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Mitochondrial transport and metabolism of the major methyl donor and versatile cofactor S-adenosylmethionine, and related diseases: a review^

Authors

Magnus Monné[1,2], Carlo M. T. Marobbio[1], Gennaro Agrimi[1], Luigi Palmieri[1,3,*] and Ferdinando Palmieri[1,3,*]

Affiliations

[1]Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Via E. Orabona 4, 70125 Bari, Italy. [2]Department of Sciences, University of Basilicata, Via Ateneo Lucano 10, 85100 Potenza, Italy. [3]CNR Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies (IBIOM), 70126 Bari, Italy.

*Corresponding authors: Ferdinando Palmieri and Luigi Palmieri, Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari, Via E. Orabona 4, 70125 Bari, Italy. E-mail address: ferdpalmieri@gmail.com; luigi.palmieri@uniba.it

^This paper is dedicated to the memory of Professor Vincenzo Zappia.

Abstract

S-adenosyl-L-methionine (SAM) is a coenzyme and the most commonly used methyl-group donor for the modification of metabolites, DNA, RNA and proteins. SAM biosynthesis and SAM regeneration from the methylation reaction product S-adenosyl-L-homocysteine (SAH) take place in the cytoplasm. Therefore, the intramitochondrial SAM-dependent methyltransferases require import of SAM and export of SAH for recycling. Orthologous mitochondrial transporters belonging to the mitochondrial carrier family have been identified to catalyze this antiport transport step: Sam5p in yeast, SLC25A26 (SAMC) in humans and SAMC1-2 in plants. In mitochondria SAM is used by a vast number of enzymes implicated in: the regulation of replication, transcription, translation and enzymatic activities; the maturation and assembly of mitochondrial tRNAs, ribosomes and protein complexes; and the

biosynthesis of cofactors, such as ubiquinone, lipoate and molybdopterin. Mutations in SLC25A26 and mitochondrial SAM-dependent enzymes have been found to cause human diseases, which emphasizes the physiological importance of these proteins.

Keywords: S-adenosyl-L-methionine; diseases; metabolism; methyltransferase; mitochondria; mitochondrial carrier; mitochondrial transport.

Abbreviations: AAC, ADP/ATP carrier; BCAA, branched-chain amino acid; BHMT betaine-homocysteine S-methyltransferase; DNMT, DNA methyltransferase; ETFβ, electron transfer flavoprotein subunit β; EPRA, expression purification reconstitution transport assay; HCys, homocysteine; LIAS, lipoyl synthase; MC, mitochondrial carrier; MOCO, molybdenum cofactors; MS, methionine synthase; MAT, S-adenosylmethionine synthetase; MT, methyltransferase; MTA, 5'-deoxy-5'methylthioadenosine; mtDNA, mitochondrial DNA; SAC, S-adenosylcysteine; SAH, S-adenosyl-L-homocysteine; SAHase, S-adenosylhomocysteine synthase; SAM, S-adenosyl-L-methionine; SAMC, S-adenosyl-L-methionine carrier; TCA, tricarboxylic acid; THF, tetrahydrofolate.

Introduction

S-adenosyl-L-methionine (SAM, also known as AdoMet) is a cofactor found in all known species and thought to be the second most commonly used enzyme substrate after ATP (1). It is synthesized in the cytoplasm from ATP and methionine, which in humans is an essential amino acid. SAM is formed by a covalent bond between the sulfur atom of methionine and the 5'-carbon of adenosine derived from ATP, which give rise to a positively charged sulfonium ion (**Fig. 1**). The properties of SAM used in catalysis are the electrophilic carbon centres adjacent to the positively charged sulfur atom, its ionic nature and its radical reactivity (2). SAM is the major methyl-donor reagent for essential methylation reactions of targets ranging from small metabolites to large biological macromolecules. These reactions are catalyzed by methyltransferases (MTs), which transfer the methyl group of the SAM sulfonium

ion to O, N, C or S atoms of their substrates and give rise to S-adenosyl-L-homocysteine (SAH) (3). SAH may be recycled into SAM in the cytoplasm by the methionine/SAM cycle. In various organisms SAM-dependent MTs are encoded by about 1% of the genes and are found in cytoplasm, nucleus, chloroplasts and mitochondria (4). SAM is also used as a cofactor or a cosubstrate by enzymes different from MTs for transfer of functional groups of SAM (other than the methyl group) and by the SAM-radical enzymes (2).

Approximately 30% of cellular SAM has been estimated to be located in mitochondria (5). SAM needs to be imported into mitochondria from the cytoplasm, and its uptake was first observed in isolated rat-liver mitochondria (6). Subsequently, the proteins transporting SAM across the mitochondrial membrane and their corresponding genes were identified in humans, plants and yeast (7-9). These orthologous transporters are members of the mitochondrial carrier (MC) family, which is named solute carrier family 25 (SLC25) in higher animals. MCs have characteristic sequence features, a six-transmembrane α-helical fold and transport specific substrates, such as cofactors, nucleotides, amino acids, dicarboxylates and inorganic anions (10-17). In the mitochondrial matrix SAM is used for methylating DNA, RNA and proteins, and for the biosynthesis of cofactors such as lipoate, ubiquinone (Coenzyme Q) and molybdopterin, as well as for biotin production in yeast and plants. Although the presence of methylated mitochondrial macromolecules had been known for quite some time, most of the SAM-dependent enzymes responsible for these modifications have been identified only in the last ten years (18-20). This review will focus on the mitochondrial transport and metabolism of SAM as well as on associated genetic diseases.

1. SAM biosynthesis, usage and recycling

The biosynthesis of SAM depends on the availability of the precursor methionine, which is synthesized by plants and microorganisms but not by animals and is, therefore, required in their diet. It has been suggested that the transporters SLC1A5, SLC3A1, SLC6A14, SLC6A19 and SLC6A20, among others, contribute to the intestinal absorption and cellular import of methionine (21). In the cytoplasm, methionine and ATP are condensed into SAM by S-adenosylmethionine synthetase (methionine adenosyltransferase, MAT) (**Fig. 2**) with pyrophosphate and phosphate

as byproducts (1). Many organisms contain multiple genes for MATs; in humans there are two isoforms, MATα1 is expressed only in hepatocytes (22), and MATα2, which has 84% sequence identity with the former and is found in non-hepatocyte cells together with its regulatory subunit MATβ (23,24). SAM biosynthesis takes place exclusively in the cytosol and nucleus except in hepatocytes where MATα1 is partially localized to mitochondria (24-27).

In cytoplasm, nucleus, mitochondria and chloroplasts MTs use SAM as a methyl donor for modifications of DNA, RNA, protein, lipids and metabolites, producing SAH as a byproduct. For example, in the cytoplasm SAM is used for the methylation of nitrogen atoms in various molecules to form adrenalin, phosphatidylcholine, 1-methylnicotinamide and creatine (28-30); in the nucleus it is the major donor for methylations of DNA and histones for transcriptional regulation, and of tRNA and rRNA in their maturation; in plant chloroplasts, it is imported for various roles in one-carbon metabolism (31).

SAH formed in the methylation reactions in various cellular compartments may be recycled into methionine in the cytoplasm in the so-called methionine or SAM cycle (**Fig. 2**). S-adenosylhomocysteine synthase (SAHase, also called adenosylhomocysteinase or S-adenosylhomocysteine hydrolase) breaks down SAH into adenosine and homocysteine (HCys). HCys may (i) be re-methylated by methionine synthase (MS) or betaine-HCys S-methyltransferase (BHMT), taking the methyl group from 5-methyltetrahydrofolate or betaine, respectively, to form methionine; (ii) enter the trans-sulfuration pathway leading to the synthesis of cysteine for translation and glutathione production; or (iii) be exported to the blood (32). MS has cobalamin as a cofactor, which, after being employed in a round of catalysis, requires the activity of MS reductase to be reactivated (33,34). BHMT is most strongly expressed in liver and kidney, where it probably plays a major role in methionine recycling (35,36). Methionine formed in reactions (i) can be utilized to synthesize SAM again. The SAM cycle is linked to the folate cycle through MS which transfers a methyl group from 5-methyltetrahydrofolate forming tetrahydrofolate (THF) (37). The majority of the folate cycle one-carbon units that are transferred onto HCys originally come from serine, which is converted into glycine in mitochondria by serine hydroxymethyltransferase (38). THF and its structural analogues are another example of biological methyl-group donors and are mostly involved in synthetic

reactions of nucleotides and amino acids (37). In order to control the SAM/SAH ratio many of the enzymes of the SAM and folate cycles are regulated at transcriptional and enzymatic activity level through covalent modifications and allosteric interactions with SAM (37, 39-41).

Chemical groups of SAM other than the methyl group are utilized by other enzymes (**Fig. 1**) (2). The SAM-radical enzymes, which often contain iron sulfur clusters, split the cofactor into a methionine and 5'-deoxyadenosine radical that is required in their catalysis. Most MTs bind SAM with a "SAM-dependent MT fold" consisting of a core structure of a mixed seven-stranded β-sheet, that is similar to the parallel 5-stranded NAD(P)-binding Rossmann fold (42-44). The SAM radical enzymes on the other hand have a TIM-barrel (Triose-phosphate Isomerase Mutase barrel) protein structure (45). Furthermore, for the maturation of tRNAs, SAM-dependent tRNA ribosyltransferase-isomerase ribosylates tRNA leaving adenine and methionine as byproducts. In the biosynthesis of polyamines (spermidine and spermine), the polyamine elongation enzymes transfer the propylamine group of decarboxylated SAM giving rise to 5'-deoxy-5'-methylthioadenosine (MTA) (46). In addition, in biotin biosynthesis the C_{α}-amino group of the SAM methionine is donated in an amination reaction.

2. Mitochondrial SAM transporters

2.1 The mitochondrial SAM transporters are members of the MC family

Besides SAM, MCs have been found to transport various substrates across the mitochondrial inner membrane: cofactors (e.g. CoA, thiamine pyrophosphate, NAD^+ and FAD), nucleotides (e.g. ADP, ATP and several dNTPs and (d)AMPs), amino acids (e.g. aspartate, glutamate, ornithine and arginine), carboxylated metabolites (e.g. 2-oxoglutarate, malate and citrate) and small inorganic ions (phosphate and sulfate) (47-51). The substrates of most characterized MCs, including those of SAM transporters, have been identified by the expression purification reconstitution transport assay (EPRA) approach, in which the protein is recombinantly expressed, purified and reconstituted into liposomes that are used in transport assays (50,52,53). This approach has also been used to determine other transport properties, among them the kinetic parameters and transport modes, i. e. if the MC catalyzes unidirectional (uniport) and/or exchange (antiport) transport of its

substrates (48). Actually, most MCs prefer the antiport mode of transport (54). The EPRA approach has also been used to study the functional consequences of the many MC mutations or variants found in humans (55-57).

The mitochondrial SAM carriers contain the typical sequence features of MCs. Indeed, they consist of about 300 amino acid residues, that are divided into three, almost equally long, sequence repeats each containing two transmembrane segments linked by a signature motif sequence PX[DE]XX[KR]X[KR]X_{20-30}[DE]GXXXX[WYF][KR]G (PROSITE PS50920, PFAM PF00153 and IPR00193) (58,59). Atomic-resolution X-ray crystal structures of the ADP/ATP carrier (AAC), which is an extensively studied member of the MC family, presumably represent the 3D-fold of all MCs (60-62). The AAC structures show that the 300-residue MC fold consists of six transmembrane α-helices (H1-H6) in a barrel with a central substrate translocation pore. The pore possesses two alternatively opened or closed gates: one towards the intermembrane space (cytoplasmic c-gate) and the other towards the matrix (m-gate). Based on transport experiments with several MC and the AAC structures, the substrate is thought to i) enter through the open gate from one side of the membrane, ii) bind the substrate binding site located centrally in the translocation pore, iii) trigger opening of the closed gate (and closing of the gate where it entered) and iv) exit on the opposite side of the membrane (63-69).

The substrate binding site of MCs has been proposed to enclose centrally-located residues in the translocation pore at the so-called contact points (I, II and III) on H2, H4 and H6, respectively, and surrounding residues (70-72). In particular, contact point II residues co-variate with the major classes of MC substrates: G[IVLM] for nucleotides, R[QHNT] for carboxylated metabolites and R[DE] for amino acids. The latter motif has been suggested to bind the C_α carboxylic and amino groups of amino acid substrates (70,73). Notably the SAM-transporting MCs, which have R[DE] in contact point II, cluster together with the MCs for amino acids rather than those for nucleotides in phylogenetic analysis (74). Their evolutionary path would therefore be associated with the methionine part of SAM and not the adenosine portion of SAM, which in fact is a nucleoside and not a nucleotide.

2.2 The yeast mitochondrial SAM carrier

One of the 35 MCs in *S. cerevisiae*, which is encoded by YNL003c (PET8), has been identified as a SAM transporter by the EPRA approach and named Sam5p (7). Besides SAM (Km of about 75 μM), Sam5p was shown to transport SAH and the non-physiological, structurally related substrates S-adenosylcysteine (SAC) and sinefungin (adenosylornithine), but not other cofactors, nucleotides, amino acids and carboxylated metabolites. Both uniport and antiport transport of SAM were observed, although the activity of the antiport reaction was much higher. The subcellular localization and physiological role of Sam5p in yeast were also investigated. The transporter is localized in mitochondria as shown by analysis of expressed Sam5p-GFP fusion protein in yeast. *Sam5* knockout cells displayed a petite phenotype when grown on non-fermentable carbon sources, whereas they were biotin auxotrophic on fermentable carbon sources because biotin synthesis requires mitochondrial SAM. Both phenotypes could be complemented by expressing a mitochondrially-targeted version of Sam1p (one of the two yeast SAM synthetases localized in the cytoplasm). It was concluded that Sam5p imports SAM, by uniport or in exchange for SAH, into mitochondria (7).

2.3 The human mitochondrial SAM carrier

The human homologue of yeast Sam5p with 43% sequence identity, SLC25A26 (SAMC), which is one of the 53 SLC25-members in humans, has also been characterized by the EPRA method (8). In common with the substrate specificity of Sam5p, SAMC also transports SAM, SAH, SAC and sinefungin. However, at variance with its yeast counterpart, SAMC has a higher affinity for SAM (Km of about 23 μM) and seems capable of almost only antiport transport of substrates. The mitochondrial localization of SAMC was demonstrated in CHO cells expressing a GFP conjugate of the protein. The organ distribution of SAMC was examined and its mRNA was shown to be expressed widely in human tissues, at very high levels in testis and at lower levels in liver, brain, heart, kidney, lung, skeletal muscle, pancreas, small intestine and spleen. Based on the biochemical characterization of SAMC, its physiological role was assigned as mitochondrial SAM import in exchange for SAH (8).

2.4 The plant mitochondrial SAM carriers

The transport properties of *A. thaliana* SAMC1 (At4g39460) and SAMC2 (At1g34065), which are homologues of Sam5p and SAMC (31-34% sequence identity), have also been investigated (9). By using the EPRA method it was shown that AtSAMC1 has a similar substrate specificity to those of Sam5p and SAMC, a Km of 95 μM for SAM and the ability of transporting its substrates by mainly a counter-substrate mechanism. AtSAMC2 could not be reconstituted functionally into liposomes; however, it can be assumed that it is a SAM transporter too, due to its high sequence identity with AtSAMC1 (64%) (9). Another study, where 6His-tagged AtSAMC1 was expressed in yeast, purified and reconstituted into liposomes that were used in transport assays, also showed that AtSAMC1 transports SAM and SAH, and has a Km for SAM of about 130 μM (75). The expression patterns of AtSAMC1 and AtSAMC2 in different plant tissues were analyzed by real-time reverse transcription PCR (9). AtSAMC1 mRNA was found in leaves, flowers, stems, roots and, at particularly high levels, in seedlings, whereas AtSAMC2 mRNA was found at lower levels than AtSAMC1 in almost all organs analyzed. Furthermore, the promoter region of At*SAMC1* and At*SAMC2* was fused to the gene reporter β-glucuronidase. This approach showed abundant expression of AtSAMC1 in the roots of seedlings, the first leaves, the sepals of flowers, the stigma of the pollen tubes and the silique vasculature, and no significant expression AtSAMC2 (9).

The subcellular localization of AtSAMC1 has been suggested to be mitochondrial by analysis of the GFP-fused protein (9). However, other reports have suggested that AtSAMC1 is found in the chloroplast envelope membrane based on: i) the prediction of an N-terminal chloroplast target peptide in AtSAMC1 by ChloroP/TargetP (76,77), ii) proteomic approaches (77,78) and iii) mutations in At*SAMC1* causing a chloroplast pigment-defective phenotype (79). The chloroplast envelope localization of AtSAMC1 was further supported by the plastidic targeting of expressed GFP fused to the N-terminal 80 residues of AtSAMC1, which contain the predicted plastid targeting sequence, and by the fact that knockout of At*SAMC1* leads to defects in prenyllipid and chlorophyll biosynthesis, which are chloroplastic processes (75). Moreover, immunoblots of AtSAMC1 in isolated subfractionated organelles suggested chloroplast localization, but the same protein band was also detected in the mitochondrial fraction (75). This latter finding is in line with another proteomic study that suggested dual targeting of AtSAMC1 to mitochondria and

plastids (80). In conclusion, AtSAMC1 may be localized to both mitochondria and plastids where it would mediate SAM import in exchange for SAH (9).

3. Mitochondrial SAM metabolism

In the mitochondrial matrix imported SAM is used by many MTs to methylate mitochondrial DNA, RNA, proteins and metabolites, especially for cofactor biosynthesis, producing SAH, which has to be exported to the cytosol to be regenerated into SAM in the SAM cycle. Furthermore, mitochondria also contain other SAM-dependent enzymes that do not produce SAH. The mitochondrial MTs and other SAM-dependent enzymes are listed in **Table 1**, whereas the metabolism in which they are involved is illustrated in **Fig. 2**.

3.1 Methylation of mitochondrial DNA

The human mitochondrial DNA (mtDNA) encodes 2 rRNAs, 22 tRNAs and 13 polypeptides, which are all subunits of the respiratory chain complexes and of ATP synthase. The methylations found in mtDNA are C5-methyldeoxycytidine (5mC) and in particular abundance N6-methyldeoxyadenosine (6mA), which is usually widespread in prokaryotes but less frequent in the nuclear genome of mammals (19,81). DNA MTs (DNMT) also found in nucleus have been proved to methylate mtDNA: DNMT1, DNMT3a and DNMT3b for 5mC; and METTL4 for 6mA (**Fig. 2 and Table 1**) (19,82-84). Many DNA MTs use a base flipping mechanism to access the base to be methylated, similarly to base excision repair enzymes (42). The level of mtDNA methylation is increased in certain conditions, e.g. in hypoxia. Some mtDNA methylations regulate mtDNA replication, by affecting the copy number of mtDNA/mitochondrion, and transcription, which thereby alters mitochondrial activity; they may also work as epigenetic markers. Altered mtDNA methylation patterns are associated with human disorders, such as cancer, cardiovascular and neurodegenerative diseases, as well as aging (20,85).

3.2 SAM-dependent methylation of mitochondrial RNA

Similar to what has been found in nuclear RNA, the methylation of mitochondrial RNA mainly has a regulatory function in mRNA, and structural function in tRNA and rRNA. N1-methyladenosine (m1A) is prevalent in the mitochondrial-encoded mRNA, tRNA and rRNA transcripts, and is formed by the action of the SAM-dependent

TRMT61B and TRMT10C (**Fig. 2 and Table 1**) (86,87). Interestingly, methylation of mitochondrial mRNA may regulate translation, e. g. m1A modification of ND5 mRNA causes repression of its translation (87). The m1A modification is found at specific positions in mitochondrial tRNAs. Furthermore, TRMT10C/5, NSUN2-3, TRMT2B and CDK5RAP1 add site-specific methylations to N1-guanine (forming m1G), C5-cytosine (m5C), C5-uridine (m5U) and 2-thio-N6-(dimethylallyl)adenosine (ms2i6A), respectively, of which some are found in the wobble base position of the tRNAs (**Fig. 2 and Table 1**). Methylation of mitochondrial tRNA along with other post-transcriptional modifications are required for correct maturation and function (88).

Compared to nuclear and bacterial rRNAs, mammalian mitochondrial rRNAs have only nine methylated sites, i.e. m5U429, m4C839, m5C841, m6A936 and m6A937 in the small ribosomal subunit 12S rRNA; m1A947 and 2′-O-ribose methylations of G1145, U1369 and G1370 in the large subunit 16S rRNA (89,90). Specific SAM-dependent MTs have been identified to be responsible for each position (**Table 1**). It is noteworthy that: i) TRMT61B also methylates mRNA and tRNA; and ii) CDK5RAP1 catalyzes a radical SAM reaction with one of the two molecules of SAM used splitting the S-C(5') bond (**Fig. 1**) and giving rise to 5'-deoxyadenosine and methionine (90). The rRNA methylations have been found at the functionally important open cleft between the small and large subunits of the mitochondrial ribosome: where mRNA interacts with the 12S rRNA, in the Aminoacyl-site and in the Peptidyl-site (18). The action of several mitochondrial rRNA MTs is coordinated with ribosomal assembly factors for the orchestrated maturation of the mitochondrial ribosome (91-93).

3.3 SAM-dependent methylation of mitochondrial proteins

Methylations of mitochondrial proteins by specific SAM-dependent MTs play roles in protein complex assembly and protein-protein/protein-RNA interactions (**Fig. 2 and Table 1**). Most of these MTs belong to the 7β-strand protein family (94) and many of them were originally called METTL (MT like); later their names were changed according to the abbreviation of their protein substrate followed by the type of residue they methylate (e.g. K in case of lysine) and MT. Here, the mitochondrial protein MTs and their roles are briefly described approximately in the order of their methylation targets in catabolism. CSKMT (citrate synthase lysine (K) MT or METTL12) predominantly trimethylates citrate synthase residue p.Lys395, which is

close to the active site, causing a small reduction of activity that might contribute to the tricarboxylic acid (TCA) cycle regulation (95,96). NDUFAF7 (NADH dehydrogenase ubiquinone assembly factor 7), which is one of the many complex I assembly factors, dimethylates p.Arg85 in the NDUFS2 subunit symmetrically (both the terminal nitrogens of the side chain guanidino group); this is an essential and early step in the assembly of complex I by forming the initial nucleus of the peripheral arm and its juncture with the membrane arm (94,97). ETFBKMT (METTL20) trimethylates p.Lys199 and p.Lys202 in ETFβ (electron transfer flavoprotein subunit β) and these modifications are important for electron transfer, the recognition and binding of the fatty acid oxidation and one-carbon metabolism dehydrogenases (98,99). ATPSCKMT (FAM173B) trimethylates p.Lys43 of the ATP synthase subunit C, this being essential for the correct incorporation of the subunit into the ATP synthase complex (100,101). ANTKMT (FAM173A) trimethylates the ADP/ATP carriers SLC25A5 and SLC25A6 (and most probably also SLC25A4) at p.Lys52, located in the mitochondrial matrix loop between H1 and H2, giving rise to reduced respiration rate, which may be explained by a diminished transport activity of the ADP/ATP carrier (102). The MT HEMK1 methylates the mitochondrial translation release factor (MTRF1L) on the glutamine residues in the peptide anticodon GGQ motif, which binds the mRNA UAA and UAG stop codons (103).

3.4 SAM-dependent mitochondrial biosynthesis of cofactors

Mitochondrial SAM is required for methylating several metabolites that are intermediates in the biosynthesis of various cofactors, such as lipoate, ubiquinone (Coenzyme Q), molybdopterin, heme and biotin (the latter only in yeast and plant) **(Fig. 2 and Table 1).**

3.4.1 Lipoate biosynthesis

The cofactor lipoate is synthesized in mitochondria by lipoyl synthase (LIAS) from octanoate (derived from type II fatty acid synthesis), sulfur (donated from an iron-sulfur cluster within LIAS) and SAM, which is used in a radical reaction breaking it down to 5'-deoxyadenosine and methionine (104,105). In mitochondria lipoate is subsequently covalently linked to the terminal amino group of specific lysines in the H-protein of glycine dehydrogenase (decarboxylase of the glycine cleavage system) and the E2 components of the four different mitochondrial dehydrogenase

complexes that couple 2-oxoacids to CoA: pyruvate dehydrogenase, oxoglutarate dehydrogenase, 2-oxoadipate dehydrogenase and branched-chain α-ketoacid dehydrogenase.

3.4.2 Ubiquinone biosynthesis

Human biosynthesis of ubiquinone involves at least 10 polypeptides: PDSS1-2 (phenyl diphosphate synthase subunit 1 and 2, corresponding to Coq1p in yeast) and COQ2-COQ10 (106). PDSS1-2 and COQ2 produce the benzoquinone ring condensed with an isoprenoid chain, which, through various methylation, decarboxylation, hydroxylation and deamination reactions catalyzed by COQ3-COQ10, finally results in ubiquinone in the inner mitochondrial membrane. In the human ubiquinone biosynthesis two SAM-dependent enzymes are involved: COQ3 and COQ5. COQ3 is an O-methyltransferase, which substitutes the hydrogen on the 3-hydroxyl group of 3,4-dihydroxy-5-polyprenylbenzoate with a methyl group, and COQ5 methylates carbon-3 of the benzoquinol ring (107,108). Ubiquinone is a component of the mitochondrial respiratory chain which transfers electrons from complex I, II and electron transfer flavoprotein ubiquinone oxidoreductase (ETFQO) to complex III.

3.4.3 Molybdopterin biosynthesis

The first step in the synthesis of molybdenum cofactors (MOCO) is catalyzed by mitochondrial MOCS1, which requires SAM for the conversion of GTP to cyclic pyranopterin monophosphate (cPMP) by yet another radical SAM reaction mechanism involving two iron sulfur clusters (109,110). The corresponding *A. thaliana* SAM-dependent enzyme CNX2 is also found in mitochondria (111). The mitochondrially-produced cPMP is exported by an ABC transporter (in plant ABCB25) to the cytosol, where MOCS2-3 and GPHN finalize the biosynthesis of the organic pterin moiety that binds molybdenum. Four molybdopterin-dependent enzymes have been identified in mammals and they are all oxidases: the mitochondrial intermembrane space sulfite oxidase (sulfur metabolism), the outer mitochondrial membrane amidoxime-reducing component (reduction of N-oxygenated molecules), the cytoplasmic xanthine oxidase (purine catabolism) and aldehyde oxidase (aromatic azaheterocycles and xenobiotic metabolism).

3.4.4 Heme-protein assembly

Human RSAD1 is a radical SAM enzyme (together with CDK5RAP1 and LIAS), which, based on the characterization of its bacterial homologue, is thought to function as a heme chaperone involved in the heme-insertion into enzymes (112).

3.4.5 Biotin biosynthesis

In microorganisms and plants, SAM is also used for the biosynthesis of biotin (vitamin H or vitamin B7) required for the action of two enzymes called Bio2p and Bio3p (out of six Bio1p-6p) in yeast (113). In yeast, the last reaction of biotin biosynthesis is catalyzed by biotin synthase (Bio2p), which is a mitochondrial radical SAM enzyme containing an iron-sulfur cluster (114). In Arabidopsis, the enzyme corresponding to Bio2p is also found in the mitochondrial matrix (115) together with Bio3p, which catalyzes an earlier step in the pathway: the substitution of a keto group in a biotin intermediate with the α-amino group of the SAM methionine (an unusual mechanism) leaving S-adenosyl-4-methylsulfanyl-2-oxobutanoate as a rest product (116-118). In contrast, it is not clear whether yeast Bio3p is mitochondrial. Animals are not capable of biotin biosynthesis and take it up through absorption in the digestive system by the sodium-dependent multivitamin transporter SLC5A6, which also transports precursors for other cofactors, such as pantothenate and lipoate (119). In cells, biotin is added covalently onto specific lysine residues (in Met-Lys-Met sequences) of carboxylases by biotin-protein ligase (holocarboxylase synthetase), which is thought to be present both in the cytoplasm and in mitochondria (120). Five carboxylases are known to contain biotin, which is used as a cofactor: pyruvate carboxylase (gluconeogenesis and lipogenesis), 3-methylcrotonyl-CoA carboxylase (BCAA catabolism), propionyl-CoA carboxylase (BCAA and fatty acid catabolism), and acetyl-CoA carboxylases 1 and 2 (fatty acid biosynthesis) (121). Of note, although these carboxylases have mitochondrial localization with exception of acetyl-CoA carboxylase 1 isoform, which is cytoplasmic, it is yet not known how biotin is imported into animal mitochondria.

4. Diseases associated with mitochondrial SAM transport and metabolism

Some genetic diseases are due to defects in the cytoplasmic SAM-cycle enzymes. For example, mutations in MS and MS reductase cause Homocystinuria-megaloblastic anemia (122,123), and in MAT1A and SAHase cause

hypermethioninemia (124-126). In addition, several diseases have been reported to be associated to alterations of genes encoding proteins involved in mitochondrial SAM transport and metabolism (**Table 2**).

4.1 Disorders associated with mitochondrial SAM transport

Disease-causing mutations in SLC25A26 have been identified in three unrelated children, who exhibited symptoms of different severity ranging from mild muscle weakness, lactic acidosis, cardiorespiratory insufficiency and developmental delay to respiratory/multiple organ failure and death (127). The biochemical analysis of the affected patients revealed several mitochondrial defects in SAM dependent processes (reduced 12S rRNA stability; methylation of ETFβ and the AACs SLC25A5 and SLC25A6; diminished ubiquinone and lipoic acid biosynthesis) leading to dysfunctional translation and respiratory chain activity. The disease caused by mutations in SLC25A26 has been classified as combined oxidative phosphorylation deficiency 28 (COXPD28, Table 2) and follows an autosomal recessive inheritance pattern. The functional consequences of the three disease-causing mutations on SAM transport were investigated thoroughly by introducing them into recombinant SLC25A26 constructs and examining i) the complementation of the growth defects of the *Sam5* knockout *S. cerevisiae* strain, and ii) the transport activity through the EPRA method (127). In the first approach the qualitative consequences of the mutations were evaluated in the yeast heterologous system. The expression of SLC25A26 wild-type and the p.Ala102Val, p.Val148Gly, p.Pro199Leu or short SAMC variants in *S. cerevisiae SAM5* null mutant could rescue the growth defects observed when grown on non-fermentable carbon sources to various degrees (section 2.2) (7,127). Wild-type SLC25A26 almost completely restored the growth rate whereas the p.Val148Gly variant only partially rescued it, and the p.Ala102Val, p.Pro199Leu and short SAMC did not affect the phenotype. It was also shown that the latter variant was not targeted to mitochondria. The second approach using the EPRA method, provides a quantitative measure of the mutation effect on the transport capacity and has been employed to assess the effects of many disease-causing mutations in other MCs (128-139). The recombinantly expressed SLC25A26 mutants p.Ala102Val, p.Pro199Leu and the truncated variant displayed virtually abolished transport activity, whereas p.Val148Gly was about 15% active compared with the wild-type protein (127). The results of the first and second approach are therefore

fairly well in agreement. The measured transport activities of the three SLC25A26 deficiency point mutations appear to be correlated with their position in the SLC25A26 homology model: the inactive mutations are found inside the substrate translocation pore (p.Ala102Val) and in the third signature motif sequence (p.Pro199Leu), whereas the somewhat active variant (p.Val148Gly) is located outside of the pore. All three mutated residues are conserved and are predicted to be of functional importance based on the high single-nucleotide evolutionary rate in these positions (127,140). In addition, cysteine mutations of the residues corresponding to p.Ala102 and p.Pro199 of SLC25A26 in the 2-oxoglutarate carrier (SLC25A11) have been found to be inactive, whereas the substitution with cysteine of the V148 counterpart in the 2-oxoglutarate carrier had about 50% activity (141-143).

Later, the effects of the p.Ala102Val, p.Val148Gly and p.Pro199Leu mutations in SLC25A26 were also evaluated in knockout organisms (144). In similarity to SLC25A26 deficiency, the knockout of SLC25A26 in *Drosophila melanogaster* and mouse causes decreased mitochondrial SAM levels, diminished biosynthesis of SAM-dependent iron-sulfur clusters, cofactors and metabolites as well as impaired complex I stability and assembly of the oxidative phosphorylation system (144). Mitochondrial SAM import and SAM-related processes in the matrix of the knockout fly were partially restored by complementation with *D. melanogaster* SLC25A26 containing the corresponding disease-causing mutations of p.Ala102Val and p.Val148Gly, whereas the p.Pro199Leu variant hardly affected the phenotype characteristics.

Since the discovery of the first three cases with COXPD28 (127), another three patients have been found. Similar symptoms have been observed in a fourth patient with the compound heterozygous SLC25A26 mutations p.Ala12Pro and p.Ala66Glu (145). These two mutations are located in the interface between H1 and H6, and between H2 and H3, respectively, and are predicted to be pathogenic by *in silico* analysis. Recently, with whole-exome sequencing also a fifth and a sixth patient have been found, which are adults carrying homozygous p.Glu135Gly and p.Arg142Gln mutations, respectively (146). In these two patients the symptoms, such as abdominal pain, lactic acidosis, exercise intolerance and mitochondrial myopathy, partly overlap with those of the phenotype described in the previously reported cases, but they are milder. The two mutated residues are located in the last

part of the second signature motif of MCs ([DE]GXXXX[WYF][KR]G) and in MC structures they form a salt bridge between them. In addition, the arginine is implicated in binding cardiolipin, which is necessary for MC activity (147-150). Notably, the latter two mutant variants were expressed in mouse embryonic fibroblasts deficient of SLC25A26 rescuing the phenotype, and the *D. melanogaster* SLC25A26 variant corresponding to p.Arg142Gln was also expressed in the knockout Drosophila model, which died at early larvae developmental stage (146). Because the two mutations apparently did no effect the uptake of SAM by isolated mitochondria, as measured in the authors' experimental setups, it was hypothesized that they had specific effects on mitochondrial SAH export and not on SAM import. However, the precise alterations in the transport properties of the last four identified SLC25A26 mutations that trigger COXPD28 pathogenesis have not been investigated with purified recombinant proteins, such as in reconstituted liposomes using the EPRA method.

Besides the mutated variants being responsible of COXPD28, SLC25A26 has been found to be down-regulated in cervical cancer cell lines through mechanisms that involve promoter region methylations and the transcription repressor FOXD3 (151,152). Most probably the reduced expression of SLC25A26 decreases mitochondrial SAM import with the consequences of diminished mtDNA methylation, biosynthesis of iron-sulfur clusters, cofactors, etc. These effects are corroborated by somewhat opposite effects of SLC25A26 overexpression in CaSki cells, where the levels of mitochondrial SAM and mtDNA methylation increase leading to decreased expression of respiratory complex subunits (151). In addition, SLC25A26 overexpression causes impairment of the cytoplasmic SAM cycle through accumulation of HCys and increased production of glutathione (151). Interestingly, a similar situation has been observed when SLC25A26 expression was increased by the copper containing compound [Cu(ttpy-tpp)Br_2]Br (153). Therefore, it is likely that altered SLC25A26 expression leads to an imbalance of SAM levels in both mitochondria and cytoplasm with effects on SAM-dependent processes inside and outside mitochondria.

4.2 Disorders associated with mitochondrial SAM metabolism

Some disorders are caused by mutations in the genes encoding for DNMTs methylating mtDNA, DNMT1, DNMT3a and DNMT3b. These enzymes, as well as the mitochondrial tRNA MT NSUN2, appear to be localized both in mitochondria and nucleus/cytosol. Autosomal dominant cerebellar ataxia, deafness and narcolepsy (ADCADN) and Hereditary sensory neuropathy type IE (HSN1E) (**Table 2**) are neurological and neurodegenerative pathologies associated with mutations in DNMT1. ADCADN is characterized by mitochondrial dysfunction with decreased ATP production (154) and both ADCADN and HSN1E bears hallmarks common in mitochondrial diseases. However, given that DNMT1 is also localized outside the mitochondria, it is difficult to say whether the symptoms are caused by reduced methylation in the mitochondria (155). This is also true for the diseases caused by mutations in DNMT3a and DNMT3b. DNMT3a often contains somatic mutations associated with acute myeloid leukemia (AML) (156). Mutations in the DNMT3a gene cause the autosomal dominant genetic diseases Heyn-Sproul-Jackson syndrome (HESJAS) and Tatton-Brown-Rahman syndrome (TBRS) that are both characterized by an impaired intellectual development dependent from the reciprocally-related phenotypes of microcephalic dwarfism and macrocephalic overgrowth, respectively (157,158). Immunodeficiency-centromeric instability-facial anomalies syndrome 1 (ICF1) and Facioscapulohumeral muscular dystrophy 4 (FSHD4) are caused by mutations in DNMT3b, which also methylates nuclear DNA (159,160). Moreover, various NSUN2 mutations have been reported to cause the autosomal recessive mental retardation-5 (MRT5) phenotype, which is characterized by intellectual disability, facial dysmorphic features, delayed psychomotor and speech development (161-163). However, since NSUN2 is localized to the cytoplasm and nucleolus, it is not clear whether mitochondrial tRNA methylation / mitochondrial translation has a role in higher cognitive function.

Three of the about 50 forms of Combined oxidative phosphorylation deficiency (COXPD) (**Table 2**) are linked to mutations in genes encoding mitochondrial RNA MTs: NSUN3, TRMT5 and TRMT10C, which are all thought to be exclusively located to mitochondria. Mutations in NSUN3 found in two patients cause COXPD48, which exhibits microcephaly, developmental delay, muscle weakness, external ophthalmoplegia and lactic acidosis (164,165). COXPD48 patient fibroblasts showed lack of methylation m5C in the anticodon of the mitochondrial $tRNA^{Met}$, leading to impaired mitochondrial translation and subsequent defects in the mitochondrial

respiratory chain and oxygen consumption. In two patients mutations in TRMT5 have been thought to be responsible for COXPD26, which is characterized by lactic acidosis, hypertrophic cardiomyopathy or exercise intolerance and deficiency of respiratory complexes I, III and IV (88). Furthermore, in these patients skeletal muscle hypomethylation of G37 in mitochondrial tRNAs was observed. Moreover, mutations in the gene encoding TRMT10C give rise to COXPD30 (166). This disease has only been reported in two patients, who presented hypotonia, feeding difficulties, deafness, lactic acidosis, increased cerebrospinal fluid lactate levels, and both died of respiratory failure at 5 months of age. In addition, defective TRMT10C, which methylates both mitochondrial tRNA and mRNA, leads to reduced assembly of respiratory complexes I, III and IV due to impaired mitochondrial translation.

A single case of a homozygous missense mutation in the MRM2 gene has been described as Mitochondrial DNA depletion syndrome 17 (MTDPS17) (167). The mutation in MRM2, which is a mitochondrial MT involved in the maturation of mitochondrial rRNA, leads to multiple defects in the oxidative phosphorylation system and mtDNA loss. MTDPS17 is a MELAS-like (mitochondrial encephalopathy, lactic acidosis and stroke-like episodes) syndrome exhibiting childhood-onset, rapidly progressive encephalomyopathy and stroke-like episodes. MELAS is caused by mutations in several different mtDNA genes, among them the genes for many mitochondrial tRNAs, and also in some nuclear genes encoding mitochondrial proteins; it is primarily characterized by defects in oxidative phosphorylation.

There are other rare genetic diseases, which are connected to SAM-dependent cofactor enzymes: LIAS, COQ5 and MOCS1. Three cases have been identified with mutations in the gene encoding for LIAS, presenting Hyperglycinemia, lactic acidosis, and seizures (HGCLAS) (168,169). Other symptoms of HGCLAS are increased serum glycine and lactate levels in newborns and severely delayed psychomotor development or encephalopathy, which may lead to childhood death. Furthermore, decreased lipoate production and decreased levels of the E2 components of PDHc and OGDHc as well as reduced activity of the glycine cleavage enzyme system were observed. Coenzyme Q10 deficiency-9 (COQ10D9) is caused by mutations in COQ5 and has been found in three sisters (170). This disorder is characterized by cerebellar ataxia associated with cerebellar atrophy, and often also by intellectual disability and seizures. In cells from the patients the COQ5 mRNA and protein levels as well as the ubiquinone levels were diminished, and defects in

respiratory complex II and III were observed. Like patients suffering from several other genetic diseases associated with ubiquinone biosynthesis, which commonly exhibit various neurological and muscular manifestations, patients with COQ10D9 responded to oral ubiquinone treatment positively. *MOCS1* mutations cause molybdenum cofactor deficiency of complementation group A (MOCODA), a disease observed in several cases (171,172). The symptoms of MOCODA appear in infancy and are severe; they mainly consist of poor feeding, intractable seizures and severe psychomotor retardation, which most often lead to death in early childhood. Dysfunctional molybdenum cofactor biosynthesis leads to decreased serum uric acid and increased urine sulfite levels due to deficiency of xanthine dehydrogenase and sulfite oxidase, which both use molybdopterin. In addition, MOCODA patients display increased excretion of taurine, S-sulfocysteine, hypoxanthine and xanthine, of which the latter accumulates and forms urinary xanthine stones.

5. Conclusions/Perspectives

This review highlights the many crucial roles of SAM, as important methylating agent, essential cofactor and generator of free radicals, in fundamental mitochondrial processes such as replication, transcription, translation, oxidative phosphorylation and cofactor metabolism. Essential for the mitochondrial SAM-dependent enzymes is the MC-catalyzed import of SAM from the cytoplasm and export of SAH produced, inside the mitochondria, in the methylation reactions. In agreement with its importance in mitochondrial metabolism, SAMC is expressed widely in human tissues and appears to be widespread in eukaryotes ranging from fungi, plant and animals. Moreover, the genetic diseases associated with mitochondrial SAM transport and metabolism underlines the important roles of the cofactor in vital processes of this organelle and the rest of the cell.

It is worth noting that the cytosolic/nuclear metabolism and expenditure of SAM are connected with the SAM-dependent processes in mitochondria through the SAM / SAH ratio. Firstly, the cytosolic SAM cycle largely depends, besides on new "input" of methionine, on the recycling of SAH (derived from the various cellular compartments) for the regeneration of SAM. Obviously, the carriers catalyzing the translocation of SAM and SAH across the mitochondrial membrane play a pivotal role in this regard by directly linking the mitochondrial matrix and cytosolic pools of these

two compounds. Secondly, the SAM cycle is also dependent on the other branches of the one-carbon metabolism through its connection to the folate cycle (which is partly confined in the mitochondrial matrix), and the biosynthesis of cysteine and glutathione from HCys. Unfortunately, the relationships between the above-mentioned cytosolic/nuclear processes**,** the SAM-dependent reactions within the mitochondria and the SAM / SAH ratios in these compartments still need to be fully investigated.

Some issues of mitochondrial SAM transport and metabolism are peculiar and not yet well understood.

(A) Whereas yeast Sam5p, plant SAMC1 and SAMC2 are capable of importing SAM into mitochondria via uniport transport, human SAMC appears to be catalyzing almost exclusively antiport transport. Therefore, given that not all matrix SAM is converted into SAH for counter exchange, it is difficult to see how net transfer of SAM into mitochondria is achieved. One might speculate that i) the extremely low SAMC uniport activity is enough to satisfy the required quantities of SAM that are consumed by the mitochondrial SAM radical enzymes, ii) the proton motif force of energized mitochondria *in vivo* facilitates uniport import of SAM having a net positive charge, iii) there is another yet unidentified counter substrate of SAMC; however, the most likely candidate, the byproduct of SAM radical enzymes, 5'-deoxyadenosine, is not transported by SAMC, or iv) there exists another not yet known mitochondrial transporter for SAM import.

(B) Another unresolved problem is the possible dual localization of Arabidopsis SAMC1 and SAMC2 in mitochondria and chloroplasts. All methods used so far for protein sub-cellular localization have their drawbacks: isolated organelles may be contaminated; proteomic identification in one organelle does not exclude that the same protein is localized to another organelle; and, using the N-terminal extension of MCs or whole proteins fused to GFP may exclude, conceal or obstruct targeting information. It should be possible to clearly determine the organellar localization of SAMC1 and SAMC2 with alternative approaches.

(C) The mitochondrial MTs and other SAM-dependent enzymes are very specific for their substrates and the majority of them seem to have only one single target, perhaps with the exception of the mtDNA MTs and some of the RNA MTs. The

substrate specificity (nucleic acid sequences or structural motifs) of the latter enzymes and how they are regulated, especially those for the regulatory methylations of mtDNA and mRNA, have not yet determined satisfactorily. Furthermore, it is not clear in which physiological circumstances and for which purposes citrate synthase and the ADP/ATP carrier are methylated.

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Figure legends

Figure 1. SAM represented in ball-and-stick.

Figure 2. SAM metabolism and transport in a typical human cell. The schematic overview shows SAM transporters (yellow), SAM-using enzymes (red), enzymes metabolizing SAM (olive green), enzymatic reactions (black arrows), reactions with yet unidentified enzymes (grey arrows) and SAM distribution (red arrows). Abbreviations: 5'dAd, 5'-deoxyadenosine; ANTKMT, adenine nucleotide translocase lysine (K) methyltransferase; ATPSCKMT, ATP synthase subunit C lysine methyltransferase; BHMT, betaine-homocysteine S-methyltransferase; CDK5RAP1, CDK5 regulatory subunit associated protein; COQ, Coenzyme Q O-methyltransferase; cPMP, cyclic pyranopterin monophosphate; CS, citrate synthase; CSKMT, citrate synthase lysine methyltransferase; DNMT, DNA methyltransferase; ETFβ, electron transfer flavoprotein subunit β; ETFBKMT, electron transfer flavoprotein subunit β lysine (K) methyltransferase; FPP, farnesyl pyrophosphate; HEMK1, MTRF1L glutamine (E) methyltransferase; LIAS, lipoyl synthase; METTL, methyltransferase-like protein; MOCS1, molybdenum cofactor synthesis step 1; MRM, mitochondrial rRNA methyltransferase; MTR, methionine synthase; MTRF1L, mitochondrial translational release factor 1-like; NDUFAF, NADH dehydrogenase

ubiquinone complex I assembly factor; NSUN, NOP2/Sun RNA methyltransferase; RSAD1, radical SAM domain-containing protein 1; SAHase, S-adenosylhomocysteine synthase; SAMS, SAM synthase; TFB, mitochondrial transcription factor B; TRMT, tRNA methyl transferase.

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Table 1. Mitochondrial enzymes that use SAM.

SUBSTRATE	GENE NAME	PROTEIN NAME	REFERENCES
DNA			
C5-deoxycytidine	DNMT1	C5-deoxycytidine methyltransferase	(82)
C5-deoxycytidine	DNMT3a/3b	C5-deoxycytidine methyltransferase	(83,84)
N6-deoxyadenosine	METTL4	N6-adenine methyltransferase	(19)
mRNA/tRNA/rRNA			
U54 of tRNAs, U429 of 12S rRNA	TRMT2B	C5-uridine-methyltransferase	(173)
G9 and A9 of tRNAs, and adenines of mRNA for ND5	TRMT10C	N1-purine-methyltransferase	(87,174)
A59 of tRNAs, A947 in 16S rRNA and adenines of mRNA for ND5	TRMT61B	N1-adenine-methyltransferase	(86,175,176)
tRNA			
cytosine of tRNAs	NSUN2	tRNA C5-cytosine methyltransferase	(177,178)
C34 (wobble base) of $tRNA^{Met}$	NSUN3	tRNA C5-cytosine-methyltransferase	(164,179,180)
G37 of tRNAs	TRMT5	N1-guanine-methyltransferase	(181)
N6-(dimethylallyl)-A37 of tRNAs	CDK5RAP1	tRNA C2-methylthiotransferase	(182)
rRNA			
G1145 of 16S rRNA	MRM1	rRNA 2'O-methyltransferase 1	(183)
U1369 of 16S rRNA	MRM2	rRNA 2'O-methyltransferase 2	(89,183)
G1370 of 16S rRNA	MRM3	rRNA 2'O-methyltransferase 3	(89,183)
C841 of 12S rRNA	NSUN4	rRNA C5-cytosine methyltransferase	(184,185)
A936 and A937 of 12S rRNA	TFB1M	N6-dimethyladenosine transferase 1	(186)
A936 and A937 of 12S rRNA	TFB2M	N6-dimethyladenosine transferase 2	(187)
C839 of 12S rRNA	METTL15	N4-cytidine- methyltransferase	(188)
m4C839 and m5C841 of 12S rRNA	METTL17	N4/C5-methyltransferase-like protein 17	(189)
protein			
K395 of citrate synthase	CSKMT	Lysine (tri)methyltransferase	(95,96)
R85 of Complex I subunit NDUFS2	NDUFAF7	Arginine dimethyltransferase	(94)
K199 and K202 of ETFβ	ETFBKMT	Lysine trimethyltransferase	(98,99)
K43 of ATP synthase subunit C	ATPSCKMT	Lysine trimethyltransferase	(101)
K52 of AACs	ANTKMT	Lysine trimethyltransferase	(102)
translation release factor MTRF1L	HEMK1	MTRF1L glutamine methyltransferase	(103)
lipoate			
N6-octanoyl-L-lysyl-[protein]	LIAS	Lipoyl synthase	(104)
ubiquinone			
3,4-dihydroxy-5-all-trans-polyprenylbenzoate	COQ3	Ubiquinone biosynthesis O-methyltransferase	(107)
2-polyprenyl-6-methoxy-1,4-benzoquinol	COQ5	2-methoxy-6-polyprenyl-1,4-benzoquinol methylase	(108)
molybdenum cofactor			
GTP	MOCS1	GTP 3',8-cyclase	(110)
heme			

heme assembly	RSAD1	Radical S-adenosyl methionine domain-containing protein 1	(112)
	(RSAD2) (not exclusively mitochondrial)	Radical S-adenosyl methionine domain-containing protein 2	(190)
biotin			
(4R,5S)-dethiobiotin	BIO2 (yeast and plant)	Biotin synthase	(114)
(S)-8-amino-7-oxononanoate	BIO3 (plant)	DAPA aminotransferase	(118)

Table 2. Genetic diseases associated to mitochondrial SAM-dependent enzymes.

Mutated protein	Disorder name	OMIM number / inheritance	References of first report
SLC25A26*	Combined oxidative phosphorylation deficiency 28 (COXPD28)	616794 / AR	(127)
DNMT1	AD Cerebellar ataxia, deafness, and narcolepsy (ADCADN)	604121 / AD	(191)
	Neuropathy, hereditary sensory, type IE (HSN1E)	614116 / AR	(192)
DNMT3a	Somatic acute myeloid leukemia (AML)	601626	(156)
	Heyn-Sproul-Jackson syndrome (HESJAS)	618724 / AD	(158)
	Tatton-Brown-Rahman syndrome (TBRS)	615879 / AD	(157)
DNMT3b	Immunodeficiency-centromeric instability-facial anomalies syndrome 1 (ICF1)	242860 / AR	(160)
	Facioscapulohumeral muscular dystrophy 4 (FSHD4)	619478 / DD	(159)
NSUN2	AR mental retardation 5 (MRT5)	611091 / AR	(161)
NSUN3*	Combined oxidative phosphorylation deficiency 48 (COXPD48)	619012 / AR	(164)
TRMT5*	Combined oxidative phosphorylation deficiency 26 (COXPD26)	616539 / AR	(88)
TRMT10C*	Combined oxidative phosphorylation deficiency 30 (COXPD30)	616974 / AR	(166)
MRM2*	Mitochondrial DNA depletion syndrome 17 (MTDPS17)	618567 / AR	(167)
LIAS*	Hyperglycinemia, lactic acidosis and seizures (HGCLAS)	614462 / AR	(168)
COQ5*	Coenzyme Q10 deficiency 9 (COQ10D9)	619028 / AR	(170)
MOCS1*	Molybdenum cofactor deficiency A (MOCODA)	252150 / AR	(171)

*, thought to be exclusively mitochondrial; AD, autosomal dominant; AR, autosomal recessive; DD, digenic dominant.

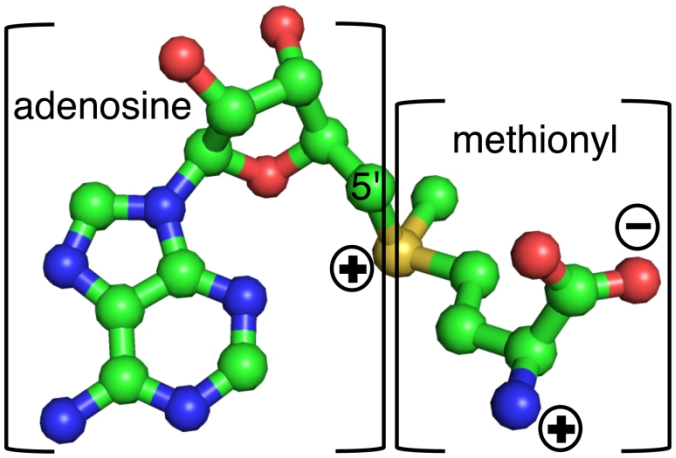

Figure 1

209x297mm (300 x 300 DPI)

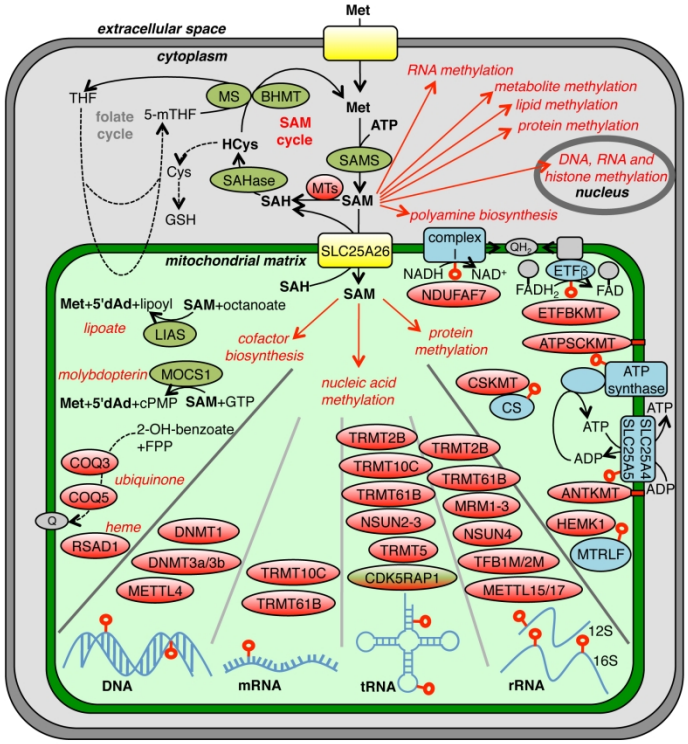

Figure 2

209x297mm (300 x 300 DPI)