Mitochondrial Fatty Acids and Neurodegenerative Disorders

Alexander J Kastaniotis[1*], Kaija J Autio[1] and Remya R Nair[2]

[1] Faculty of Biochemistry and Molecular Medicine, University of Oulu, P.O. Box 5400, FI-90014 University of Oulu, Finland; [2] Mammalian Genetics Unit, MRC Harwell Institute, Oxfordshire, OX11 0RD, UK.

*To whom correspondence should be addressed: Faculty of Biochemistry and Molecular Medicine, University of Oulu, P.O. Box 5400, FI-90014 Oulu, Finland; Tel. +358294481196; Email: alexander.kastaniotis@oulu.fi

Abstract

Fatty acids in mitochondria, in *sensu stricto*, arise either as β-oxidation substrates imported *via* the carnitine shuttle or through *de novo* synthesis by the mitochondrial fatty acid synthesis (mtFAS) pathway. Defects in mtFAS or processes involved in the generation of the mtFAS product derivative lipoic acid (LA), including iron-sulfur cluster synthesis required for functional LA synthase, have emerged only recently as etiology for neurodegenerative disease. Intriguingly, mtFAS deficiencies very specifically affect CNS function, while LA synthesis and attachment defects have a pleiotropic presentation beyond neurodegeneration. Typical mtFAS defect presentation include optical atrophy, as well as basal ganglia defects associated with dystonia. The phenotype display of patients with mtFAS defects can resemble the presentation of disorders associated with Coenzyme A (CoA) synthesis. A recent publication links these processes together based on the requirement of CoA for ACP maturation. MtFAS defects, CoA synthesis- as well as Fe-S cluster-deficiencies share lack of LA as a common symptom.

Introduction to mitochondrial fatty acids

Two of the central processes of lipid metabolism in living organisms are the synthesis of fatty acids (FAS) and breakdown/β-oxidation of fatty acids. FAS results in *de novo* generation of longer fatty acids from acetyl-CoA moieties. In mammals, this process takes place in two cellular compartments, in the cytosol and in mitochondria, while elongation to acyl chain lengths exceeding 16-18 carbons as well as secondary modifications of fatty acids occurs in the endoplasmic reticulum. During β-oxidation, dietary/ storage derived fatty acids are ultimately broken down to their 2 carbon residues (or a terminal 3-carbon moiety from rarely occurring odd numbered fatty acids), producing for example substrates to feed the tricarboxylic acid cycle (TCA) or hepatic ketogenesis in liver during fasting. In many eukaryotes, including mammals, fatty acid catabolism takes place in both peroxisomes and mitochondria. Consequently, mitochondria are the only cellular compartment participating in both processes, the synthetic as well as the degradative routes of fatty acid metabolism and have established a mechanism to maintain these two metabolic routes separated in the mitochondrial matrix. Several comprehensive reviews on mitochondrial β-oxidation defects, also addressing related disorders and associated animal models have been published recently (e.g. (Adeva-Andany and others, 2019; Merritt and others, 2018; Tucci, 2017)). We will therefore touch on this topic only briefly and focus on new developments concerning mitochondrial fatty acid synthesis (mtFAS) and its role in neurodegenerative disease.

Mitochondrial β-oxidation

The major route of fatty acid degradation in mammals is the mitochondrial β-oxidation pathway. Fatty acids in the cytosol are activated by acyl-CoA synthases to form acyl-coenzyme

A (CoA) esters which are subsequently converted to acyl carnitines to allow transportation through mitochondrial membranes. This transport system consists of two acyltransferases: carnitine palmitoyl transferase 1 and 2 (CPT1 and CPT2) and carnitine acyl-carnitine translocase (CACT) and requires L-carnitine. β-Oxidation in mitochondria follows the canonical route of acyl-group shortening by two carbon atoms per breakdown cycle in a process with four enzyme activities. In brief, dehydrogenase converts the saturated fatty acid to a trans-2-enoyl-CoA moiety, followed by addition of water by enoyl CoA hydratase, resulting in 3-hydroxyacyl-CoA. This product is further converted to 3-ketoacyl-CoA by 3-hydroxyacyl-CoA dehydrogenase. Finally, thiolase breaks 3-ketoacyl-CoA to acyl-CoA shortened by two carbons and acetyl-CoA.

Fatty acid oxidation is an essential pathway for maintaining energy balance in the body. During fasting, certain tissues like heart, skeletal muscle and kidney rely on fatty acid β-oxidation as major ATP source. Although ketone bodies can satisfy a substantial part of the energy demand in brain during fasting, mitochondrial β-oxidation levels in neurons are much lower than in other tissues (Yang and others, 1987). In neural tissue, the mitochondrial β-oxidation occurs mainly in astrocytes, and the rate of β-oxidation in neurons is regarded as negligible (Edmond and others, 1987; Panov and others, 2014). Transcriptomic analyses of mouse and human brain have revealed higher expression levels of mitochondrial β-oxidation genes in astrocytes than neurons (Eraso-Pichot and others, 2018). Congruently, investigations of mitochondrial proteomes of different cerebellar cells of mouse have revealed that β-oxidation enzymes and rate are elevated in astrocytes compared to neurons like Purkinje cells (PCs) or granule cells (Fecher and others, 2019). It was shown in a recent study that fatty acids from neurons can be transported as ApoE-positive lipid particles to astrocytes to be

consumed by mitochondrial β-oxidation. This is regarded as a mechanism to avoid toxic effects of fatty acid accumulation in neurons (Ioannou and others, 2019).

Genetic defects in mitochondrial β-oxidation can cause hypoketotic hypoglycaemia, cardiomyopathy, arrhythmia and rhabdomyolysis (Houten and others, 2016) and also neurological manifestations like peripheral neuropathy or epilepsy during acute metabolic derangement due to severe hypoglycaemia and secondary ammonia accumulation (Fahnehjelm and others, 2016; Fraser and others, 2019; Immonen and others, 2016; Spiekerkoetter, 2010).

Overview of mitochondrial fatty acid synthesis

In eukaryotes, the bulk of *de novo* fatty acid synthesis takes place in cytosol and is catalysed by a multifunctional polypeptide enzyme (FAS type I) (Smith and others, 2003). Fatty acids synthesized in the cytoplasm have vital roles in cellular biochemical processes; as components of triacylglycerols in energy storage, as substrate for fatty chain elongation and as contributors to cellular integrity by serving as structural component of cell membranes. In contrast to cytosolic FAS, mtFAS occurs in the mitochondrial matrix and closely resembles the prokaryotic pathway (FAS type II), which employs individual polypeptides to perform the enzymatic steps involved in the generation of fatty acids. The first component of the mtFAS pathway and FAS activity in mitochondria of eukaryotes were already reported three decades back (Brody and Mikolajczyk, 1988; Mikolajczyk and Brody, 1990). However, our understanding of the purpose of the strict evolutionary conservation of FAS in mitochondria, beyond providing the precursor for the endogenous synthesis of the lipoic acid (LA) cofactor essential for several important mitochondrial enzyme complexes (Solmonson and

DeBerardinis, 2018), has improved only recently. Nevertheless, it has been evident from early on based on the phenotypes of cytosolic FAS and mtFAS mutants that loss of cytosolic FAS cannot be compensated by mtFAS and vice versa, indicating that the pathways are not redundant (Harington and others, 1993; A. J. Kastaniotis and others, 2017).

With exception of the acyl carrier protein (ACP), which was identified from *Neurospora crassa* mitochondria (Brody and Mikolajczyk, 1988), all of the components involved in this pathway were initially identified in the eukaryotic model organism *Saccharomyces cerevisiae* (bakers' yeast). Deletion of any one of the mtFAS components in yeast results in respiratory deficiency (Hiltunen and others, 2009). The corresponding mammalian enzymes (Table I) were subsequently found based on their homology to yeast and bacterial proteins and their ability to complement respective yeast knock-out (KO) mutants. All the genes encoding the enzymes are nuclear encoded (Hiltunen and others, 2009; A. J. Kastaniotis and others, 2017), and the complete set of enzymes required for the function of the mtFAS pathway have been characterised (Hiltunen and others, 2010). Like for cytosolic FAS, the fundamental building block of mtFAS is acetyl-CoA. As initiating events, the carboxylation of acetyl-CoA is followed by the subsequent replacement of the CoA group with ACP, yielding in malonyl-ACP (Fig.1). The process then commences by the condensation reaction with another acetyl group with malonyl-ACP, under concomitant release of CO_2. From here on, the pathway enters a cycle or repeating steps of reduction, dehydration and another reduction, yielding butyryl-ACP as the first saturated fatty acid product (Fig.2). Repetitive cycling results in addition of two carbon atoms after each condensation-reduction-dehydration-reduction reaction (Hiltunen and others, 2010).

The first discovery elucidating the physiological role of mtFAS was achieved in plant (Wada and others, 1997) and few months later confirmed in *S. cerevisiae* (Brody and others,

1997). Both groups demonstrated a role of mtFAS in the production of octanoic acid (C8) required for the intramitochondrial generation of LA. For nearly two decades, C8 remained the only product of mtFAS in eukaryotes with a confirmed cellular function. Although it was already reported in 1988 that *in vivo* radioactively labelled *N. crassa* ACP was found to carry hydroxymyristic acid (Brody and Mikolajczyk, 1988), a physiological role for longer fatty acid products of mtFAS has not been clearly determined. In yeast, the phenotype of mutants with loss of mtFAS function is more severe than in mutants with defects in the sulphur insertion or transfer steps of LA synthesis impairment alone (Kursu and others, 2013), and a suppressor variant of a mitochondrially mislocalized peroxisomal fatty acyl-CoA ligase was described that rescued the respiratory defect of yeast mutants, but not the lipoylation defect (Harington and others, 1994; Kursu and others, 2013; Van Vranken and others, 2018). All these pieces of evidence point to the existence of physiologically relevant fatty acids longer than C8. By now, mtFAS has been implicated in playing roles in respiratory chain integrity, mitochondrial dynamics, iron-sulphur (Fe-S) cluster biogenesis and mitochondrial translation (Fig.2). In 2013, Kursu and others proposed that mtFAS operates as a metabolic sensing circuit, providing mitochondria with information on acetyl-CoA availability and accordingly coordinating mitochondrial biogenesis events inside the mitochondria (internal mitochondrial biogenesis events/IMBE) with nuclear-controlled gene expression associated with mitochondrial biogenesis. In this model, the amount of synthesized fatty acid was suggested to be proportional to the concentration of acetyl-CoA in the mitochondrial matrix and serve as a signal for respiratory chain synthesis. A few years later, Van Vranken and others (2018) provided evidence re-enforcing this model and demonstrated that the actual IMBE signal is likely to be acylated ACP.

Lipoylation

LA is a fatty acid-derived cofactor essential for the function of pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (KDH), the glycine cleavage system (GCS), branched chain dehydrogenase (BCD) and α-ketoadipate dehydrogenase (KAD), residing in mitochondria in mammals. Until the late 1990s, LA was considered a vitamin for humans. However, it is now evident that a conserved intramitochondrial LA synthesis-and-attachment pathway exists in human cells and most other eukaryotes (Cronan, 2016). According to the most recent model, C8 synthesised on ACP is initially transferred to the H protein of the glycine cleavage system by octanoyl transferase LIPT2 (Lip2 in yeast). The thiol groups on positions 6 and 8 of the C8 molecule are then inserted by the Fe-S cluster containing enzyme LA synthase LIAS/LASY (Lip5), and the lipoylated H protein then serves as substrate for transfer of the lipoyl moiety to the E2 subunits of the remaining lipoate-bearing enzymes by LIPT1 (Lip3). The pathway is required for respiratory competence in yeast and is essential in mammals.

Although it was reported that the detrimental effects of inhibition of the human mtFAS condensing enzyme OXSM on function of PDH and KDH in human cultured cells were counteracted by LA supplementation in the growth media (C. Chen and others, 2014), there is currently no solid evidence for the use of LA obtained from dietary sources in mitochondrial enzyme lipoylation. Poignantly, it was recently shown conclusively that Lip3/LIPT1 lacks the ability to activate free lipoic acid for transfer to the target complexes (Cao and others, 2018).

Acyl carrier protein (ACP)

ACP was the first component of the mtFAS that was identified and assumes a key position in the pathway as platform of the synthesis process itself and in relaying the

metabolic state information to relevant effector molecules (Van Vranken and others, 2018). Mitochondrial ACP is a small protein with a molecular mass of approximately 10 kDa. The polypeptide by itself (apo-ACP) is unable to support fatty acid synthesis and needs to be converted into the holo-form by addition of a 4'-phosphopantetheine (4'PP) group derived from CoA (reviewed in (Masud and others, 2019)). In mammals, this process carried out by a phosphopantetheinyl-ACP transferase (PPTase), encoded by the *AASDHPPT* gene in mammals (Joshi and others, 2003). Yeast uses a dedicated mitochondrial PPTase named Ppt2 (Stuible and others, 1998), but a mitochondrial localization of the mammalian PPTase has not been documented. Association of the nascent fatty acid chain with holo-ACP ensures specificity of the substrate for enzymes of the synthetic pathway. CoA-bound fatty acids, in contrast, are preferred substrates for the β-oxidation route of fatty acid metabolism. Although both acyl-ACP and acyl-CoA are activated thioester forms of carboxylic acid sharing the 4'PP moiety, the association of the fatty acid with the polypeptide is responsible for metabolic isolation of the substrates in the anabolic and catabolic direction, respectively.

In addition to serving as a FAS platform, Acyl-ACP has been found to be physically interacting with proteins of the Lysine-Tyrosine-Arginine-Motif (LYRM) family (Angerer, 2015) that play important roles in mitochondrial Fe-S cluster synthesis and assembly and function of respiratory chain (Masud and others, 2019).

Composition and physiological roles of mtFAS

The mammalian and yeast mtFAS pathway are structured very similarly and resemble the prokaryotic FAS type II pathway (Table I). Malonyl-CoA-ACP transacylase (MCAT), the condensing enzyme/3-ketoacyl synthase (OXSM) and the 2-enoyl thioester reductase (MECR)

can be identified by homology searches in human protein databases using yeast Mct1, Cem1 and Etr1 protein sequences, respectively, as templates. The former two are also direct orthologs of the corresponding bacterial enzymes. The remainder of the members of the mtFAS pathway, however, show an intriguing increase in complexity, and a few puzzling observations concerning mtFAS remain. Yeast has one dedicated mitochondrial acetyl-CoA carboxylase (Hfa1). In contrast, malonyl-CoA synthetase ACSF3 (Witkowski and others, 2011) and a conserved mitochondrial isoform of cytosolic acetyl-CoA carboxylase ACC1 together appear to serve as providers of the malonyl-CoA for acyl chain extension by mtFAS in mammals (Monteuuis and others, 2017). Moreover, unlike the yeast and bacterial equivalents, the mammalian 3-ketoacyl reductase is composed as a "dimer of dimers" of two distinct polypeptides, both evolutionarily related to microbial ketoreductases. The CBR4 protein acts as the catalytic subunit, while 17βHSD8 provides a scaffolding function and is required for the stability of CBR4 (Z. Chen and others, 2009; Venkatesan and others, 2014). Finally, the ORF encoding human mitochondrial 3-hydroxyacyl-thioester dehydratase 2 (HTD2) is transcribed into a bicistronic mRNA, preceded by RPP14, encoding a subunit of the human ribonuclease P (RNase P) complex (Autio and others, 2008). This bicistronic arrangement is conserved in vertebrates (Autio and others, 2008). In yeast, mtFAS is essential for mitochondrial RNAse P-dependent tRNA maturation. However, it has been conclusively shown by others that mammalian mitochondrial RNAse P does not contain an RNA subunit (Holzmann and others, 2008), so the significance of the RPP14-HsHTD2 arrangement remains obscure.

Apart from the role as provider of the C8 precursor for LA synthesis, mtFAS has been implicated in several aspects of IMBE (Fig.3A). We have previously provided evidence that a product of mtFAS longer than eight carbons must play a role in IMBE in yeast, acting as a

signal molecule in the metabolic sensing function proposed as a physiological function of mtFAS (Kursu and others, 2013). The possible role of C8 as a cofactor of mitochondrial enzyme complexes in such a signalling circuit is evident, and recently published data indicates that also acylated ACP is likely to serve as IMBE-promoting signal (Van Vranken and others, 2018). It is now well established that mitochondrial ACP does not merely serve as mtFAS platform, but also partakes as a structural component of several mitochondrial protein complexes. ACP was found present in the mammalian mitochondrial ribosome (Brown and others, 2017), and acylated ACP has been identified as stoichiometric subunit (NDUFAB1) of respiratory complex I, where it is present in two distinct locations, interacting with LYRM proteins (Runswick and others, 1991; Sackmann and others, 1991; Zhu and others, 2016). Depletion of ACP leads to loss of respiratory complex I in both the *N. crassa* model and in mammalian cultured cells (Feng and others, 2009; Schneider and others, 1995). Acylated ACP was also identified to be physically associated with several respiratory chain assembly factors in yeast and has been shown to be required for the function of the eukaryotic mitochondrial Fe-S cluster synthesis (ISC) machinery. Unacylated holo-or acyl-ACP was found as a subunit of the ISC protein complex, interacting with the Nfs1 cysteine desulphurase and LYRM protein Isd11 (Van Vranken and others, 2016). Similar to the effect on complex I integrity, the depletion of ACP resulted in destabilization of ISC and depletion of Fe-S clusters in the cells (Van Vranken and others, 2016). Interestingly, LA synthetase is an iron sulphur cluster dependent protein. Consequently, ISC biogenesis defects can affect LA biosynthesis and have been shown to result in neurological symptoms (Lebigot and others, 2017). Depletion of mtFAS in mammalian cells has shown to result in mitochondrial fission in mammals (Monteuuis and others, 2017; Nair and others, 2018) and hence mitochondrial morphology dysregulation appears to be an additional phenotype of mtFAS dysfunction.

Both yeast Etr1 and mammalian MECR have been reported to also localize to the nucleus, and it has been suggested that they participate in regulation of nuclear gene expression (Masuda and others, 1998; Yamazoe and others, 1994). However, no data has been put forth to date supporting such a function. Expression of a heterologous bacterial enoyl reductase alone is sufficient to rescue the respiratory defect of the *S. cerevisiae etr1* deletion strain, but the rescue is not complete (Kursu and others, 2013; Torkko and others, 2001).

Mammalian models to study mitochondrial fatty acid synthesis and associated pathways

In bovine heart mitochondrial matrix preparations, the carbon flow from a two-carbon precursor to LA to the H protein (apoH) of the glycine cleavage system was demonstrated by incubating the preparation with labelled acetyl-ACP and malonyl-CoA. The predominant fatty acyl species associated with ACP identified in these experiments was octanoic acid. However, myristic acid was found as the longest fatty acyl chain species attached to ACP (Witkowski and others, 2007).

A few ex-vivo models for mtFAS dysfunction have been generated using mammalian cultured cells. Inactivation of mtFAS by RNAi knockdown of ACP/NDUFAB1 resulted in cells death (Clay and others, 2016; Feng and others, 2009) accompanied by reduced level of lipoylated proteins, reduced activity of respiratory complex I, lowered mitochondrial membrane potential, and oxidative stress. These phenotypes match the defects expected from mtFAS disruption, according to the suggested roles in respiratory complex assembly in interaction with LYRM proteins. Although supplementation of LA in the medium helped to reduce the oxidative damage, it did not restore the levels of lipoylated proteins (Feng and others, 2009). Congruently, we and others have not been able to obtain viable cell lines with

complete inactivation of the genes involved in this pathway (Monteuuis and others, 2017; Shvetsova and others, 2017) indicating that mtFAS is essential for mammalian cell survival.

The results of studies concerned with either overexpression or knockdown of mammalian mtFAS components inspired studies on the effect of these changes on cellular transcriptional activities and metabolism. Overexpression of MECR in HeLa cells increased PPAR pathway-mediated transcriptional activities. Since MECR was found to be localised in mitochondria in this over-expression model, the authors favoured a model where PPAR activities were modulated through the products of mtFAS pathway. However, the authors were not able to rule out an effect mediated by a the previously published nuclear variant of MECR (see above) (Kim and others, 2014; Parl and others, 2013) (Parl and others, 2013). In another study published by the same group, the effect of overexpression of MECR and a knockdown of ACP on metabolism was investigated. Here, mtFAS products contributed little to the mitochondrial lipids that were analysed, but mtFAS function was found to play a regulatory role on the level of bioactive lipids involved in various mitochondrial biochemical pathways (Clay and others, 2016).

Based on the observation that overexpression of the Etr1 mitochondrial enoyl-thioester reductase in yeast results in massive expansion of the mitochondrial compartment (Torkko and others, 2001), the first mtFAS-defect animal model generated was a mouse with heart-specific overexpression of MECR. Elevated levels of MECR protein resulted in reduced cardiac mechanical function, decreased performance in an endurance tolerance test and enlarged mitochondria with focal mitochondrial clumping in the myocardium (Z. Chen, Leskinen and others, 2009), resembling the effect of overexpression of Etr1 in yeast. A few years later, a conditional *Mcat* KO mouse model exhibited loss of weight, lowered activity, kyphosis, hypothermia, alopecia and reduced lifespan. The phenotypic changes such as loss

of white adipose tissue, reduced muscle strength, diminished TCA cycle, reduced level of lipoylated proteins and compromised mitochondrial respiration were consistent with a systemic energy disequilibrium (Smith and others, 2012). It was later reported by our group that the generation of *Mecr* null mutant mice resulted in embryonic lethality in mid-gestation phase due at least in part to placental developmental failure (Nair and others, 2017) although the embryos exhibited pleiotropic defects.

The analysis of MECR expression in different tissues revealed high levels of the protein in cerebellar PCs, prompting the initiation of studies of the function of MECR in neurons. Towards this goal, a PC-specific KO of Mecr was generated where inactivation of Mecr occurred postnatally. Gait abnormalities were observed in these conditional KO mice around 7 months of age. Detailed characterization of this model revealed functional and cellular ultrastructural changes preceding progressive loss of PCs and ataxic symptoms (Nair and others, 2018). Reduction of the PC lining is already detectable in cerebellar sections of 6 months old mice, where concomitant microgliosis and astrogliosis indicated ongoing neuron damage, progressing to almost complete loss of PCs by 11 months of age. It was not possible to confirm a statistically significant reduction of lipoylation in the PCs still present at earlier stages, and no evidence for oxidative damage, or a clear mechanism for neuron death could be established in this model. Ultrastructural analyses revealed an electronlucent mitochondrial matrix and dilated endoplasmic reticulum in PCs of the KO mice at 6 months of age. In addition, loss of activity of respiratory complexes I and II could be detected, while analysis of respiratory complex IV activity displayed a mosaic pattern. Induction of KO of MECR in primary neurons obtained from mice carrying a homozygous floxed *MECR* allele, followed by fluorescence microscopy analyses, revealed mitochondrial fragmentation.

Subsequently, two reports published by the Wang group in 2019 (Hou and others, 2019; R. Zhang and others, 2019) addressed the question concerning the consequences of a *NDUFAB1*/ACP KO in mice. Complete inactivation of ACP resulted in early embryonic lethality (Hou and others, 2019) and cardiomyocyte-specific ablation of *NDUFAB1*, using an Mlc2v-Cre driver construct that starts expressing at around day 8 of embryonic development, lead to severe heart defect phenotypes. The authors observed disturbed assembly of Fe-S cluster containing respiratory complexes in the cardiac tissue. In contrast, overexpression of ACP in the mouse heart resulted in higher respiratory complex I activity, increased respiratory supercomplex abundance, and protected against ischemia-reperfusion injury. However, no increase in mtFAS activity was detected as judged by LA content in these hearts (Hou et al 2019). These results differ from the data obtained from mice overexpressing MECR in the heart (Z. Chen and others, 2009) and may indicate a separation of roles of ACP and the acyl chain tethered to ACP. The follow up work by this research group indicated an important role of ACP in skeletal muscle glucose metabolism (R. Zhang and others, 2019), and systemic overexpression of ACP protected the transgenic mice from obesity and insulin resistance, leading to the conclusion that ACP may play a fundamental role in the mitochondrial response to metabolic challenge.

Based on the optical atrophy phenotype observed in MCAT deficiency patients (see below), Li and co-workers generated a siRNA mediated knockdown of *Mcat* in the mouse retinal cell line 661W and observed a disturbed mitochondrial morphology and reduced uptake of Mitotracker dye, consistent with a loss of membrane potential (Li and others, 2020). A retinal ganglion cell (RGC) specific *Mcat* KO mouse line established by the same group (Li and others, 2020) revealed attenuation of the neurofibre layer in the proximity of Cre-positive RGCs in the retina of the affected mice. In addition, the consecutive neurofibre was found to

be disturbed in many cases. These results indicated that the depletion of MCAT in RGC mitochondria disrupts maintenance of axons, consistent with a role of the enzyme in survival of optical nerve neurons.

Homozygous *Lias* KO mice exhibited embryonic lethality resulting from gastrulation defects (Yi and Maeda, 2005). The supplementation of LA in the diet of heterozygous pregnant females did not rescue the embryonic lethality. A hypomorphic mutation of LIAS resulted in nearly headless mouse embryos that lacked most of the forebrain at mid-gestation (Zhou and Anderson, 2010). Defects in these synthesis and transfer processes lead to severe human disorders (Mayr and others, 2014; Solmonson and DeBerardinis, 2018; Tort and others, 2016). Functional analyses of some LIPT1 mutations found in patients (see below) were carried out in cell and mouse models (Ni and others, 2019)

Human patients with defects in mtFAS and related pathways

Defects in mtFAS can affect multiple mitochondrial functions (Fig.3B). In common perception, the clinical symptoms develop due to deficiency of lipoylated proteins involved in carbohydrate and amino acid metabolism. However, several studies in yeast implicate other roles of mtFAS (Cory and others, 2017; Kursu and others, 2013; Van Vranken and others, 2018) and acyl-ACP (Masud and others, 2019). As a result, the clinical symptoms observed can be heterogeneous, making it a challenge to diagnose mtFAS defect cases based on the patient display of the disorder alone (Table II). However, as whole exome sequencing of patient samples is getting more common, more cases related to mtFAS impairment will be identified in the future. The existing reports show that the age of onset of symptoms can vary between neonatal stage to young adulthood. Although most of the patients develop symptoms during

the neonatal period, the mutations observed do not appear to affect the normal course of development during pregnancy.

a) Human disorders due to defects in the mtFAS pathway

The first report about mtFAS deficiency patients was published in 2016 by Heimer and collaborators (Heimer and others, 2016). The patients described within this paper carried missense, nonsense, or frameshift mutations, or nucleotide changes resulting in splice site aberrations in *MECR* (genomic location 1p35.3). The three latter types generated truncated variants of the MECR protein, and all mutations described thus far are recessive and cause complete or partial loss of enzyme function. All patients appear to maintain at least one allele encoding a MECR enzyme with partial function. These defects lead to childhood onset dystonia and optic atrophy. In most of the patients, intellectual capacity is preserved. Brain imaging by MRI typically reveals basal ganglia abnormalities, with bilateral hyperintense T2-weighted signal in caudate, putamen, or pallidum, or a combination thereof, in affected individuals. These alterations are evident at the time of onset of dystonia (Heimer and others, 1993; Heimer and others, 2016). Optic atrophy may appear with a delay of several years after the first indications of dystonia are detected in MEPAN patients. The oldest identified MEPAN patient is currently 52 years of age, and was initially diagnosed to display sea blue histiocyte associated neurodegeneration (Swaiman and others, 1983), suspected to be form of PKAN (pantothenate kinase associated neurodegeneration, formerly Hallervorden-Spatz syndrome), although iron accumulation characteristic for this disorder could not be confirmed in follow up studies. The condition was termed MEPAN (mitochondrial enoyl CoA reductase protein-associated neurodegeneration) syndrome and can also be found under the OMIM designation DYTOABG (dystonia with optic atrophy and basal ganglia abnormalities). Currently, there are at least 17 known MEPAN cases ((Heimer and others, 2016), and the

disorder is rare (<1:100000 according to ORPHANET) but slightly more frequent in the Ashkenazi Jewish population, where two mutation variants (c.695G>A /p.Gly232Glu and c.830+2InsT) appear to be more common (Heimer and others, 1993; Heimer and others, 2016).

In a new case of MECR-dysfunction related disease reported from Turkey in 2019, the affected child developed gait abnormalities, epilepsy, dystonia and optic atrophy. MRI analyses revealed basal ganglia signal abnormalities, cerebral and cerebellar atrophy. The patient was found to carry compound heterozygous mutation in *MECR* (Gorukmez and others, 2019). The cerebellar atrophy and epilepsy features were not previously reported in MECR defect patients but are common in disorders caused by defects in LA synthesis or attachment (Mayr and others, 2014).

Neurological phenotypes can also often be detected associated with a metabolic disorder termed “combined malonic and methylmalonic aciduria (CMAMMA) (Wehbe and others, 2019), although in many cases the consequences of ACSF3 defects are rather benign (Levtova and others, 2019). Patients with this disorder were found to harbour mutations in the *ACSF3* gene, which has been implicated as producer of intramitochondrial malonyl-CoA, thereby linking CMAMMA to mtFAS. Especially older patients with ACSF3 mutations exhibit neurological symptoms. Dysregulation of mitochondrial energy metabolism due to mtFAS dysfunction has recently been suggested to lie at the root of these brain function related phenotypes (Tucci, 2020). Most recently, a novel report on an autosomal recessive mtFAS defect disorder caused by mutations in the *MCAT* gene, encoding mitochondrial malonyl-CoA::ACP transacylase, was published (Li and others, 2020). Both patients, siblings from a Chinese consanguineous family, were diagnosed at an age of about 8 years. Analysis of the pathogenic *MCAT* mutation variants in HEK293 cells indicated that they probably result in

unstable MCAT protein. The patients were described to suffer from progressive non-syndromic bilateral optic neuropathy, accompanied by nystagmus. Ophthalmoscopic inspection revealed that the capillaries of the optic discs in these patients were attenuated, and the optic disc pallor appeared diffuse in these individuals. A closer investigation of the optic disc region by optical coherence tomography indicated a thinning of the fibre layer around this structure, consistent with neurodegenerative damage.

b) Disorders caused by defects in the LA synthesis and attachment pathway

There are many case reports associated with defects in LA synthesis or lipoylation pathways, published during the last decade. LA deficiency disorders in humans include mtFAS defects, *LIAS* defect, Fe-S cluster biogenesis defects, *LIPT1 or LIPT2* mutations, dihydrolipoamide dehydrogenase deficiency as well as *GCSH* (GCS H protein) deficiency (Mayr and others, 2014). LA biosynthesis and mitochondrial oxidative metabolism by lipoylated proteins were reviewed recently (Solmonson and DeBerardinis, 2018). Homozygous point mutation in *LIAS* resulted in neonatal epilepsy, muscular hypotonia, lactic acidosis and hyperglycinemia (Mayr and others, 2011). In a 21 year old patient who had been diagnosed with hyperglycinemia in serum and cerebrospinal fluid already as a toddler, a combination of missense and splice site mutations in *LIAS* was identified (Tsurusaki and others, 2015). Mutations in *LIPT1,* encoding the enzyme responsible for transfer of the lipoyl moiety from GCDH to other 2-ketoacid dehydrogenases have also been identified (Cao and others, 2018). *LIPT1* defect patients typically exhibited metabolic acidosis, encephalopathy, epilepsy, pulmonary hypertension and biochemical analysis showed deficiency of PDH and KDH (Tache and others, 2016; Tort and others, 2014). LIPT1 deficiencies can also present as Leigh's disease (Soreze and others, 2013) and are often associated with developmental delay and early death in humans. Habarou and others reported cases of children carrying biallelic mutation in *LIPT2*.

The patients presented with severe neonatal onset encephalopathy, exhibited hyperlactaemia, increased plasma glycine, alanine and decreased branched chain dehydrogenase and combined 2-ketoacid dehydrogenase deficiency (Habarou and others, 2017). The non-ketotic hyperglycinemia in patients with LIPT2 mutations, cause by lack of lipoylated H protein of GCS in addition to dysfunction of other lipoylated complexes, separates this disorder from LIPT1-deficiencies.

c) Iron sulphur cluster biogenesis pathways

A subset of LA deficiencies were associated with ISC biogenesis defects. There are few excellent recent reviews about the Fe-S cluster biogenesis and functions, pathophysiological changes, clinical outcomes and diagnosis of Fe-S cluster biogenesis defects (Alfadhel and others, 2017; Vanlander and Van Coster, 2018). Fe-S cluster synthesis defects causing neurological disorder have been identified in *GLRX5, BOLA3* and *LIAS*, (Baker and others, 2014) as well as in *IBA57* (Ishiyama and others, 2017), which all affect 4Fe-4S cluster formation required for LA synthase function. Mutations in *NFU1* stand out of the Fe-S deficiency group of disorders, as they specifically affected LA synthesis in 9 unrelated neonatal patients and many of them displayed neurological symptoms like hypotonia and irritability (Navarro-Sastre and others, 2011). Seven new patients carrying compound heterozygous and homozygous mutations in *NFU1* exhibited fatal infantile encephalopathy and pulmonary hypertension. The patient fibroblasts and skeletal muscle samples revealed combined respiratory complex and PDH deficiencies, metabolic acidosis and hyperglycinemia (Ahting and others, 2015). NFU1 has since been characterized as a LIAS-rejuvenation factor that replaces a Fe-S cluster in the enzyme that serves as a sulphur donor and is sacrificed during the thiolation process (McCarthy and Booker, 2017).

d) A role of mtFAS in Parkinson's disease?

In a study related to Parkinson's disease, seeding of primary neuron cultures with recombinant preformed α synuclein promoted recruitment of endogenous α synuclein and its conversion to fibrillar and nonfibrillar phosphorylated α synuclein forms. The nonfibrillar form is reported as a product of autophagosomal activity on fibrillar form and it produced mitotoxic lesions like reduced membrane potential, oxidative stress, recruitment of phosphorylated (inactive) ACC1 followed by reduced lipoylation, mitochondrial fragmentation and cell death. The study shows link between mitotoxicity and Parkinson's disease (Grassi and others, 2018) and possible connection to mtFAS. There has been no systematic investigation correlating expression levels of the transcript isoform 1 of *ACACA,* encoding the proposed mitochondrial isoform of ACC1 and mitochondrial function.

e) Acyl-ACP deficiency as a factor linking several neurodegenerative disorders

The first MEPAN/MECR deficiency patient identified was initially suspected to display a variant of PKAN, as some of the clinical symptoms between PKAN and MEPAN are overlapping. A recent publication now has presented a compelling body of evidence linking MEPAN/mtFAS deficiencies, PKAN and CoPAN (Coenzyme A synthase Protein associated neurodegeneration) through an effect on acyl-ACP availability (Lambrechts and others, 2019). While defects in mtFAS result in hypoacylated ACP, PKAN or CoPAN-related mutations will always result in a decrease of available holo-ACP due to the lack of CoA, the substrate donating the 4'PP group required by PPTase to generate the functional form of ACP (Fig.3C). According to the model presented in this publication and supported by the data within, these disorders share a shortage of C8/LA and possibly also longer holo-ACP-associated acyl groups as a phenotype, with similar pathological consequences. In spite of similarity of phenotypes between these disorders, a fundamental difference between MEPAN and CoPAN as well as

PKAN is that the latter two disorders show more pleiotropic phenotypes and belong to the group of “Neurodegeneration with Brain Iron Accumulation” (NBIA) diseases. The model proposed by Lambrechts et al now offers an explanation for this discrepancy in disorder display. All of these disorders lead (or have been inferred to lead) to lack of mitochondrial fatty acids and LA in particular, but MEPAN patients still have normal levels of (holo-)ACP, while CoPAN and PKAN is associated with a severe reduction of ACP in the patients’ cells. ACP is associated with the Fe-S cluster synthesis machinery. ACP deficiency in yeast is lethal (Van Vranken and others, 2016) and lack of PPTase is very detrimental to yeast growth, while mutations in mtFAS enzymes only cause respiratory deficiency (Merz and Westermann, 2009), indicating that only (holo-)ACP is absolutely required for Fe-S synthesis. Loss of the 4’PP of ACP moiety results in strongly reduced interaction with the Nsf1-Isd11 complex of the Fe-S cluster synthesis machinery in yeast, and a marked reduction of the Nsf1-Isd11 itself, accompanied by lower activity of Fe-S cluster dependent enzymes (Van Vranken and others, 2016). The 4’PP moiety, albeit not essential for Fe-S cluster generation, is therefore required for efficient function and stability of the Fe-S cluster synthesis machinery. The role of the fatty acyl moiety in Fe-S cluster function, if any, is not yet clear.

In human disease, Fe-S cluster deficiency results in mitochondrial iron overload. The model by Lambrechts and others therefore implies that the iron accumulation phenotype may not be a primary defect in many NBIA disorders, but rather a mitochondrial response to Fe-S cluster deficiency.

Conclusions and future challenges

In contrast to disorders caused by mitochondrial β-oxidation defects, mtFAS or LA synthesis/attachment deficiencies have only recently entered the databases as diseases

causing neurological defects. There is no doubt that the list of mtFAS disease alleles will expand to cover all or nearly all of the contributing factors, in addition to ACSF3, MCAT and MECR pathological variants currently known. With more data available in the near future, a clearer understanding of the processes leading to neurodegeneration may be achieved.

ACSF3 clearly has other roles in addition to providing malonyl-CoA for mtFAS leading to the more pleiotropic presentation of CMAMMA. But for the MCAT and MECR defect cases, the very strict neurological phenotype is remarkable. Neurons require a high level of oxidative phosphorylation. It may be argued that at least one of the alleles carried by mtFAS patients still has some residual function, which may be sufficient to support cell viability in other cell types. Nevertheless, if the role of mtFAS is so essential in mitochondrial function, why are we not able to observe severe effects on other patient tissues with a high energy demand like skeletal muscle or heart? Owing to the central roles of CoA in metabolism and Fe-S clusters in the function of multiple important enzymes, it is evident why defects in these processes have much more pleiotropic consequences than mtFAS dysfunctions. But why are mutations that affect LA synthesis and attachment so much more pleiotropic? There is currently no answer to these questions, but there is a suspicion. Unlike yeast, where mtFAS is essential for respiration, most mammalian cells carry out β-oxidation in mitochondria. The notable exception from this observation are neurons. Hence, the question must be asked if there is a route for fatty acid breakdown intermediates to ACP in tissues carrying out mitochondrial β-oxidation. There may be alternative explanations, for example reduced motility of mitochondria with mtFAS defects. Mitochondria in mtFAS deficient cells both in yeast an mammals display severe morphological changes (A. Kastaniotis and others, 2004; Monteuuis and others, 2017; Torkko and others, 2001), which may also be associated with a diminished ability to move around in the cell. In neurons, mitochondria often are transported for long

distances along the axons (Saxton and Hollenbeck, 2012), and disturbed transport processes may be an alternative explanation for the neuron specificity of mtFAS disorders. Research addressing these hypotheses is necessary.

The remarkable contribution of Lambrechts and others in 2019 has been able to help in our understanding of the similarities of CoPAN, PKAN, MEPAN and other mtFAS deficiency disorders. The existence of a common denominator in these disorders – holo-ACP – also points to a common defect that may in the future be addressed pharmacologically: LA deficiency. In light of our current understanding that free LA provided in the diet is not available for attachment to ketoacid dehydrogenase complexes, there is no doubt that it will be a challenge to find a compound that may be used for enzyme lipoylation. But such a molecule – being accepted as transfer substrate by LIPT1 and LIPT2, able to cross the cell and mitochondrial membranes and to overcome the blood-brain-barrier – would likely be of tremendous help for many patients. No candidate molecule meeting all these challenges has been found to date, and efforts towards finding promising compounds are necessary.

ACP has emerged one of the key players shared by CoPAN, PKAN and MEPAN syndromes. Acylation of ACP must clearly play a role in its proposed function to coordinate IMBE. But more profoundly, the 4'phosphopantetheinylation of ACP appears to be one of the most critical steps in cellular function. If Lambrechts and co-workers are correct, this process must be very sensitive to the concentration of CoA in the cell. Hence, if mtFAS is an intramitochondrial sensor of cellular energy state, could ACP serve as a mitochondrial sensor for CoA, one of the most central compounds of cellular metabolism?

Acknowledgements

We thank professor Kalervo Hiltunen for the proofreading and critical comments for this manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Academy of Finland (grant number 314925) and the Sigrid Juselius Foundation.

References

Adeva-Andany MM, Carneiro-Freire N, Seco-Filgueira M, Fernandez-Fernandez C and Mourino-Bayolo D (2019) Mitochondrial beta-oxidation of saturated fatty acids in humans. *Mitochondrion* 46: 73-90.

Ahting U, Mayr JA, Vanlander AV, Hardy SA, Santra S, Makowski C, and others (2015) Clinical, biochemical, and genetic spectrum of seven patients with NFU1 deficiency. *Frontiers in Genetics* 6: 123.

Alfadhel M, Nashabat M, Abu Ali Q and Hundallah K (2017) Mitochondrial iron-sulfur cluster biogenesis from molecular understanding to clinical disease. *Neurosciences (Riyadh, Saudi Arabia)* 22(1): 4-13.

Angerer H (2015) Eukaryotic LYR Proteins Interact with Mitochondrial Protein Complexes. *Biology* 4(1): 133-150.

Autio KJ, Kastaniotis AJ, Pospiech H, Miinalainen IJ, Schonauer MS, Dieckmann CL, and others (2008) An ancient genetic link between vertebrate mitochondrial fatty acid synthesis and RNA processing. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 22(2): 569-578.

Baker PR,2nd, Friederich MW, Swanson MA, Shaikh T, Bhattacharya K, Scharer GH, and others (2014) Variant non ketotic hyperglycinemia is caused by mutations in LIAS, BOLA3 and the novel gene GLRX5. *Brain : A Journal of Neurology* 137(Pt 2): 366-379.

Brody S and Mikolajczyk S (1988) Neurospora mitochondria contain an acyl-carrier protein. *European Journal of Biochemistry / FEBS* 173(2): 353-359.

Brody S, Oh C, Hoja U and Schweizer E (1997) Mitochondrial acyl carrier protein is involved in lipoic acid synthesis in Saccharomyces cerevisiae. *FEBS Lett* 408(2): 217-220.

Brown A, Rathore S, Kimanius D, Aibara S, Bai XC, Rorbach J, and others (2017) Structures of the human mitochondrial ribosome in native states of assembly. *Nature Structural & Molecular Biology* 24(10): 866-869.

Cao X, Zhu L, Song X, Hu Z and Cronan JE (2018) Protein moonlighting elucidates the essential human pathway catalyzing lipoic acid assembly on its cognate enzymes. *Proceedings of the National Academy of Sciences of the United States of America* 115(30): E7063-E7072.

Chen C, Han X, Zou X, Li Y, Yang L, Cao K, and others (2014) 4-methylene-2-octyl-5-oxotetrahydrofuran-3-carboxylic acid (C75), an inhibitor of fatty-acid synthase, suppresses the mitochondrial fatty acid synthesis pathway and impairs mitochondrial function. *The Journal of Biological Chemistry* 289(24): 17184-17194.

Chen Z, Kastaniotis AJ, Miinalainen IJ, Rajaram V, Wierenga RK and Hiltunen JK (2009) 17beta-hydroxysteroid dehydrogenase type 8 and carbonyl reductase type 4 assemble as a ketoacyl reductase of human mitochondrial FAS. *The FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 23(11): 3682-3691.

Chen Z, Leskinen H, Liimatta E, Sormunen RT, Miinalainen IJ, Hassinen IE, and others (2009) Myocardial overexpression of Mecr, a gene of mitochondrial FAS II leads to cardiac dysfunction in mouse. *PloS One* 4(5): e5589.

Clay HB, Parl AK, Mitchell SL, Singh L, Bell LN and Murdock DG (2016) Altering the Mitochondrial Fatty Acid Synthesis (mtFASII) Pathway Modulates Cellular Metabolic States and Bioactive Lipid Profiles as Revealed by Metabolomic Profiling. *PloS One* 11(3): e0151171.

Cory SA, Van Vranken JG, Brignole EJ, Patra S, Winge DR, Drennan CL, and others (2017) Structure of human Fe-S assembly subcomplex reveals unexpected cysteine desulfurase architecture and acyl-ACP-ISD11 interactions. *Proceedings of the National Academy of Sciences of the United States of America* 114(27): E5325-E5334.

Cronan JE (2016) Assembly of Lipoic Acid on Its Cognate Enzymes: an Extraordinary and Essential Biosynthetic Pathway. *Microbiology and Molecular Biology Reviews : MMBR* 80(2): 429-450.

Edmond J, Robbins RA, Bergstrom JD, Cole RA and de Vellis J (1987) Capacity for substrate utilization in oxidative metabolism by neurons, astrocytes, and oligodendrocytes from developing brain in primary culture. *Journal of Neuroscience Research* 18(4): 551-561.

Eraso-Pichot A, Braso-Vives M, Golbano A, Menacho C, Claro E, Galea E, and others (2018) GSEA of mouse and human mitochondriomes reveals fatty acid oxidation in astrocytes. *Glia* 66(8): 1724-1735.

Fahnehjelm KT, Liu Y, Olsson D, Amren U, Haglind CB, Holmstrom G, and others (2016) Most patients with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency develop pathological or subnormal retinal function. *Acta Paediatrica (Oslo, Norway : 1992)* 105(12): 1451-1460.

Fecher C, Trovo L, Muller SA, Snaidero N, Wettmarshausen J, Heink S, and others (2019) Cell-type-specific profiling of brain mitochondria reveals functional and molecular diversity. *Nature Neuroscience* 22(10): 1731-1742.

Feng D, Witkowski A and Smith S (2009) Down-regulation of mitochondrial acyl carrier protein in mammalian cells compromises protein lipoylation and respiratory complex I and results in cell death. *The Journal of Biological Chemistry* 284(17): 11436-11445.

Fraser H, Geppert J, Johnson R, Johnson S, Connock M, Clarke A, and others (2019) Evaluation of earlier versus later dietary management in long-chain 3-hydroxyacyl-CoA dehydrogenase or mitochondrial trifunctional protein deficiency: a systematic review. *Orphanet Journal of Rare Diseases* 14(1): 258-019-1226-y.

Gorukmez O, Gorukmez O and Havali C (2019) Novel MECR Mutation in Childhood-Onset Dystonia, Optic Atrophy, and Basal Ganglia Signal Abnormalities. *Neuropediatrics* 50(5): 336-337.

Grassi D, Howard S, Zhou M, Diaz-Perez N, Urban NT, Guerrero-Given D, and others (2018) Identification of a highly neurotoxic alpha-synuclein species inducing mitochondrial damage and mitophagy in Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America* 115(11): E2634-E2643.

Habarou F, Hamel Y, Haack TB, Feichtinger RG, Lebigot E, Marquardt I, and others (2017) Biallelic Mutations in LIPT2 Cause a Mitochondrial Lipoylation Defect Associated with Severe Neonatal Encephalopathy. *American Journal of Human Genetics* 101(2): 283-290.

Harington A, Schwarz E, Slonimski PP and Herbert CJ (1994) Subcellular relocalization of a long-chain fatty acid CoA ligase by a suppressor mutation alleviates a respiration deficiency in Saccharomyces cerevisiae. *The EMBO Journal* 13(23): 5531-5538.

Harington A, Herbert CJ, Tung B, Getz GS and Slonimski PP (1993) Identification of a new nuclear gene (CEM1) encoding a protein homologous to a beta-keto-acyl synthase which is essential for mitochondrial respiration in Saccharomyces cerevisiae. *Molecular Microbiology* 9(3): 545-555.

Heimer G, Gregory A, Hogarth P, Hayflick S and Ben Zeev B (1993) MECR-Related Neurologic Disorder. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, and others (eds) *GeneReviews((R)).* Seattle (WA): University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.

Heimer G, Keratar JM, Riley LG, Balasubramaniam S, Eyal E, Pietikainen LP, and others (2016) MECR Mutations Cause Childhood-Onset Dystonia and Optic Atrophy, a Mitochondrial Fatty Acid Synthesis Disorder. *American Journal of Human Genetics* 99(6): 1229-1244.

Hiltunen JK, Chen Z, Haapalainen AM, Wierenga RK and Kastaniotis AJ (2010) Mitochondrial fatty acid synthesis--an adopted set of enzymes making a pathway of major importance for the cellular metabolism. *Progress in Lipid Research* 49(1): 27-45.

Hiltunen JK, Schonauer MS, Autio KJ, Mittelmeier TM, Kastaniotis AJ and Dieckmann CL (2009) Mitochondrial fatty acid synthesis type II: more than just fatty acids. *The Journal of Biological Chemistry* 284(14): 9011-9015.

Holzmann J, Frank P, Loffler E, Bennett KL, Gerner C and Rossmanith W (2008) RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. *Cell* 135(3): 462-474.

Hou T, Zhang R, Jian C, Ding W, Wang Y, Ling S, and others (2019) NDUFAB1 confers cardio-protection by enhancing mitochondrial bioenergetics through coordination of respiratory complex and supercomplex assembly. *Cell Research* 29(9): 754-766.

Houten SM, Violante S, Ventura FV and Wanders RJ (2016) The Biochemistry and Physiology of Mitochondrial Fatty Acid beta-Oxidation and Its Genetic Disorders. *Annual Review of Physiology* 78: 23-44.

Immonen T, Turanlahti M, Paganus A, Keskinen P, Tyni T and Lapatto R (2016) Earlier diagnosis and strict diets improve the survival rate and clinical course of long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *Acta Paediatrica (Oslo, Norway : 1992)* 105(5): 549-554.

Ioannou MS, Jackson J, Sheu SH, Chang CL, Weigel AV, Liu H, and others (2019) Neuron-Astrocyte Metabolic Coupling Protects against Activity-Induced Fatty Acid Toxicity. *Cell* 177(6): 1522-1535.e14.

Ishiyama A, Sakai C, Matsushima Y, Noguchi S, Mitsuhashi S, Endo Y, and others (2017) IBA57 mutations abrogate iron-sulfur cluster assembly leading to cavitating leukoencephalopathy. *Neurology.Genetics* 3(5): e184.

Joshi AK, Zhang L, Rangan VS and Smith S (2003) Cloning, expression, and characterization of a human 4'-phosphopantetheinyl transferase with broad substrate specificity. *The Journal of Biological Chemistry* 278(35): 33142-33149.

Kastaniotis A, Autio K, Sormunen R and Hiltunen J (2004) Htd2p/Yhr067p is a yeast 3-hydroxyacyl-ACP dehydratase essential for mitochondrial function and morphology. *Mol Microbiol* 53(5): 1407-1421.

Kastaniotis AJ, Autio KJ, Keratar JM, Monteuuis G, Makela AM, Nair RR, and others (2017) Mitochondrial fatty acid synthesis, fatty acids and mitochondrial physiology. *Biochimica Et Biophysica Acta* 1862(1): 39-48.

Kim DG, Yoo JC, Kim E, Lee YS, Yarishkin OV, Lee DY, and others (2014) A Novel Cytosolic Isoform of Mitochondrial Trans-2-Enoyl-CoA Reductase Enhances Peroxisome Proliferator-Activated Receptor alpha Activity. *Endocrinology and Metabolism (Seoul, Korea)* 29(2): 185-194.

Kursu VA, Pietikainen LP, Fontanesi F, Aaltonen MJ, Suomi F, Raghavan Nair R, and others (2013) Defects in mitochondrial fatty acid synthesis result in failure of multiple aspects of mitochondrial biogenesis in Saccharomyces cerevisiae. *Molecular Microbiology* 90(4): 824-840.

Lambrechts RA, Schepers H, Yu Y, van der Zwaag M, Autio KJ, Vieira-Lara MA, and others (2019) CoA-dependent activation of mitochondrial acyl carrier protein links four neurodegenerative diseases. *EMBO Molecular Medicine*: e10488.

Lebigot E, Gaignard P, Dorboz I, Slama A, Rio M, de Lonlay P, and others (2017) Impact of mutations within the [Fe-S] cluster or the lipoic acid biosynthesis pathways on mitochondrial protein expression profiles in fibroblasts from patients. *Molecular Genetics and Metabolism* 122(3): 85-94.

Levtova A, Waters PJ, Buhas D, Levesque S, Auray-Blais C, Clarke JTR, and others (2019) Combined malonic and methylmalonic aciduria due to ACSF3 mutations: Benign clinical course in an unselected cohort. *Journal of Inherited Metabolic Disease* 42(1): 107-116.

Li H, Yuan S, Minegishi Y, Suga A, Yoshitake K, Sheng X, and others (2020) Novel mutations in malonyl-CoA-acyl carrier protein transacylase provoke autosomal recessive optic neuropathy. *Human Molecular Genetics* 29(3): 444-458.

Masud AJ, Kastaniotis AJ, Rahman MT, Autio KJ and Hiltunen JK (2019) Mitochondrial acyl carrier protein (ACP) at the interface of metabolic state sensing and mitochondrial function. *Biochimica Et Biophysica Acta.Molecular Cell Research* 1866(12): 118540.

Masuda N, Yasumo H, Furusawa T, Tsukamoto T, Sadano H and Osumi T (1998) Nuclear receptor binding factor-1 (NRBF-1), a protein interacting with a wide spectrum of nuclear hormone receptors. *Gene* 221(2): 225-233.

Mayr JA, Feichtinger RG, Tort F, Ribes A and Sperl W (2014) Lipoic acid biosynthesis defects. *Journal of Inherited Metabolic Disease* 37(4): 553-563.

Mayr JA, Zimmermann FA, Fauth C, Bergheim C, Meierhofer D, Radmayr D, and others (2011) Lipoic acid synthetase deficiency causes neonatal-onset epilepsy, defective mitochondrial energy metabolism, and glycine elevation. *American Journal of Human Genetics* 89(6): 792-797.

McCarthy EL and Booker SJ (2017) Destruction and reformation of an iron-sulfur cluster during catalysis by lipoyl synthase. *Science (New York, N.Y.)* 358(6361): 373-377.

Merritt JL,2nd, Norris M and Kanungo S (2018) Fatty acid oxidation disorders. *Annals of Translational Medicine* 6(24): 473.

Merz S and Westermann B (2009) Genome-wide deletion mutant analysis reveals genes required for respiratory growth, mitochondrial genome maintenance and mitochondrial protein synthesis in Saccharomyces cerevisiae. *Genome Biology* 10(9): R95-2009-10-9-r95. Epub 2009 Sep 14.

Miinalainen I, Chen Z, Torkko J, Pirila P, Sormunen R, Bergmann U, and others (2003) Characterization of 2-enoyl thioester reductase from mammals. An ortholog of YBR026p/MRF1'p of the yeast mitochondrial fatty acid synthesis type II. *J Biol Chem* 278(22): 20154-20161.

Mikolajczyk S and Brody S (1990) De novo fatty acid synthesis mediated by acyl-carrier protein in Neurospora crassa mitochondria. *European Journal of Biochemistry / FEBS* 187(2): 431-437.

Monteuuis G, Suomi F, Keratar JM, Masud AJ and Kastaniotis AJ (2017) A conserved mammalian mitochondrial isoform of acetyl-CoA carboxylase ACC1 provides the malonyl-CoA essential for mitochondrial biogenesis in tandem with ACSF3. *The Biochemical Journal* 474(22): 3783-3797.

Nair RR, Koivisto H, Jokivarsi K, Miinalainen IJ, Autio KJ, Manninen A, and others (2018) Impaired Mitochondrial Fatty Acid Synthesis Leads to Neurodegeneration in Mice. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 38(45): 9781-9800.

Nair RR, Keratar JM, Autio KJ, Masud AJ, Finnila MAJ, Autio-Harmainen HI, and others (2017) Genetic modifications of Mecr reveal a role for mitochondrial 2-enoyl-CoA/ACP reductase in placental development in mice. *Human Molecular Genetics* 26(11): 2104-2117.

Navarro-Sastre A, Tort F, Stehling O, Uzarska MA, Arranz JA, Del Toro M, and others (2011) A fatal mitochondrial disease is associated with defective NFU1 function in the maturation of a subset of mitochondrial Fe-S proteins. *American Journal of Human Genetics* 89(5): 656-667.

Ni M, Solmonson A, Pan C, Yang C, Li D, Notzon A, and others (2019) Functional Assessment of Lipoyltransferase-1 Deficiency in Cells, Mice, and Humans. *Cell Reports* 27(5): 1376-1386.e6.

Panov A, Orynbayeva Z, Vavilin V and Lyakhovich V (2014) Fatty acids in energy metabolism of the central nervous system. *BioMed Research International* 2014: 472459.

Parl A, Mitchell SL, Clay HB, Reiss S, Li Z and Murdock DG (2013) The mitochondrial fatty acid synthesis (mtFASII) pathway is capable of mediating nuclear-mitochondrial cross talk through the PPAR system of transcriptional activation. *Biochemical and Biophysical Research Communications* 441(2): 418-424.

Runswick MJ, Fearnley IM, Skehel JM and Walker JE (1991) Presence of an acyl carrier protein in NADH:ubiquinone oxidoreductase from bovine heart mitochondria. *FEBS Letters* 286(1-2): 121-124.

Sackmann U, Zensen R, Rohlen D, Jahnke U and Weiss H (1991) The acyl-carrier protein in Neurospora crassa mitochondria is a subunit of NADH:ubiquinone reductase (complex I). *European Journal of Biochemistry* 200(2): 463-469.

Saxton WM and Hollenbeck PJ (2012) The axonal transport of mitochondria. *Journal of Cell Science* 125(Pt 9): 2095-2104.

Schneider R, Massow M, Lisowsky T and Weiss H (1995) Different respiratory-defective phenotypes of Neurospora crassa and Saccharomyces cerevisiae after inactivation of the gene encoding the mitochondrial acyl carrier protein. *Current Genetics* 29(1): 10-17.

Shvetsova AN, Mennerich D, Keratar JM, Hiltunen JK and Kietzmann T (2017) Non-electron transfer chain mitochondrial defects differently regulate HIF-1alpha degradation and transcription. *Redox Biology* 12: 1052-1061.

Smith S, Witkowski A and Joshi AK (2003) Structural and functional organization of the animal fatty acid synthase. *Progress in Lipid Research* 42(4): 289-317.

Smith S, Witkowski A, Moghul A, Yoshinaga Y, Nefedov M, de Jong P, and others (2012) Compromised mitochondrial fatty acid synthesis in transgenic mice results in defective protein lipoylation and energy disequilibrium. *PloS One* 7(10): e47196.

Solmonson A and DeBerardinis RJ (2018) Lipoic acid metabolism and mitochondrial redox regulation. *The Journal of Biological Chemistry* 293(20): 7522-7530.

Soreze Y, Boutron A, Habarou F, Barnerias C, Nonnenmacher L, Delpech H, and others (2013) Mutations in human lipoyltransferase gene LIPT1 cause a Leigh disease with secondary deficiency for pyruvate and alpha-ketoglutarate dehydrogenase. *Orphanet Journal of Rare Diseases* 8: 192-1172-8-192.

Spiekerkoetter U (2010) Mitochondrial fatty acid oxidation disorders: clinical presentation of long-chain fatty acid oxidation defects before and after newborn screening. *Journal of Inherited Metabolic Disease* 33(5): 527-532.

Stuible HP, Meier S, Wagner C, Hannappel E and Schweizer E (1998) A novel phosphopantetheine:protein transferase activating yeast mitochondrial acyl carrier protein. *The Journal of Biological Chemistry* 273(35): 22334-22339.

Swaiman KF, Smith SA, Trock GL and Siddiqui AR (1983) Sea-blue histiocytes, lymphocytic cytosomes, movement disorder and 59Fe-uptake in basal ganglia: Hallervorden-Spatz disease or ceroid storage disease with abnormal isotope scan? *Neurology* 33(3): 301-305.

Tache V, Bivina L, White S, Gregg J, Deignan J, Boyadjievd SA, and others (2016) Lipoyltransferase 1 Gene Defect Resulting in Fatal Lactic Acidosis in Two Siblings. *Case Reports in Obstetrics and Gynecology* 2016: 6520148.

Torkko JM, Koivuranta KT, Miinalainen IJ, Yagi AI, Schmitz W, Kastaniotis AJ, and others (2001) Candida tropicalis Etr1p and Saccharomyces cerevisiae Ybr026p (Mrf1'p), 2-enoyl thioester reductases essential for mitochondrial respiratory competence. *Molecular and Cellular Biology* 21(18): 6243-6253.

Tort F, Ferrer-Cortes X and Ribes A (2016) Differential diagnosis of lipoic acid synthesis defects. *Journal of Inherited Metabolic Disease* 39(6): 781-793.

Tort F, Ferrer-Cortes X, Thio M, Navarro-Sastre A, Matalonga L, Quintana E, and others (2014) Mutations in the lipoyltransferase LIPT1 gene cause a fatal disease associated with a specific

lipoylation defect of the 2-ketoacid dehydrogenase complexes. *Human Molecular Genetics* 23(7): 1907-1915.

Tsurusaki Y, Tanaka R, Shimada S, Shimojima K, Shiina M, Nakashima M, and others (2015) Novel compound heterozygous LIAS mutations cause glycine encephalopathy. *Journal of Human Genetics* 60(10): 631-635.

Tucci S (2020) Brain metabolism and neurological symptoms in combined malonic and methylmalonic aciduria. *Orphanet Journal of Rare Diseases* 15(1): 27-020-1299-7.

Tucci S (2017) Very long-chain acyl-CoA dehydrogenase (VLCAD-) deficiency-studies on treatment effects and long-term outcomes in mouse models. *Journal of Inherited Metabolic Disease* 40(3): 317-323.

Van Vranken JG, Jeong MY, Wei P, Chen YC, Gygi SP, Winge DR, and others (2016) The mitochondrial acyl carrier protein (ACP) coordinates mitochondrial fatty acid synthesis with iron sulfur cluster biogenesis. *eLife* 5: 10.7554/eLife.17828.

Van Vranken JG, Nowinski SM, Clowers KJ, Jeong MY, Ouyang Y, Berg JA, and others (2018) ACP Acylation Is an Acetyl-CoA-Dependent Modification Required for Electron Transport Chain Assembly. *Molecular Cell* 71(4): 567-580.e4.

Vanlander AV and Van Coster R (2018) Clinical and genetic aspects of defects in the mitochondrial iron-sulfur cluster synthesis pathway. *Journal of Biological Inorganic Chemistry : JBIC : A Publication of the Society of Biological Inorganic Chemistry* 23(4): 495-506.

Venkatesan R, Sah-Teli SK, Awoniyi LO, Jiang G, Prus P, Kastaniotis AJ, and others (2014) Insights into mitochondrial fatty acid synthesis from the structure of heterotetrameric 3-ketoacyl-ACP reductase/3R-hydroxyacyl-CoA dehydrogenase. *Nature Communications* 5: 4805.

Wada H, Shintani D and Ohlrogge J (1997) Why do mitochondria synthesize fatty acids? Evidence for involvement in lipoic acid production. *Proceedings of the National Academy of Sciences of the United States of America* 94(4): 1591-1596.

Wehbe Z, Behringer S, Alatibi K, Watkins D, Rosenblatt D, Spiekerkoetter U, and others (2019) The emerging role of the mitochondrial fatty-acid synthase (mtFASII) in the regulation of energy metabolism. *Biochimica Et Biophysica Acta.Molecular and Cell Biology of Lipids* 1864(11): 1629-1643.

Witkowski A, Thweatt J and Smith S (2011) Mammalian ACSF3 protein is a malonyl-CoA synthetase that supplies the chain extender units for mitochondrial fatty acid synthesis. *The Journal of Biological Chemistry* 286(39): 33729-33736.

Witkowski A, Joshi AK and Smith S (2007) Coupling of the de novo fatty acid biosynthesis and lipoylation pathways in mammalian mitochondria. *The Journal of Biological Chemistry* 282(19): 14178-14185.

Yamazoe M, Shirahige K, Rashid MB, Kaneko Y, Nakayama T, Ogasawara N, and others (1994) A protein which binds preferentially to single-stranded core sequence of autonomously replicating sequence is essential for respiratory function in mitochondrial of Saccharomyces cerevisiae. *The Journal of Biological Chemistry* 269(21): 15244-15252.

Yang SY, He XY and Schulz H (1987) Fatty acid oxidation in rat brain is limited by the low activity of 3-ketoacyl-coenzyme A thiolase. *The Journal of Biological Chemistry* 262(27): 13027-13032.

Yi X and Maeda N (2005) Endogenous production of lipoic acid is essential for mouse development. *Molecular and Cellular Biology* 25(18): 8387-8392.

Zhang L, Joshi AK and Smith S (2003) Cloning, expression, characterization, and interaction of two components of a human mitochondrial fatty acid synthase. Malonyltransferase and acyl carrier protein. *The Journal of Biological Chemistry* 278(41): 40067-40074.

Zhang L, Joshi AK, Hofmann J, Schweizer E and Smith S (2005) Cloning, expression, and characterization of the human mitochondrial beta-ketoacyl synthase. Complementation of the yeast CEM1 knock-out strain. *The Journal of Biological Chemistry* 280(13): 12422-12429.

Zhang R, Hou T, Cheng H and Wang X (2019) NDUFAB1 protects against obesity and insulin resistance by enhancing mitochondrial metabolism. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* 33(12): 13310-13322.

Zhou X and Anderson KV (2010) Development of head organizer of the mouse embryo depends on a high level of mitochondrial metabolism. *Developmental Biology* 344(1): 185-195.

Zhu J, Vinothkumar KR and Hirst J (2016) Structure of mammalian respiratory complex I. *Nature* 536(7616): 354-358.

Table I. Human genes encoding mtFAS components

Chromosomal locations according to NCBI Gene resources database

Gene	Chromosomal location	Function of gene product	Comments	References
AASDHPPT (*ACPS*)	Chromosome 11, NC_000011.10 (106077605..106098695)	4'phosphopantetheinyl transferase/ holo-ACP synthase of mtFAS	homology to aminoadipate-semialdehyde dehydrogenase -phosphopantetheinyl transferase *LYS5* in yeast; however, aminoadipate-semialdehyde dehydrogenase does not exist in humans and ACPS (holo-ACP synthase) may be a more appropriate designation	(Joshi and others, 2003)
ACACA (*ACC1*)	Chromosome 17, NC_000017.11 (37084992..37406836, complement)	acetyl-CoA carboxylase of mtFAS	the mitochondrial isoform of ACC1 is encoded by transcript isoform 1 of *ACACA*; redundant with ACSF3 in providing malonyl-CoA to mtFAS	(Monteuuis and others, 2017)
ACSF3	Chromosome 16, NC_000016.10 (89093847..89160556)	malonyl-CoA synthetase of mtFAS	redundant with ACC1 in providing malonyl-CoA to mtFAS, associated human disorder CMAMMA	(Witkowski and others, 2011)
CBR4	Chromosome 4, NC_000004.12 (168894484..169010284, complement)	3-ketoacyl-ACP reductase of mtFAS, β-subunit	Initially characterized as carbonyl reductase, CBR4 requires 17BHSD8 protein to form the active 3-ketoacyl-ACP reductase heterotetramer	(Z. Chen and others, 2009)
HSD17B8	Chromosome 6, NC_000006.12 (33204655..33206831)	3-ketoacyl-ACP reductase of mtFAS, α-subunit	initially characterized as 17β-hydroxysteroid dehydrogenase type 8, HSD17B8 requires CBR4 protein to form the active 3-ketoacyl-ACP reductase heterotetramer	(Z. Chen and others, 2009)

HTD2	Chromosome 3, NC_000003.12 (58306245..58320193)	hydroxyacyl-thioester (ACP) dehydratase type 2 of mtFAS	encoded by a bicistronic transcript preceded by the ORF encoding RPP14, a subunit of human nucleolar RNase P	(Autio and others, 2008)
MCAT	Chromosome 22, NC_000022.11 (43132206..43143398, complement)	malonyl-CoA::ACP transacylase of mtFAS	associated human mtFAS disorder MCAT deficiency	(L. Zhang and others, 2003)
MECR	Chromosome 1, NC_000001.11 (29192657..29230948, complement)	mitochondrial trans-2-enoyl-CoA reductase/ trans-2-enoyl-ACP reductase of mtFAS	associated human disorder (MEPAN), possible nuclear isoform reported	(Miinalainen and others, 2003)
NDUFAB1 (*ACP*)	Chromosome 16, NC_000016.10 (23581012..23596329, complement)	acyl-carrier protein (ACP) of mtFAS	must be converted into the holo-ACP form by phosphopantetheinyl transferase in order to be functional; structural subunit of mitochondrial NADH dehydrogenase complex (respiratory complex I), structural component of mitochondrial ribosomes	(L. Zhang and others, 2003)
OXSM	Chromosome 3, NC_000003.12 (25788283..25794534)	3-ketoacyl-ACP synthase (condensing enzyme) of mtFAS		(L. Zhang and others, 2005)

Table II. Human patient mutations reported from mtFAS or LA biosynthesis or ISC biogenesis.
The references cited may include more than one patient; only neurological symptoms listed in the table.

Condition/Disorder	Gene	Neurological symptoms	Reference
MEPAN/DYTOABG	*MECR*	Dystonia, reduced visual activity, dysarthria, gait abnormalities, epilepsy	(Gorukmez and others, 2019; Heimer and others, 2016)
CMAMMA	*ACSF3*	Seizures	(Wehbe and others, 2019)
MCAT deficiency (Optic neuropathy)	*MCAT*	Visual impairment	(Li and others, 2020)
LA synthesis and attachment defects	*LIAS*	Neonatal epilepsy, muscular dystonia, myoclonic movements	(Mayr and others, 2011; Tsurusaki and others, 2015)
	LIPT 1	Dystonia, hypertonia/hypotonia, delayed psychomotor development	(Soreze and others, 2013; Tort and others, 2014)
	LIPT 2	Encepahalopathy	(Habarou and others, 2017)
Fe-S cluster biogenesis defects	*NFU1*	Neurological regression, fatal infantile encephalopathy	(Ahting and others, 2015; Navarro-Sastre and others, 2011)

	GLRX5, BOLA3,	Spastic paraplegia, optic atrophy, hypotonia, seizures	(Baker and others, 2014)
	IBA57	Psychomotor retardation triggered by infection	(Ishiyama and others, 2017)
PKAN, CoPAN	*PANK COASY*	Progressive motor dysfunction and dystonia	(Lambrechts and others, 2019)

Figure legends

Figure 1. Initiating reactions of mtFAS and functions of ACP in human cells. The processes are simplified, and some molecular detail has been omitted. In mammals, the malonyl-CoA priming/extender unit can apparently be provided by either a malonyl-CoA synthase (ASCSF3) or by condensation of acetyl-CoA with CO2 by a mitochondrial isoform of acetyl-CoA carboxylase ACC1 (mACC1). The apo-ACP polypeptide is converted to the holo-form by attachment of a 4'-phosphopantetheine moiety transferred from CoA. A phosphopantetheinyl transferase (PPTase) encoded by the *AASDHPPT/ACPS* has been suggested to carry out this task in mammals. While yeast cells have a dedicated mitochondrial PPTase, mitochondrial localization of the likely mammalian mitochondrial ACP-modifying PPTase has not yet been reported. Holo-ACP is malonylated by malonyl-CoA transferase MCAT to yield malonyl-ACP, one of the two reactants required to enter the FAS cycle. *Unacylated holo-ACP has been reported to be associated with the mammalian mitochondrial ribosome as well as with the Fe-S cluster synthesis apparatus. Evidence from yeast indicates that phosphopantetheinylation is crucial for ACP function in Fe-S cluster synthesis, but acylation of ACP may not be essential for this function.

Figure 2. The mtFAS cycle and functions of acyl-ACP in mammalian mitochondria. Malonyl-ACP undergoes a condensation reaction with acyl-ACP, carried out by the OXSM ketoacyl synthase (KAS) under release of CO_2. The heterotetrameric CBR4/HSD17B8 ketoacyl reductase (KAR) then converts the 3-ketogroup to a hydroxy moiety, which is subsequently removed by 3-hydroxyacyl-thioester dehydratase HTD2 (DH), leading to formation of the 2-enoyl-ACP intermediate. The 2-enoyl-thioester reductase (ER) MECR carries out the final

reduction step in the FAS cycle to produce the saturated, ACP-conjugated fatty acid. Unlike the NADH-dependent processes in β-oxidation, the reduction steps in FAS use the NADPH cofactor. The enzyme generating the acetyl-ACP molecule required for the first condensation reaction has not yet been reported. (1.) Octanoyl-APC is utilized in the formation of lipoic acid (LA) Fatty acyl chains of up to 16 carbons have been reported to be generated by mtFAS. (2.) Also acylated ACP has been shown to interact with the Fe-S cluster synthesis machinery, and mammalian ACP is required for the integrity of Fe-S cluster containing respiratory complexes I (NAHD dehydrogenase, C I), II (succinate dehydrogenase, C II) and III (cytochrome c reductase, C III). (3.) ACP has also been found as an essential structural component of respiratory complex I (C I). (4.) The phenotype of mtFAS mutants indicates that acylated ACP is important for assembly and/or integrity of C I and C II. (5.) In yeast, also C IV (cytochrome c oxidase) and C V (ATP synthase) are affected by mtFAS defects, but no such relationship has been reported in mammalian mitochondria. MIM: mitochondrial inner membrane.

Figure 3. Simplified graphic model of consequences of mtFAS of CoA synthesis deficiency disorders on ACP acylation/4'pantetheinylation and states and mitochondrial functions. Participating components are stylized, some molecular details are omitted. A) In healthy cells, sufficient CoA is available to support the required level of 4'pantetheinylation of mitochondrial ACP. The Fe-S cluster synthesis complexes ribosomes and respiratory complex I (C I) incorporate ACP in order to function. The respiratory chain assembles correctly and is fully functional (C IV and C V, which in mammalian cells have not been reported to be affected by ACP or mtFAS deficiencies, have omitted for clarity). CI incorporates acyl-ACPs, while the Fe-S synthesis machinery has been reported to associate with acyl-ACP as well as unacylated

holo-ACP moieties. To date, only unacylated holo-ACP has been reported to participate in the mitochondrial ribosome structure. B) Defects in mtFAS affect the abundance of acylated ACP. In patients with some remaining mtFAS activity, acyl-ACPs with the appropriate chain lengths are still produced, albeit at lower levels. With exception of PPTase defects, even complete loss of function mtFAS mutants are likely to carry a short chain fatty acid adduct [R] with two-, three- or four-carbon chains attached to the 4'PP moiety. Fe-S cluster synthesis complexes are affected only mildly or not at all. An effect of mtFAS deficiencies on ribosome function in mammals has not been reported to date. Due to shortage of octanoyl-ACP, the LA (lipoic acid) abundance is reduced, resulting in reduction of ketoacid dehydrogenase (PDH, KGD, GCS, BCD, KAD) activities. Respiratory complex I abundance is reduced due to lack of acyl-ACP, while respiratory complex II presence in the membrane is lowered due to faulty complex assembly. Unlike in the yeast model, effects on complexes III, IV and V have not been reported in the available models or in patients with compromised mtFAS. C) Defects in the CoA synthesis pathway result in a decrease in available CoA, leading to a reduction of available holo-ACP. Consequently, according to the hypothesis supported by the data from Lambrechts and others, a reduction of CoA leads to a decrease in the amount of functional Fe-S synthesis complexes, which is interpreted by the mitochondria as a lack of iron, triggering a compensatory reaction that results in mitochondrial iron overload. As an immediate result of holo-ACP shortage, Fe-S cluster containing respiratory complexes I, II and III are destabilized, in addition to defects in the assembly of complexes I and II and structural defects of C I due to the lack of the acyl- ACP (NDUFAB1) subunit. Consequently, respiratory complexes abundance is reduced. Due to the lack of functional Fe-cluster-dependent LA synthase as well as reduction of available octanoyl-ACP, LA decreases, affecting the activity of ketoacid dehydrogenase complexes. Lastly, the number of mitoribosomes may be affected due to

structural problems resulting from the lack of holo-ACP. CoA plays central roles in many other cellular metabolic pathways, and CoA deficiency causes a pleiotropic disease presentation. For clarity, this figure focuses on the effects on ACP abundance and the ensuing consequences.

Figure 1

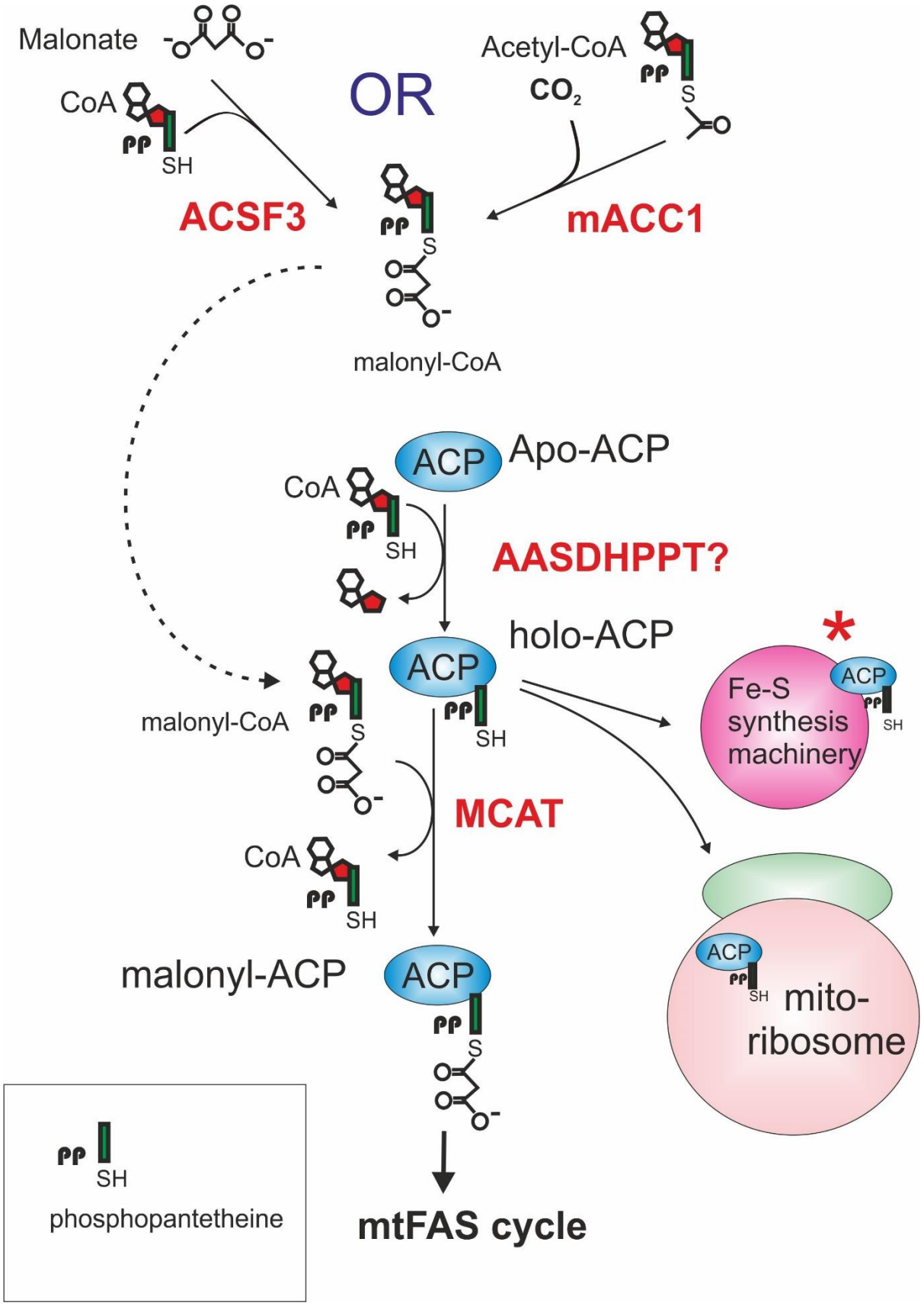

Malonate
CoA
SH
OR
Acetyl-CoA
CO_2
S
ACSF3
mACC1
malonyl-CoA
ACP
Apo-ACP
CoA
SH
AASDHPPT?
holo-ACP
ACP
SH
malonyl-CoA
S
MCAT
CoA
SH
malonyl-ACP
ACP
S
mtFAS cycle
Fe-S synthesis machinery
ACP
SH
*
ACP
SH
mito-ribosome
SH
phosphopantetheine

Figure 2

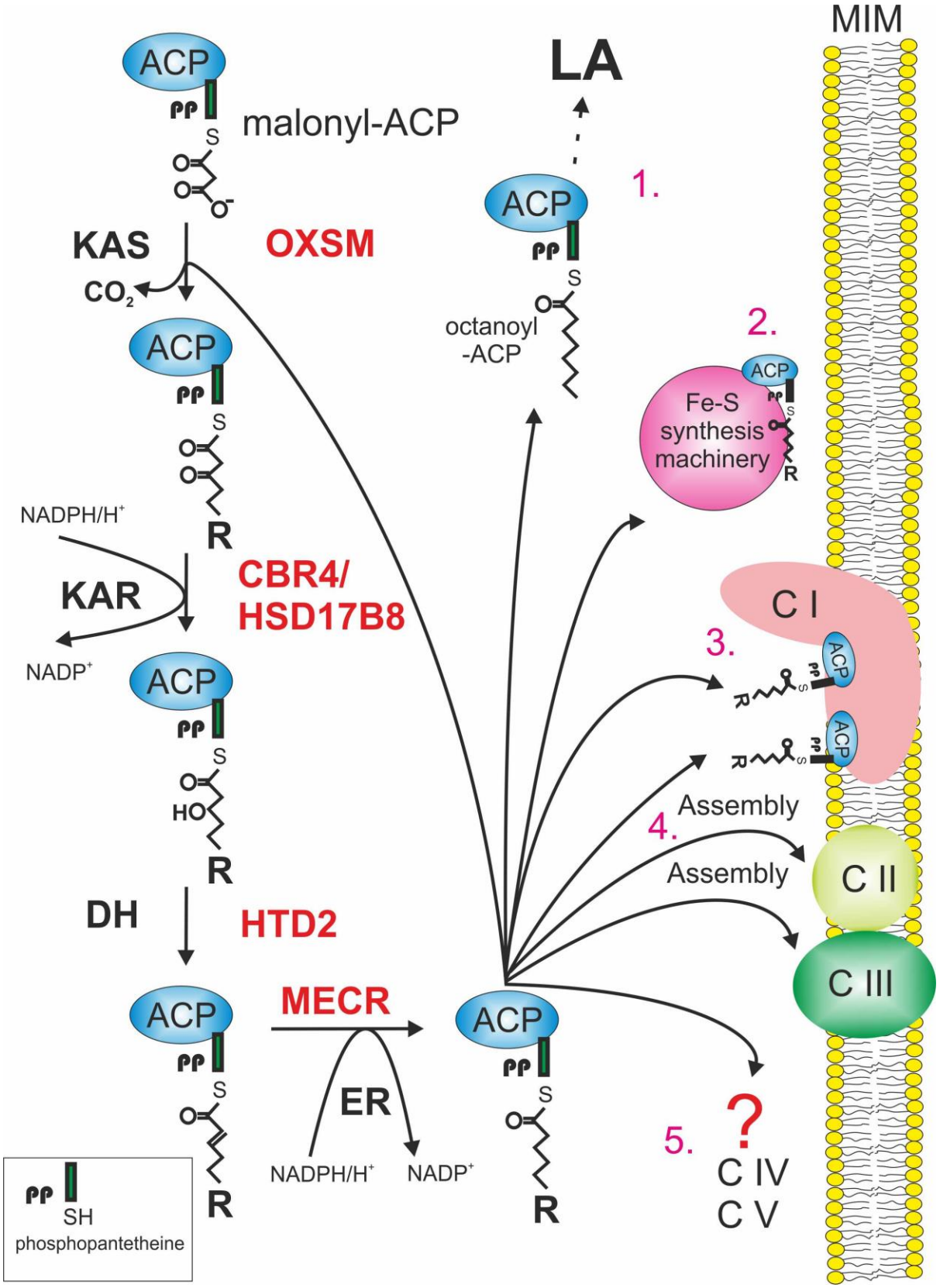

MIM
LA
ACP
malonyl-ACP
KAS
OXSM
CO2
octanoyl
-ACP
1.
2.
Fe-S
synthesis
machinery
NADPH/H+
KAR
CBR4/
HSD17B8
NADP+
C I
3.
Assembly
4.
Assembly
C II
C III
DH
HTD2
MECR
ER
NADPH/H+
NADP+
5.
?
C IV
C V
SH
phosphopantetheine

Figure 3A

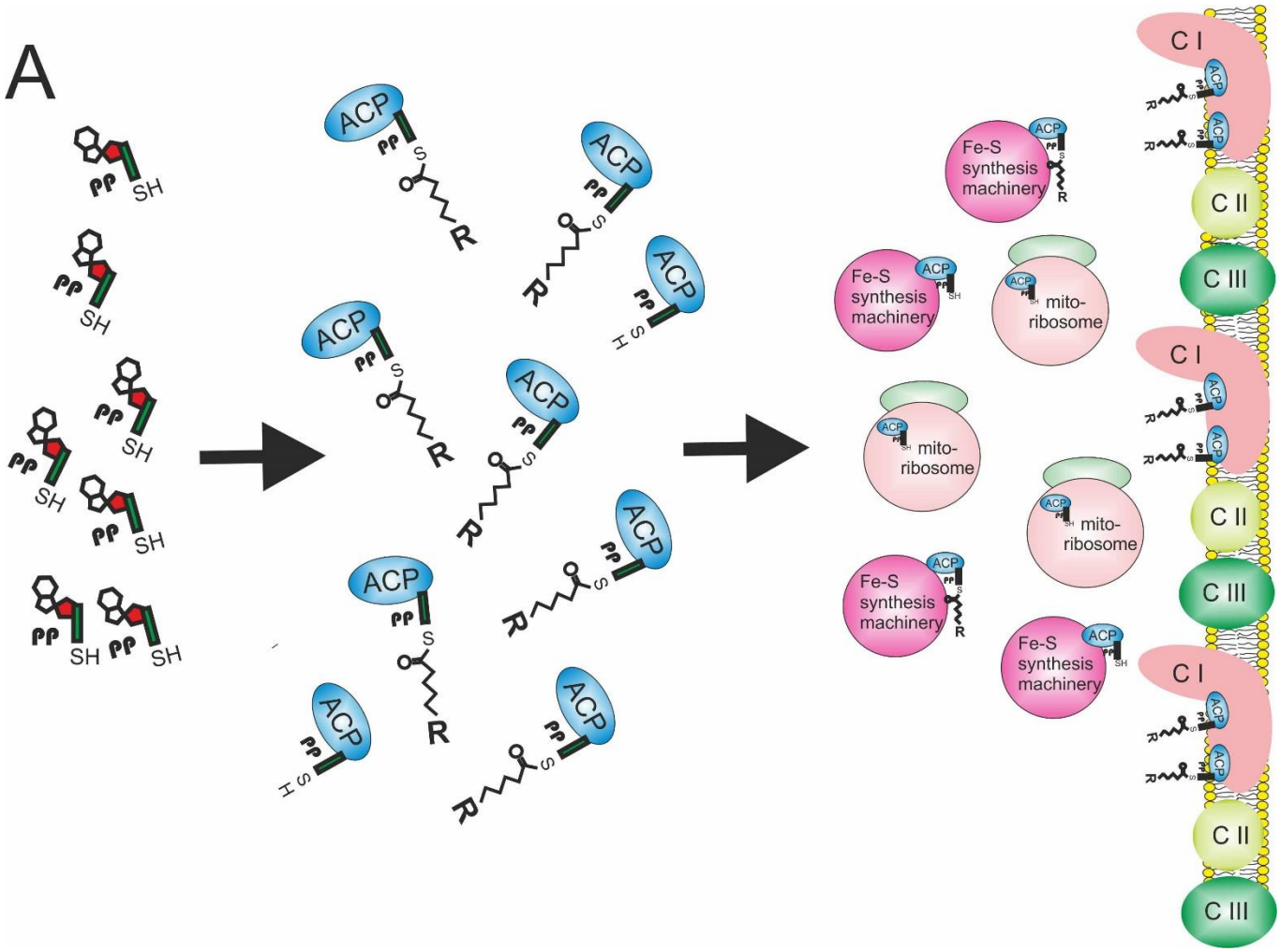


Figure 3B

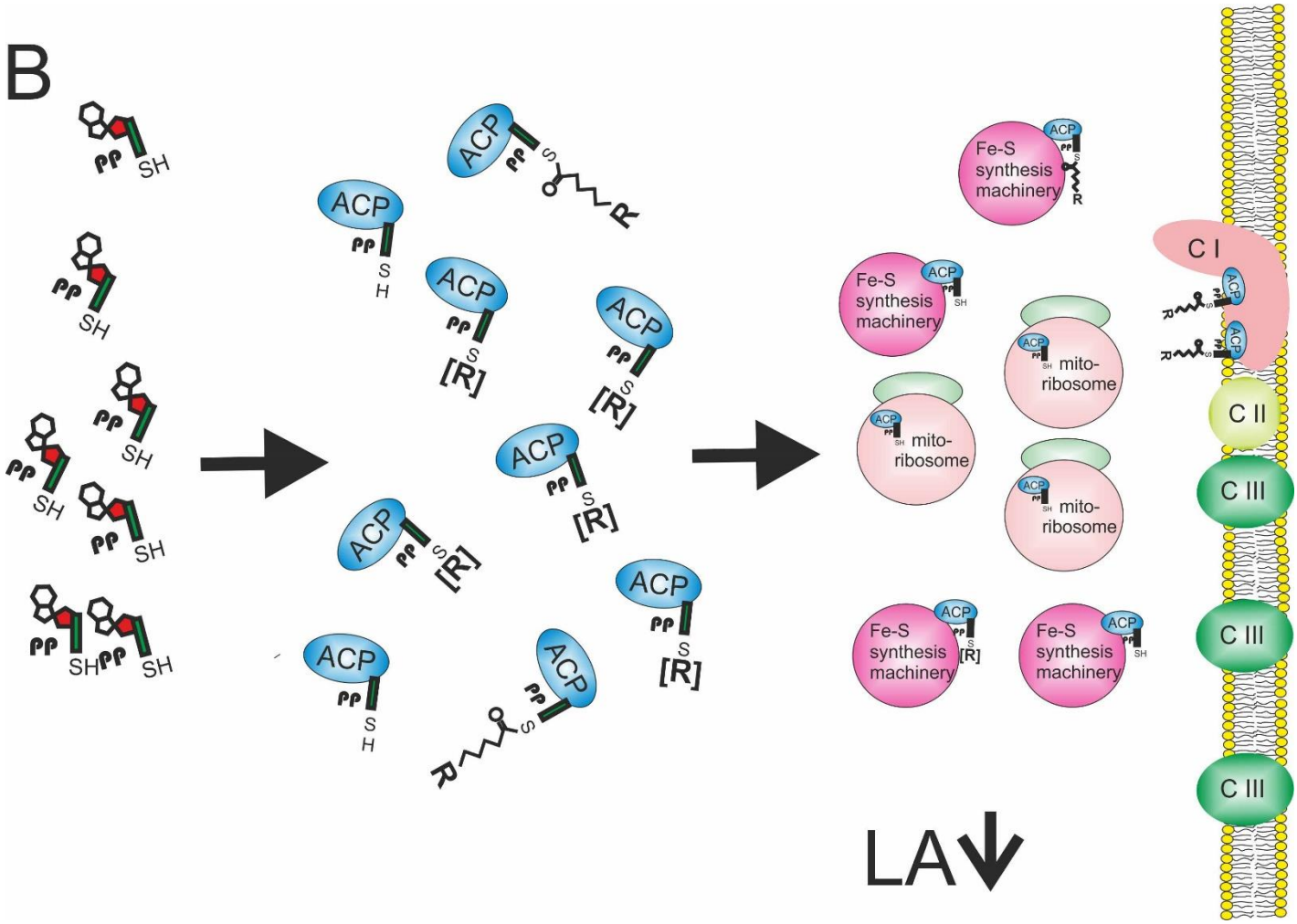
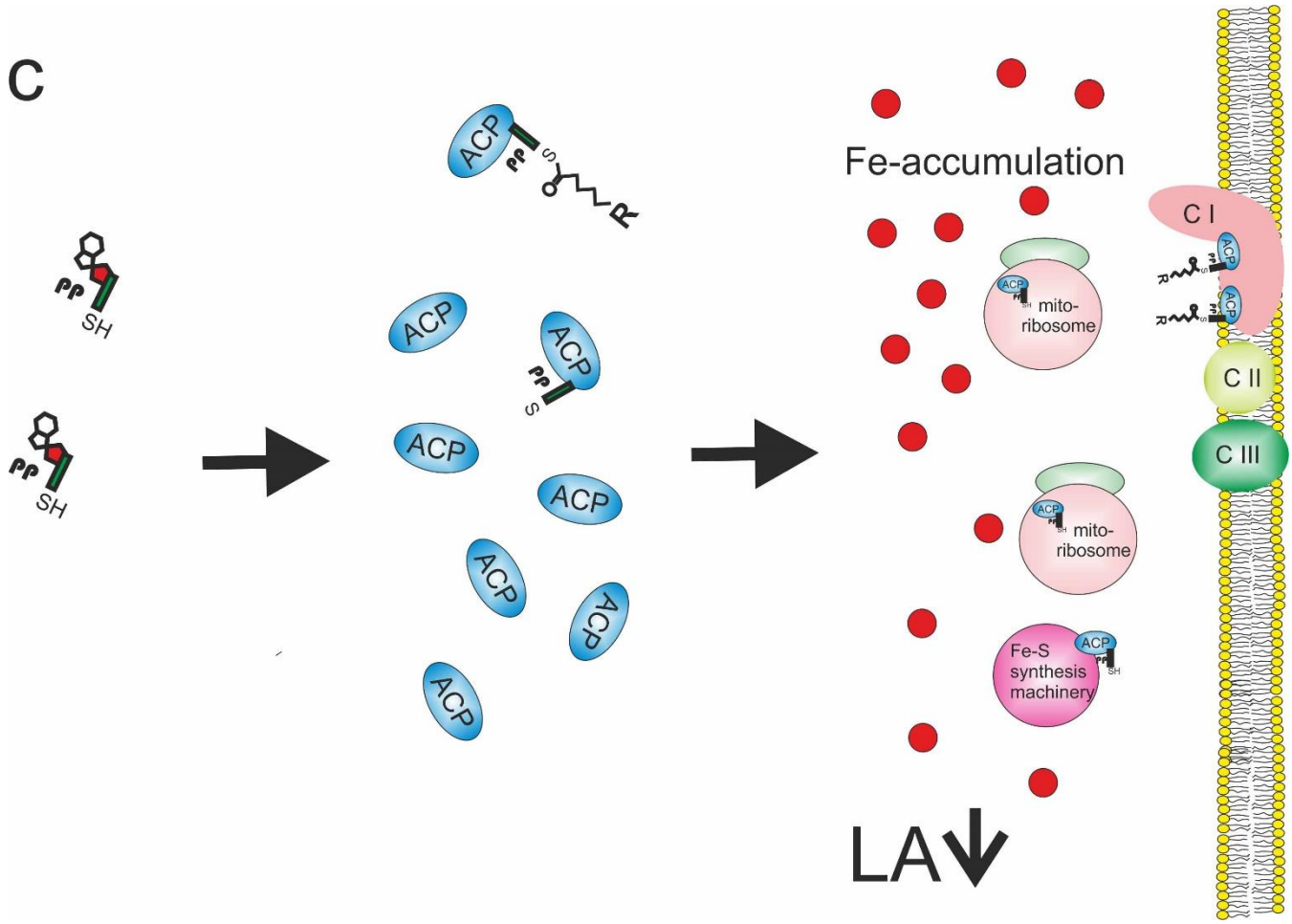

B
SH
ACP
S
H
[R]
Fe-S synthesis machinery
mito-ribosome
C I
C II
C III
C III
C III
LA

Figure 3C


C
SH
SH
ACP
ACP
ACP
ACP
ACP
ACP
ACP
ACP
R
Fe-accumulation
mito-
ribosome
mito-
ribosome
Fe-S
synthesis
machinery
C I
C II
C III
LA