Minireview

Mitochondrial fatty acid synthesis: a relic of endosymbiontic origin and a specialized means for respiration

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Abstract Genes that encode mitochondrial homologues to the bacterial enzymes of fatty acid synthesis were found in various eukaryotic species. Inactivation of these genes leads to a disturbed mitochondrial respiration and an increase in mitochondrial lysophospholipids. We postulate that there is a mitochondrial biosynthetic system providing fatty acids for phospholipid repair. The mitochondrial acyl carrier protein may also play another role, supporting the formation of the respiratory NADH:ubiquinone oxidoreductase.

Key words: Fatty acid synthesis; Acyl carrier protein; Mitochondrial respiration; Mitochondrial biogenesis; Lipid repair; NADH:ubiquinone oxidoreductase (EC 1.6.99.3)

1. Introduction

The synthesis of fatty acids is performed by a universal cycle of enzymatic reactions. The cycle starts by transferring a malonyl group from CoA to the acyl carrier protein (ACP), and an acetyl group from CoA via the ACP to the β-ketoacyl-ACP synthase. This enzyme catalyzes the condensation of the two groups resulting in a 3-ketobutyryl residue attached to the ACP. The reaction is followed by the reduction of the β-keto group to a β-hydroxy group, the removal of H_2O, and the reduction of the enoyl group to a saturated acyl-ACP. All these enzymes together with the ACP are called fatty acid synthase (FAS).

This basic mechanism is found in all organisms and leads to stearic, oleic and palmitic acid. There are mainly two different forms of organization of the enzymes which are, according to Lynen [1], called FAS type I and type II. FAS type I systems are found in the cytosol of animals and fungi. As a consequence of gene fusion [2], all enzymatic activities are located on one or two multifunctional polypeptide chains (for reviews, see [2,3]). In contrast, discrete enzymes supposed to interact only during the reactions are present in FAS type II, which is found in bacteria (for exceptions see [4]) and chloroplasts [5]. It is believed that species with a type II FAS do not usually contain a cytosolic FAS I, and vice versa, an exception being *Euglena gracilis* [6].

Recently, a prokaryotic type of ACP was found in the mitochondria of the fungus *Neurospora crassa* [7,8] and in bovine heart [9]. GeneS encoding mitochondrial ACPs has been identified in a variety of different species. Further sequences resembling those of other type II FAS enzymes can also be found in the genome databases. This was surprising, because it means that a mitochondrial fatty acid synthesis of type II coexists with cytosolic type I FAS or with type II FAS of chloroplasts.

In this review, we will focus on the possible roles of the enzymes of mitochondrial fatty acid synthesis. We will also discuss evolutionary relationships between the enzymes of the mitochondrial FAS and their bacterial counterparts.

2. The mitochondrial acyl carrier protein

A mitochondrial ACP of type II FAS was first discovered by Brody and coworkers in *N. crassa* following in vivo incorporation of [^{14}C]pantothenate [7,10]. Homologous proteins or their genes have been found later in *Saccharomyces cerevisiae* [11], *Arabidopsis thaliana* [12], *Bos taurus* [9], *Caenorhabditis elegans* [13], *Schizosaccharomyces pombe* (Acc. No. Z69380), *Homo sapiens* (TIGR Human cDNA Database, THC 141079) and *Drosophila melanogaster* (Acc. No. Y09068). All mitochondrial ACPs possess an N-terminal presequence with the characteristic feature for targeting the protein into the mitochondrion [14]. The mature ACP is, like its prokaryotic counterparts, a small, acidic protein with a molecular mass of approximately 10 kDa and contains phosphopantetheine as prosthetic group. Most surprisingly, the ACP is a subunit of the respiratory NADH:ubiquinone oxidoreductase (complex I) in *N. crassa* and *B. taurus*, and probably other species, too [8,9].

The gene of the mitochondrial ACP was disrupted in *N. crassa* and *S. cerevisiae* [15]. In the obligate aerobic fungus *N. crassa,* this resulted in a loss of the respiratory complex I which is by-passed by an alternative non-proton-pumping NADH:ubiquinone oxidoreductase. The contents and the function of the respiratory ubiquinone:cytochrome *c* oxidoreductase (complex III) and cytochrome *c* oxidase (complex IV) are unchanged in the *N. crassa* mutant. A further feature of the *N. crassa* mutant that lacks the mitochondrial ACP is a fourfold increase in the amount of mitochondrial lysophospholipids (these are derived from phospholipids by the removal of one acyl chain). Upon disruption of its ACP gene (*ACP1*), the facultative anaerobic yeast *S. cerevisiae* became respiratory deficient with a characteristic *pet* phenotype [15]. The complexes III and IV were absent. This yeast does not contain a complex I and normally respires through the alternative non-proton-pumping NADH:ubiquinone oxidoreductase which is present in the respiratory chains of most fungi and bacteria [16]. *Pet* mutants of *S. cerevisiae* [17] are unable

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to grow on non-fermentable substrates and do not contain a functional system of oxidative phosphorylation.

3. The mitochondrial β-ketoacyl-ACP synthase

The β-ketoacyl-ACP synthase (KAS or condensing enzyme) catalyzes the condensation of acetyl-ACP (short and medium chain acyl-ACP) with malonyl-ACP, coupled with the decarboxylation of the latter. This is the fundamental elongation step of fatty acid synthesis. In *E. coli*, three isoenzymes (KAS I, KAS II and KAS III) showing different chain length specificities exist [18]. The gene of a mitochondrial β-ketoacyl-ACP-synthase (named *CEM1*, Condensing Enzyme with Mitochondrial function) was found first in *S. cerevisiae* [19]. The percentages of identical amino acid residues between the yeast CEM1 and the *E. coli* KAS I, II and III are 29%, 42% and 14%, respectively. In *E. coli*, KAS II is responsible for the synthesis of medium chain fatty acids [18]. The predicted CEM1 protein does not contain a N-terminal presequence.

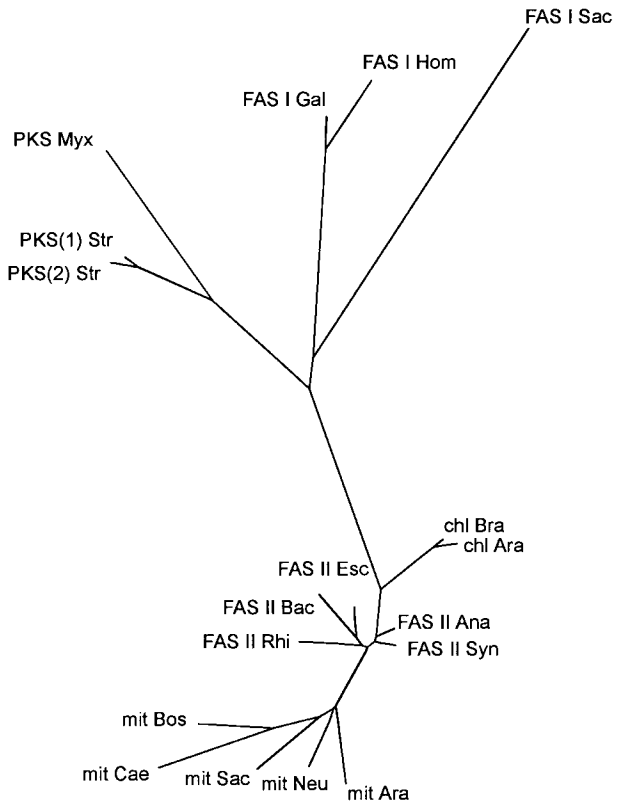

Fig. 1. Phylogenetic tree of acyl carrier proteins. FAS I and FAS II refer to fatty acid synthase type I and II, respectively, PKS to polyketide synthase, mit to mitochondrial, chl to chloroplastidial. The abbreviations for the species are: Ana *Anabaena sp.*, Ara *Arabidopsis thaliana*, Bac *Bacillus subtilis*, Bos *Bos taurus*, Bra *Brassica napus*, Cae *Caenorhabditis elegans*, Esc *Escherichia coli*, Gal *Gallus gallus*, Hom *Homo sapiens*, Myx *Sorangium cellulosum*, Neu *Neurospora crassa*, Rhi *Rhizobium meliloti*, Sac *Saccharomyces cerevisiae*, Str *Streptomyces hygroscopicus*, Syn *Synochocystis sp.* Protein sequences were aligned with the program CLUSTAL W [38], followed by manual adjustment where necessary. Only the most conserved regions were included in the analysis, presequences have been removed. Distances were calculated with Dayhoff's PAM matrix [39] and phylogenetic trees constructed with the Fitch-Margoliash algorithm. The programs PROTDIST, FITCH and DRAWTREE of the PHYLIP package [40] were applied. Tree topology was confirmed by bootstrap analysis of 100 bootstrapped replicates.

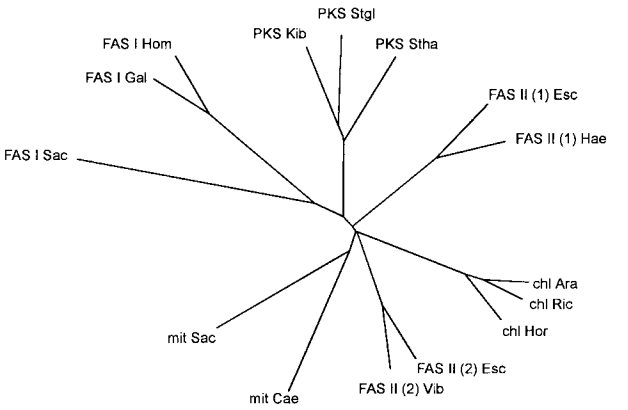

Fig. 2. Phylogenetic tree of β-ketoacyl-ACP synthases (KAS). FAS I and FAS II refer to fatty acid synthase type I and II, respectively, PKS to polyketide synthase, mit to mitochondrial and chl to chloropastidial. Arabic numerals in parentheses refer to bacterial isoenzymes. The abbreviations for the species not given in Fig. 1 are: Hae *Haemophilus influenzae*, Hor *Hordeum vulgare*, Kib *Kibdelsporangium aridum*, Ric *Ricinus communis*, Stha *Streptomyces halstedii*, Stgl *Streptomyces glaucescens*, Vib *Vibrio harveyi*. Tree construction was performed as described in the legend to Fig. 1.

On *CEM1* disruption, the yeast mutant showed a respiratory deficient *pet* phenotype [19] similar to that of the *ACP1* disruption mutants [15]. Most remarkably, the *CEM1* disruption mutant could be complemented by a long chain acyl-coenzyme A ligase (*FAM1-1*) redirected to mitochondria by a mutation that provided it with a target sequence [20]. Complementation, however, works only on rich medium.

Our group attempted to isolate KAS from the mitochondrial matrix of *N. crassa*. The enzymatic assay consists of the condensation of acetyl-ACP with malonyl-ACP to β-ketobutyryl-ACP followed by its NADH dependent reduction with a commercially available reductase [21]. The ACP was obtained by overexpressing the gene of the *N. crassa* mitochondrial ACP in *E. coli*. A preparation with kinetic properties resembling those of *E. coli* KAS II was obtained, accepting acyl-ACPs from butyryl-ACP to myristoyl-ACP with K_M values of about 50 μM while not accepting palmitoyl-ACP and being inhibited with high affinity by cerulinin [22]. Unfortunately, the assignment of a polypeptide in the preparation to a bacterial KAS by means of partial sequences was not yet possible.

4. Further enzymes of the putative mitochondrial fatty acid synthesis

Upon searching the *S. cerevisiae* genome for similarities to conserved regions of bacterial FAS type II genes, we identified YOR50-11 (EMBL Acc. No. X92441) to encode a malonyl-CoA:ACP transferase and ORF YKL055c (EMBL Acc. No. X75781) to encode a 3-oxoacyl-ACP reductase [23,24]. We propose to call these genes *MCT1* (malonyl-CoA:ACP transferase) and *OAR1* (3-oxoacyl-ACP reductase). While the *OAR1* product does not possess a cleavable N-terminal signal sequence, the N-terminal region of the *MCT1* product contains an arginine in position −10 belonging to a putative cleavage site, followed by a leucine in position −8. However, there seems to be no amphiphilic helix in the putative presequence. A gene encoding a protein with sequence similarity to bacterial β-ketoacyl-ACP synthases is also present in the genome database of *C. elegans* (EMBL Z80216) [13].

Inactivation of *MCT1* and *OAR1* in yeast leads to a respiratory deficient phenotype [25], similar to those of the *Δacp1* and *Δcem1* mutants of *S. cerevisiae* [15,19]. Apart from homology to prokaryotic enzymes, this provides further evidence that the organelle of function of these two enzymes is the mitochondrion.

Genes encoding 3-hydroxy-acyl dehydratases cannot be detected yet in the *S. cerevisiae* database due to the small number of species from which the corresponding genes have been sequenced. In the case of the enoyl reductase, homology between proteins from different species is rather low. While the tertiary structure of the enzyme is believed to be more stringently conserved, there seem to be no blocks of conserved residues in the primary structure that would allow the corresponding gene to be identified in a genome database [26].

5. The endosymbiontic origin of mitochondrial fatty acid synthesis

The endosymbiont hypothesis which states that the ancestors of eukaryotic mitochondria were free living bacteria, most probably ancestors of the α-subdivision of purple bacteria, is now generally accepted [27,28]. Therefore, it is no surprise that mitochondria, besides their specialized nucleic acid and protein synthesis systems, should also possess a prokaryote-type FAS system. As revealed by phylogenetic analysis, the sequences of the mitochondrial FAS enzymes form their own outgroup, which means that they are descended from only one prokaryotic ancestor. This outgroup shows the closest relationship to bacterial and plastidial FAS systems followed by the relationship to bacterial polyketide synthesizing enzymes [29], but is only distantly related to the cytosolic FAS type I systems even in the same organism. Representative phylogenetic trees for the ACP and the KAS are shown in Figs. 1 and 2. Clearly, the mitochondrial enzymes are more closely related to bacterial enzymes than to homologous domains of the cytosolic FAS type I of the same organism.

6. Possible role of the mitochondrial fatty acid synthesis in phospholipid repair

It has long been known that fatty acids are transported from the cytosol into the mitochondrion by the carnitine carrier system [30]. Thus, one may ask what function a mitochondrial FAS should perform. An answer might be that mitochondria have conserved their own FAS because its specialized function could not be satisfied by the eukaryote host. Isolation of the synthesis product may help to recognize this specialized function. The acyl groups bound to the mitochondrial ACP were analyzed by different authors. Brody and coworkers [7] found 3-hydroxy-myristate by means of gas chromatography/mass spectrometry. In our group, 3-hydroxymyristate and, in smaller amounts, 3-hydroxylaurate were found by HPLC (unpublished results). Our earlier report that C_6 to C_{18} and $C_{18:1}$ fatty acids are bound to the ACP subunit of complex I was caused by a contamination [31] and must be corrected in that sense. 3-Hydroxymyristate and 3-hydroxylaurate are typical intermediates of fatty acid synthesis stressing the participation of this system in de novo synthesis of fatty acids. For the ACP subunit of the bovine complex I, a unique mass difference of 302 between acylated and deacylated ACP has been reported [9], but this difference would account neither for a 3-hydroxy fatty acid nor for a saturated fatty acid.

The increased amount of lysophospholipids in the *N. crassa* mutant lacking the mitochondrial ACP [15] points to a role of the mitochondrial FAS in providing ACP-bound fatty acids as substrates for the repair of mitochondrial phospholipids. Lysophospholipids are intermediates of phospholipid removal by phospholipases after phospholipid oxidation by reactive species which are generated by the respiratory chain. As in bacteria, the lysophospholipids may be reacylated by ACP-bound fatty acids [32]. *S. cerevisiae* might be able to prevent non-reparable oxidative injury by repressing respiration and switching over to fermentation. The obligate aerobic fungus *N. crassa*, however, cannot live by fermentation like *S. cerevisiae*.

Furthermore, ACP-bound fatty acids may be required as substrates for the remodelling of the mitochondrial inner membrane phospholipids. They are imported from the endoplasmic reticulum or the mitochondrial outer membrane [33]. Remodelling after import might be necessary due to the special duty of the mitochondrial inner membrane to preserve the proton motive force which drives ATP synthesis.

7. Does the mitochondrial ACP have a special function for complex I?

While the existence of a prokaryotic FAS system in mitochondria cannot be doubted, the role of the mitochondrial ACP as a subunit of the respiratory complex I in fungi and animals [8,9] is unclear. Complex I is a amphipathic protein with a matrix arm and a membrane intrinsic arm [34]. The former harbors all known redox groups of the complex, 1 FMN and 6–7 iron-sulfur clusters, and constitutes the NADH dehydrogenase activity. In the latter, no redox group has been described so far. The ACP subunit is located in the matrix arm. Lack of the ACP in the *N. crassa* mutant prevents not only the formation of the matrix arm, but also disturbs the assembly of the membrane arm [15]. In this way, the ACP differs from other matrix arm subunits of complex I, whose absence does not affect the assembly of the membrane arm [35,36]. Therefore, the ACP may also participate in a specialized synthetic pathway other than the mitochondrial FAS, and this pathway may deliver a product which will be needed in the assembly of the membrane arm of complex I.

There is another subunit in the matrix arm of complex I predicted to participate in a synthetic pathway [37]. This 39 kDa subunit shows sequence similarity to NAD(P)H dependent dihydroflavonol reductases, β-hydroxysteroid dehydrogenases, and isoflavonol reductases. The loss of the subunit in *N. crassa* leads to a fully assembled, but inactive complex I. The matrix NADH dehydrogenase arm of complex I operates normally while the ubiquinone reduction by the membrane arm is disturbed (unpublished results). Thus it might turn out that the ACP and the 39 kDa subunit cooperate in the synthesis of a yet unknown redox group harbored in the membrane arm of complex I.

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