25A42 or CoAPC) have already been discussed in Sections 5.1.1, 4.2.1, 4.2.4, 5.1.2, 4.2.5 and 5.2.2, respectively. Other diseases listed in Table 2 are associated with MCs that have been identified before 1996, i.e. the carriers for citrate (CIC), Pi (PiC), ADP/ATP (AAC1) and carnitine/acylcarnitine (CAC), which have not been reviewed here. Information about these diseases can be found in the references within Table 2 and in previous reviews [27,159]. The disorders related to orphan MCs, i.e. those whose substrates have not yet been identified biochemically, are SLC25A38 and SLC25A46 deficiencies. Mutations in the *SLC25A38* gene cause nonsyndromic autosomal recessive congenital sideroblastic anemia (OMIM 205950) [160], which exhibits bone marrow erythroid precursor sideroblasts with defective heme biosynthesis and iron accumulation in mitochondria. These symptoms are similar to those caused by mutations in the gene for intramitochondrially-localized δ-aminolevulinic acid synthase 2. Based on these observations it has been suggested that SLC25A38 may transport δ-aminolevulinic acid, glycine or a similar substrate. Alterations in *SLC25A46* are responsible for a neurodegenerative disorder (OMIM 616505), which is characterized by optic atrophy, axonal peripheral neuropathy and cerebellar atrophy as well as decreased *N*-acetylaspartate and increased lactate levels in the central nervous system [161]. It has been proposed that SLC25A46 is located

Table 2
Diseases associated with mitochondrial carriers.

Gene/carrier-based name	OMIM/clinical name/phenotype	Defective carrier (acronym)	Mutation	References
SLC25A1, ClC deficiency	OMIM 190315	Citrate carrier (CIC)	14	[164,165]
SLC25A3, PiC deficiency	OMIM 610773	Phosphate carrier (PiC)	5	[166,167]
SLC25A4, AAC1 deficiency	OMIM 192600	ADP/ATP carrier 1 (AAC1)	2	[168,169]
	OMIM 609283, adPEO		5	[170]
SLC25A12, AGC1 deficiency	OMIM 612949	Aspartate/glutamate carrier 1 (AGC1)	3	[105,106]
SLC25A13, AGC2 deficiency	OMIM 603471, CTLN2	Aspartate/glutamate carrier 2 (AGC2)	94	[109,110]
	OMIM 605814, NICCD			[109]
SLC25A15, ORC1 deficiency	OMIM 238970, HHH syndrome	Ornithine carrier 1 (ORC1)	34	[56,60,171,172]
SLC25A19, TPC deficiency	OMIM 607196, Amish microcephaly	Thiamine pyrophosphate carrier (TPC)	1	[66,67]
	OMIM 613710, Neuropathy with striatal necrosis		1	[68]
SLC25A20, CAC deficiency	OMIM 212138	Carnitine/acylcarnitine carrier (CAC)	40	[77,173,174]
SLC25A22, GC1 deficiency	NEESBs, OMIM 609304	Glutamate carrier 1 (GC1)	3	[114,115]
	MPSI			[116]
SLC25A26, SAMC deficiency	OMIM 616794	*S*-adenosylmethionine carrier (SAMC)	4	[71]
SLC25A38 deficiency	OMIM 205950, Congenital sideroblastic anemia	?	24	[160]
SLC25A42 deficiency		CoA and PAP carrier (CoAPC)		[126]
SLC25A46 deficiency	OMIM 616505	?	6	[161]

in the mitochondrial outermembrane, has a role in mitochondrial fission and is not involved in substrate transport [161].

8. Conclusions and perspectives

Despite the considerable progress that has been made in the last two decades in discovering and characterizing numerous MCs from model organisms, there are still many MCs whose function remains unknown, including nearly half of those encoded by the genomes of *H. sapiens* and *A. thaliana* (Figs. 2 and 5). Future work is needed to identify the substrates of the orphan MCs and to discover the transporters for substrates, such as glutamine, glutathione, α-ketoisocaproate and essential amino acids, which are known to be translocated across the inner mitochondrial membrane. However, it should be highlighted that some of these transporters may not belong to the MCF. It is also possible that some of the MCs already characterized will be found to transport other substrates of physiological importance that have been overlooked by earlier studies.

Definite progress has also been made in our understanding of how MC activity is regulated, for example, by changes in driving forces, concentrations of the substrate transported, counter-substrate, activators and/or inhibitors. Regulation of gene expression at the transcriptional and translational level is also an important mechanism of MC activity modulation found in different tissues and under various physiological conditions. Unfortunately, this issue has not been exploited extensively yet (but see [26,162].

Another relevant and intriguing aspect for future investigation is the correlation between the *in vitro* activities of characterized MCs and their specific physiological roles *in vivo* in various tissues and cell types. A complicating factor in these studies might be the fact that the substrate specificities of several MCs are partially redundant leading to the potential overlap of physiological roles, as in the case of the identified isoforms of certain MCs.

As documented by this review and the vast literature cited herein, MC identification has contributed to shedding light on metabolic pathways with unkown transport reactions and gaining insight into the physiological role of MCs in cell metabolism and special cell functions. In addition, MC identification has aided the discovery of genes responsible for diseases and deepened our understanding of the molecular bases of their symptoms. It is hoped that the elucidation of the pathogenic mechanisms underlying MC-associated diseases will pave the way to the development of appropriate treatments.

Transparency document

The Transparency document associated with this article can be found, in online version.

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