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Phylogenomic reconstruction of archaeal fatty acid metabolism

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Abstract

While certain archaea appear to synthesize and/or metabolize fatty acids, the respective pathways still remain obscure. By analyzing the genomic distribution of the key lipid-related enzymes, we were able to identify the likely components of the archaeal pathway of fatty acid metabolism, namely, a combination of the enzymes of bacterial-type β-oxidation of fatty acids (acyl-CoA-dehydrogenase, enoyl-CoA hydratase, and 3-hydroxyacyl-CoA dehydrogenase) with paralogs of the archaeal acetyl-CoA C-acetyltransferase, an enzyme of the mevalonate biosynthesis pathway. These three β-oxidation enzymes working in the reverse direction could potentially catalyze biosynthesis of fatty acids, with paralogs of acetyl-CoA C-acetyltransferase performing addition of C_2 fragments. The presence in archaea of the genes for energy-transducing membrane enzyme complexes, such as cytochrome *bc* complex, cytochrome *c* oxidase, and diverse rhodopsins, was found to correlate with the presence of the proposed system of fatty acid biosynthesis. We speculate that because these membrane complexes functionally depend on fatty acid chains, their genes could have been acquired via lateral gene transfer from bacteria only by those archaea that already possessed a system of fatty acid biosynthesis. The proposed pathway of archaeal fatty acid metabolism operates in extreme conditions and therefore might be of interest in the context of biofuel production and other industrial applications.

Keywords

biofuels; β-oxidation; halobacteria; methanogens; rhodopsin; bioenergetics

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Introduction

In archaea, whose membrane lipids are built of isoprenoids (Koga and Morii, 2007), the role(s) of long-chain fatty acids and even their very presence remain controversial. On one hand, several thorough studies of archaeal membranes found no fatty acid-based lipids (Koga *et al.*, 1993; Corcelli and Lobasso, 2006; Falb *et al.*, 2008). On the other hand, palmitate, a C_{16} fatty acid, was shown to be crucial for the functional integrity of halorhodopsin and could be synthesized by cells of *Halobacterium salinarum* even when they were grown in a defined culture medium with no added fatty acids (Corcelli *et al.*, 1996). The crystal structure of halorhodopsin from *H. salinarum* (Protein DataBank entry 1E12) showed three molecules of palmitate tightly bound between the monomers in the functionally active trimer (Kolbe *et al.*, 2000). A fatty acid synthase (FAS) activity has been detected in a cell fraction from the archaeon *H. salinarum* as early as in 1971 (Pugh *et al.*, 1971). Later, it was shown that cells of *H. salinarum* could grow on a synthetic medium with known composition (a mixture of amino acids, nucleotides, glycerol and inorganic salts) and produce functional bacteriorhodopsin and halorhodopsin (Gochnauer and Kushner, 1969; Oesterhelt and Krippahl, 1973; Helgersons *et al.*, 1992). To exclude the possibility of the growth being supported by residual amounts of nutrients transferred from the previous rich medium, the cells have been even cultured in a synthetic medium for 9 passages (Gochnauer and Kushner, 1969). Hence, cells of *H. salinarum* appear to be capable of synthesizing long-chain fatty acids, in particular, palmitate, from C_2-C_3 precursors. For several other archaea, the ability to metabolize long-chain fatty acids has been reported (Falb *et al.*, 2008; Slobodkina *et al.*, 2009). For example, growth of *Archaeoglobus fulgidus* could be sustained by the oxidation of long-chain fatty acids (Khelifi *et al.*, 2010). However, the respective enzymes have not been characterized.

In a recent paper, Lombard and coworkers (Lombard *et al.*, 2012a) reported finding in various archaeal genomes homologs of the components of the bacterial fatty acid synthase II (FAS II), except for the acyl-carrier protein (ACP) and acyl-ACP synthase (see Rock and Cronan, 1996; Rock and Jackowski, 2002, and the Supplementary Materials for a description of FAS II components). Accordingly, they suggested that archaea are able to synthesize fatty acids by using these homologous components in combination with some functional analogues of ACP and acyl-ACP synthase (Lombard *et al.*, 2012a). However, no such analogues of ACP and acyl-ACP synthase have ever been identified in archaea and the few homologs of bacterial FAS II components in archaeal genomes had a patchy distribution, insufficient to form a functional pathway in a single archaeal genome (see Table S1 of Lombard *et al.*, 2012a). Thus, the problem of fatty acid biosynthesis in archaea remained unresolved, which prompted us to re-examine this issue.

We report here a genomic reconstruction of the archaeal fatty acid metabolism based on the Clusters of Orthologous Groups (COGs) approach (Tatusov *et al.*, 1997; Koonin and Galperin, 2003). The results of this study suggest that many archaea are capable of synthesizing fatty acids by means of a chimeric pathway that includes bacterial-type enzymes of fatty acid β-oxidation in combination with (multiple paralogs of) the archaeal acetyl-CoA acetyltransferase, an enzyme of the mevalonate biosynthesis pathway. Accordingly, these results do not support the idea of an ancestral origin of fatty acid

biosynthesis and, instead, suggest that most of the respective enzymes have been acquired from bacteria via lateral gene transfer.

COG-based pathway analysis

Phylogenomic reconstruction of archaeal fatty acid metabolism was based on the COGs approach (Tatusov *et al.*, 1997; 2000; Koonin and Galperin, 2003), which improves accuracy of gene function prediction by distinguishing orthologous and paralogous genes, which is particularly important when the analyzed genomes come from different domains of life and the corresponding proteins show low sequence similarity (Makarova *et al.*, 1999; 2007). The analysis of archaeal protein families relied on the assignments in the latest public release of the COG database (Tatusov *et al.*, 2003), currently available at ftp://ftp.ncbi.nlm.nih.gov/pub/COG/COG/whog, the NCBI's RefSeq database (Pruitt *et al.*, 2012), and the recently updated version of archaeal COGs (Wolf *et al.*, 2012), which is available at ftp://ftp.ncbi.nih.gov/pub/wolf/COGs/arCOG. We also used the latest versions of the eggNOG, KEGG and MetaCyc databases (Caspi *et al.*, 2012; Powell *et al.*, 2012; Nakaya *et al.*, 2013). Pathway details were taken from KEGG and the available literature data.

An analysis of the genes involved in lipid metabolism (functional group I in the COG database) identified in the genome of *H. salinarum* strain NRC-1 genes for the four key enzymes of the bacterial pathway of β-oxidation of fatty acids (see Figure S1): acyl-CoA dehydrogenase (ACD or FadE, COG1960, EC 1.3.1.8, 1.3.8.1, 1.3.8.7, 1.3.8.8, or 1.3.8.9), enoyl-CoA hydratase (ECH or FadB_1, COG1024, EC 4.2.1.17), and 3-hydroxyacyl-CoA dehydrogenase (HDH or FadB_2, COG1250, EC 1.1.1.35), and 3-ketoacyl-CoA thiolase (BKL or FadA, COG0183, EC 2.3.1.16). Each of these genes was found in *H. salinarum* genome in several paralogous copies (6, 3, 2 and 3, respectively, see Table 1). In contrast, the KEGG scheme of fatty acid degradation (http://www.genome.jp/kegg-bin/show_pathway?mapno=00071&org_name=hal) indicated the presence of 3-ketoacyl-CoA thiolase but not of any other enzyme of this pathway. These data demonstrated that the issue of archaeal fatty acid metabolism remained unresolved and was worthy of a comprehensive analysis.

An analysis of the archaeal COGs and their genome neighborhoods (Tables 1 and S1) in 69 species representing the key lineages of archaea (listed in Table S2) revealed the presence of co-occurring genes encoding three enzymes of β-oxidation of fatty acids, ACD, ECH, and HDH in approximately half of the analyzed archaeal genomes. These enzyme sequences showed highly significant similarity to the respective bacterial enzymes and were typically assigned to the same COG. In contrast, β-ketothiolases (COG0183) were found in every archaeal genome except for the highly reduced genome of *Nanoarchaeum equitans*; in some organisms they were encoded by a single gene but often were found in several paralogous forms (Table 1). Such a biased distribution of various β-ketothiolase homologs could to be due to their involvement in several substantially different reactions (pathways). Indeed, in addition to the 3-ketoacyl-CoA thiolase, COG0183 includes another enzyme of the thiolase superfamily (Pereto *et al.*, 2005; Jiang *et al.*, 2008), archaeal acetyl-CoA C-acetyltransferase (EC 2.3.1.9). This latter enzyme catalyzes the first condensation reaction in the mevalonate

pathway of the biosynthesis of C_5 isoprenoid precursors (for a review see (Miziorko, 2011). Since mevalonate pathway is essential for the synthesis of archaeal isoprenoid lipids, at least one member of COG0183 is present in complete genomes of all free-living archaea. Remarkably, the genomes that encode the three β-oxidation enzymes (ECH, HDH and ACD) invariably encode more than one member of COG0183 (Table 1). To sort out the respective proteins and trace their evolutionary relationships, we have constructed a maximum likelihood phylogenetic tree for the potential β-ketothiolases and inspected it in order to figure out the likely functions of these enzymes.

The tree (Figure 1, see also Figure S2) revealed a clear split between mostly bacterial and archaeal sequences, with the few archaeal branches (B1 through B4) in the bacterial part representing clear-cut cases of lateral gene transfer from bacteria to archaea. Within the archaeal part, one group of sequences (clades AM_1 through AM_4) contained those members of COG0183 that are present in single copies in the majority of archaeal genomes (shaded grey in Table 1), with separate groupings of euarchaeal (AM_1) and crenarchaeal (AM_2) sequences. Analysis of the genomic neighborhoods of these genes showed that they were frequently located next to the genes that code for 3-hydroxy-3-methylglutaryl CoA synthase and hydroxymethylglutaryl-CoA reductase, enzymes of the mevalonate pathway (Table S1). This observation suggested that β-ketothiolases from clades AM_1 through AM_4 indeed function in this pathway.

Other groups of sequences (clades A1 through A9 on Figure 1) contain proteins that are encoded only in some archaeal genomes, usually in large paralogous groups. Their genes almost always co-occur with the genes of aforementioned three proteins of the β-oxidation pathway (Table 1) and are often adjacent to them (Table S1), forming predicted operons. This observation makes it increasingly likely that these enzymes participate in the same pathway.

Table 1 also shows that the correlation between the presence of three β-oxidation enzymes and multiple β-ketothiolases is not absolute. Four out of the 117 inspected archaeal genomes (*Ignicoccus hospitalis*, *Pyrolobus fumarii*, *Cenarchaeum symbiosum* and *Nitrosopumilus maritimus*) encode ECH and HCD, along with two paralogs of β-ketothiolase, but do not have the genes for ACD (COG1960). A PSI-BLAST search (Altschul *et al.*, 1997) identified members of COG2368 (annotated as ‘Aromatic ring hydroxylase’) as distant homologs of FadE that could potentially have the acyl-CoA dehydrogenase activity and therefore functionally replace the bacterial ACD. Indeed, an experimentally characterized member of this COG was shown to have 4-hydroxybutyryl-CoA dehydratase activity, and also performed isomerization of the double bond (Muh *et al.*, 1996). Archaeal members of COG2368 appear only in the genomes with other β-oxidation genes (Table 1) and appear to be plausible candidates for non-orthologous gene displacement of FadE-like acyl-CoA dehydrogenase. In two of these four organisms, *C. symbiosum* and *N. maritimus*, COG2368 members are encoded next to the genes for the NDP-forming acyl-CoA synthetase (Sanchez *et al.*, 2000), see Table S3. Therefore, COG2368 members are listed in Table 1 as ACDa (predicted alternative ACD).

Hence, in all inspected archaeal genomes, ECHs, HDHs, and either FadE-like acyl-CoA-dehydrogenases (ACD) or members of COG2368 (ACDa) co-occur with additional thiolases, either of bacterial type or paralogs of archaeal acetyl-CoA acetyltransferase. The correlation is significantly positive at any level of sampling, with the Z-score ≥ 14.6. Thus, almost a half of 69 archaeal genomes shown in Table 1 are potentially capable of fatty acid metabolism, which might be important for survival of these organisms in their natural habitats.

A search for additional enzymes that co-occurred with the β-oxidation enzymes (see Table S4) identified three more COGs that, in principle, could be involved in archaeal fatty acid metabolism. COG1607 unifies various thioesterases, including the acyl-CoA hydrolase YciA from *E. coli* (Kuznetsova *et al.*, 2005). COG1804 includes members of an additional family of CoA transferases, such as crotonobetainyl-CoA:carnitine CoA-transferase CaiB (Heider, 2001). Finally, COG2030 includes MaoC-like R-specific enoyl-CoA dehydratases (whereas the β-oxidation pathway includes S-enoyl-CoA dehydratase) (Fukui *et al.*, 1998). However, none of the enzymes listed in Table S4 appears to be able to catalyze the addition or cleavage of C_2 fragments.

Could fatty acids be synthesized by reversing the β-oxidation pathway?

In principle, the reactions of the fatty acid β-oxidation pathway are reversible, which begs the question of whether the same enzymes could be used for synthesizing fatty acids from C_2 precursors. In fact, this reverse mode of operation of β-oxidation enzymes from *Escherichia coli* has been recently realized in the context of biofuel production, yielding a pathway of carbon chain elongation (Dellomonaco *et al.*, 2011; Clomburg *et al.*, 2012). In particular, thiolase AtoB was shown to catalyze condensation of two acetyl-CoA molecules, while thiolase FadA was appeared to operate on longer substrates (Clomburg *et al.*, 2012). A similar arrangement could function in archaea, where the first step, synthesis of acetoacetyl-CoA, would be shared with the mevalonate pathway and carried out by the universal archaeal acetyl-CoA C-acetyltransferase, whereas its various paralogs would catalyze subsequent steps of fatty acid chain elongation. The proposed pathway of archaeal fatty acid synthesis is shown in Figure 2. The large number of paralogs of acetyl-CoA C-acetyltransferase in some archaeal genomes (Tables 1 and S1) may reflect their specificity to hydrocarbon chains of particular length, as shown in Figure 2.

Distribution of membrane energy-converting enzymes

In addition to the above-mentioned COG1607 (YciA), COG1804 (CaiB), and COG2030 (MaoC), a search for genes that frequently co-occur with genes of fatty acid β-oxidation genes and multiple paralogs of acetyl-CoA C-acetyltransferase revealed a correlation between the presence in archaeal genomes of these genes and the genes encoding certain membrane energy-converting proteins, namely various rhodopsins and the key membrane subunits of the cytochrome *bc* complex and the cytochrome *c* oxidase (Tables 1 and S4). Specifically, genes of these energy-converting enzymes were only found in those archaea that had the β-oxidation pathway genes and multiple paralogs of acetyl-CoA C-acetyltransferase, but not the other way around. This was quite remarkable, as no such

correlation has been observed in bacteria (data not shown). We could not come up with any explanation of why the presence of cytochrome *bc* complex, the cytochrome *c* oxidase and rhodopsins would correlate with the presence of the fatty acid ***degradation*** pathway. On the other hand, there was an easy explanation for the co-occurrence of these enzymes and the fatty acid ***biosynthesis*** pathway. Indeed, membrane subunits of rhodopsin, cytochrome *bc* complex and the cytochrome *c* oxidase often incorporate fatty acid chains as essential structural elements (Gomez and Robinson, 1999; Sedlák and Robinson, 1999; Kolbe *et al.*, 2000; Shinzawa-Itoh *et al.*, 2007; Hasan *et al.*, 2011). Therefore, proper functioning of these membrane enzymes would depend on the ability of the organisms to synthesize fatty acids, at least in free-living archaea that cannot acquire these fatty acids from the surrounding milieu.

Archaeal fatty acids metabolism: specific or bacterial-like?

The fundamental difference between the fatty acid biosynthesis pathways of bacteria and archaea has been suggested back in 1971 from an observation that fatty acid synthesis in the cell fraction of *Halobacterium cutirubrum* (currently *H. salinarum*) did not change after the addition of bacterial ACP (Pugh *et al.*, 1971), while the preparations from *E. coli* showed an almost tenfold increase in activity. A recent work (Lombard *et al.*, 2012a) did not find in archaeal genomes genes of eukaryotic fatty acid synthase (FAS I, see Smith, 1994; Maier *et al.*, 2008, for reviews) but reported finding certain components of the bacterial fatty acid synthase (FAS II). Our analysis has shown that some archaea indeed encode orthologs of certain components of the bacterial FAS II. However, with two exceptions that are discussed below, these components have a patchy distribution, incompatible with a functional fatty acid biosynthesis pathway (see Table S2). These results are consistent with the idea that archaea use a system of fatty acid synthesis that is substantially different from the bacterial one.

Fatty acid metabolism in methanogens?

The above analysis could help sorting out the often controversial data on the presence of fatty acids in archaea. For example, a single, never confirmed report claimed the presence of fatty acid-based lipids in diverse methanogenic archaea; this report was based on identification of fatty acids in membrane fractions that were obtained from archaeal cells grown on yeast extract (Gattinger *et al.*, 2002). These fatty acid-based lipids were identified in the yeast extract used in the growth medium, although at the concentration about 25 times less than that observed in frozen archaeal cells. In our opinion, these data cannot be considered as conclusive evidence for the ability of these methanogens to synthesize fatty acids. Since the genomes of methanogens do not seem to contain genes of β-oxidation of fatty acids (Table 1), fatty acid-containing hydrophobic constituents of the yeast extract would selectively accumulate in the membranes of these methanogens upon consumption of the other components of the extract. Furthermore, fatty acids of organic waste, being weak uncouplers (Korshunov *et al.*, 1998), suppress the growth of methanogenic archaea within well-studied syntrophic bacterial-archaeal consortia that are widely used for production of methane from such waste (Worm *et al.*, 2010). Accordingly, sustainability of such microbial systems depends on vigorous consumption of fatty acids by bacteria, which prevents their

accumulation in the media. Apparently, methanogens cannot deal with long-chain fatty acids.

Curiously, in an early study, an addition of bacterial ACP to the cell fraction of *Methanobacterium thermoautotrophicum* (currently *Methanothermobacter thermautotrophicus*) led to a three-fold increase in the yield of fatty acids (Pugh and Kates, 1994). This observation has been interpreted as evidence for the presence of a bacterial-type FAS in this methanogen (Pugh and Kates, 1994; Lombard *et al.*, 2012a). However, the genome of *M. thermoautotrophicus* does not encode any components of FASII that could interact with the added bacterial ACP (Table S2). Therefore, the reasons for the effect observed by Pugh and Kates (1994) remain obscure, unless their cell extract contained residual amounts of bacterial FAS components.

Accumulation from yeast extract might also account for the reported presence of long-chain fatty acids (Carballeira *et al.*, 1997) and long-chain alcohols (Nishihara *et al.*, 2000) in membrane fractions of the archaeon *Pyrococcus furiosus* which also lacks β-oxidation enzymes (Table 1). It is noteworthy that the most recent study of the total lipids of *P. furiosus* found no fatty acids or fatty acid-based lipids (Lobasso *et al.*, 2012).

Fatty acid metabolism in haloarchaea

The case of the *H. salinarum*, which is apparently capable of synthesizing long-chain fatty acids, is more complex. The ability of *H. salinarum* to oxidize long-chain fatty acids has never been demonstrated, despite the presence of a bacterial-type β-ketothiolase in the genome (Table 1). In fact, long-chain fatty acids have been shown to suppress the growth of *H. salinarum* (Gonzalez, 2009). A recent study demonstrated activated expression of the *H. salinarum* genes for β-oxidation pathway (ECH, HDH, ACD) under the conditions of a low-salt stress, 2.6 M NaCl as compared to the optimal NaCl concentration of 4.3 M (Leuko *et al.*, 2009). The genes for two β-ketothiolases, VNG0678G and VNG0931G, which belong to the archaeal clades A5 and A7, respectively (Figures 1 and S2), were also upregulated under these conditions, as were the Bop and CoxA genes that encode bacteriorhodopsin and cytochrome c oxidase subunit I (Leuko *et al.*, 2009). It is tempting to suggest that this entire set of co-expressed enzymes is responsible for energy conservation under stress conditions, when newly synthesized long-chain fatty acids are needed to stabilize the membrane-bound energy-transducing enzymes.

That would mean, however, a reversal of the typical roles of bacterial- and archaeal-type β-ketothiolases in *H. salinarum*. If the typical archaeal β-ketothiolases, VNG0678G and VNG0931G, are involved in fatty acid biosynthesis, this leaves the bacterial-type β-ketothiolase VNG2063G to participate in the mevalonate synthesis pathway, in agreement with the proposal of (Falb *et al.*, 2008). In fact, the mevalonate pathway in halobacteria is atypical since it seems to begin with the condensation of acetyl-CoA with yet unknown C_2 compound (possibly a derivative of lysine) into acetoacetyl-CoA (Ekiel *et al.*, 1986; Falb *et al.*, 2008). The proposed recruitment of the bacterial-type β-ketothiolase for this reaction, implying a change in its substrate specificity, could explain why the cells of *H. salinarum* lack the ability to oxidize long-chain fatty acids (Gonzalez, 2009).

In contrast to other archaea, genomes of two haloarchaeal species, *Halogeometricum borinquense* and *Haloterrigena turkmenica*, encode almost complete sets of FAS II genes. The former contains genes for ACP, ACP synthase, 3-oxoacyl-ACP synthase, ACP-S-malonyl-transferase, and 3- oxoacyl-ACP reductase, whereas the latter encodes all these enzymes as well as enoyl-ACP reductase (Table S2). However, neither organism encodes 3-hydroxyacyl-ACP dehydratase (FabA) or acetyl-CoA carboxylase. As a result, it is not clear whether either of these organisms has a fully functional bacterial-type FAS II. Haloarchaea are known for the high extent of genes acquired by lateral gene transfer from bacteria (Makarova *et al.*, 2007; Nelson-Sathi *et al.*, 2012; Wolf *et al.*, 2012), so the presence of certain bacterial FAS genes in certain haloarchaea is hardly surprising.

Evolutionary considerations

The organization of the archaeal fatty acid biosynthesis pathway, if any, is important for resolving a long-standing evolutionary problem of the origin of biological membranes and, more specifically, the nature of the membrane organization in the last common ancestor of bacteria and archaea (LUCA). Earlier studies made a clear distinction between the archaea with their isoprenoid-based lipids, synthesized via mevalonate pathway, and bacteria and eukaryotes, who have fatty acids-based phospholipid membranes (Koga *et al.*, 1998; Boucher *et al.*, 2004). Since the components of the mevalonate pathway, as well as isoprenoids, are found in bacteria and eukaryotes, the prevailing opinion, until recently, has been that LUCA could synthesize only isoprenoid-based lipids (Benner *et al.*, 1989; Smit and Mushegian, 2000; Mulkidjanian and Galperin, 2010; Dibrova *et al.*, 2012; 2013). The recent papers by Lombard and co-workers challenged this notion by proposing that modern archaea possess a version of the bacterial FAS II (Lombard *et al.*, 2012a). This led to an important evolutionary conjecture that the fatty acid biosynthesis pathway has already been present in LUCA and therefore that LUCA could have had modern-type, two-tail fatty acid lipids (Lombard *et al.*, 2012b; 2012c). While the likely nature of LUCA's membranes is outside the scope of this report, we would like to note here that the great majority of archaea encode only non-specific components of bacterial FAS II that do not constitute a functional pathway (see Table S2, as well as Table S1 of Lombard *et al.*, 2012a). The majority of bacterial-type fatty acid metabolism enzymes found in archaea represent likely cases of lateral gene transfer and are not relevant to the status of LUCA.

The remarkable presence of the membrane energy-converting enzymes only in those organisms that also encode the proposed fatty acid biosynthesis pathway (Table 1) can also be analyzed in evolutionary terms. There is growing evidence that proton-translocating energy-converting complexes, such as cytochrome *bc* complex and cytochrome c oxidase, have evolved in bacteria and have been acquired by archaea via lateral gene transfer (Hemp and Gennis, 2008; Hemp *et al.*, 2012; Dibrova, 2013; Dibrova *et al.*, 2013). If so, these energy-transducing enzymes could be acquired only by those archaea that were able to synthesize fatty acids. Exploitation of these enzymes by archaea would have additionally required the presence of advanced, proton tight membranes. As discussed elsewhere, different branches of archaea utilize different means to make their ether-based membranes proton-tight (Mulkidjanian *et al.*, 2008; 2009).

Concluding remarks

This work provides evidence suggesting that the archaeal pathway of fatty acids metabolism combines bacterial-like enzymes of β-oxidation and archaea-specific paralogs of the acetyl-CoA C-acetyltransferase. The core set of three enzymes with reversible ACD, ECH, and HDH activities could be involved in either synthesis or oxidation of long-chain fatty acids. Coupling of these enzymes with archaeal acetyl-CoA C-acetyltransferase would then yield a synthetic pathway, with various paralogs of the acetyl-CoA C-acetyltransferase likely to be involved in the addition of C_2 fragments to the growing carbon chain. A combination of the core set with the archaeal homologs of the bacterial β-ketothiolase, as found in some genomes (Tables 1 and S1) is expected to result in β-oxidation of fatty acids. Indeed, both archaea that have been shown to be able to oxidize long-chain fatty acids, *A. fulgidus* and *Natronomonas pharaonis* (Falb *et al.*, 2008; Khelifi *et al.*, 2010), contain bacterial-type β-ketothiolases in their genomes. In several archaea, members of COG2368 could potentially replace the missing acyl-CoA-dehydrogenases. In some organisms, members of COG2030 and COG1680 could also be involved, as substrate-specific enoyl-CoA dehydratases and acyl-CoA hydrolases, respectively.

The predicted pathway of fatty acid synthesis still needs to be experimentally verified. However, targeting of the respective genes by knock-out mutations might be complicated by the lethality of these mutations. For example, disruption of the fatty acid biosynthesis in *H. salinarum* would likely affect halorhodopsin and other fatty acid-dependent membrane enzymes.

Until now, successful biotechnological attempts to reverse the system of β-oxidation for producing biofuels centered on bacterial enzymes and, specifically, on enzymes from *E. coli* (Dellomonaco *et al.*, 2011; Clomburg *et al.*, 2012). The enzymes of the proposed archaeal fatty acid biosynthesis pathway from *Sulfolobus solfataricus* and other extremophiles can be expected to operate at higher temperatures and to remain stable at lower pH values than the corresponding mesophilic enzymes. For example, oxidation of fatty acids by *A. fulgidus* has been shown to proceed at 70°C (Khelifi *et al.*, 2010), while the cells of *Geoglobus ahangari* were capable of β-oxidizing fatty acids at temperatures as high as 85°C (Kashefi *et al.*, 2002). Thus, archaeal enzymes of fatty acid metabolism might be more suitable for biofuel production and other industrial applications than the respective bacterial enzymes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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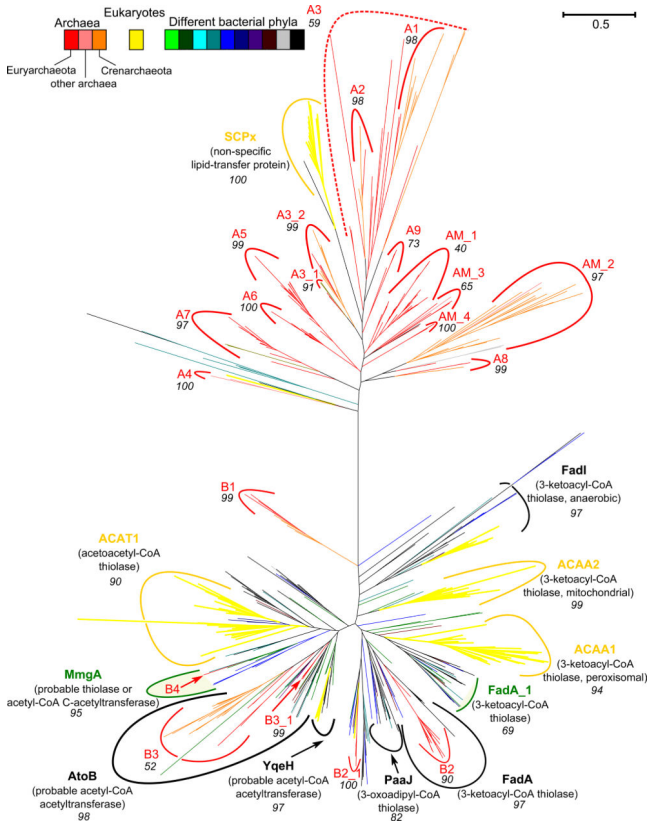

Figure 1. Schematic view of the phylogenetic tree for β-ketothiolases (acetoacetyl-CoA acyltransferases, COG0183)

Separate clades are labelled with the names of the experimentally characterized genes from model organisms. The names of *E. coli* genes are in black, names of *B. subtilis* genes are in green, names of human genes are in yellow. The branches of tree are colored according to the taxonomical identity of the source organisms; the color code is shown in the upper left corner. Clades with archaeal sequences are indicated by red arks or red arrows and named from A1 to AM_4 and from B1 to B4. The numbers underneath the clade labels indicate the reliability of respective branches (as tested using SH-like statistics implemented in PhyML). The bar in the top right corner represents the scale for the branch lengths (the expected number of substitutions per site). The tree has been constructed based on conserved blocks (total 227 positions) from 511 sequences. The sequences were aligned with Muscle (Edgar, 2004) and the alignment was manually edited using GeneDoc (Nicholas *et al.*, 1997). The phylogenetic tree was constructed by PhyML (Guindon and Gascuel, 2003) with the SPR algorithm of tree construction. The tree was visualized with MEGA5 program (Tamura *et al.*, 2011). The complete version of this tree is available in the Supplementary Materials as Figure S2.

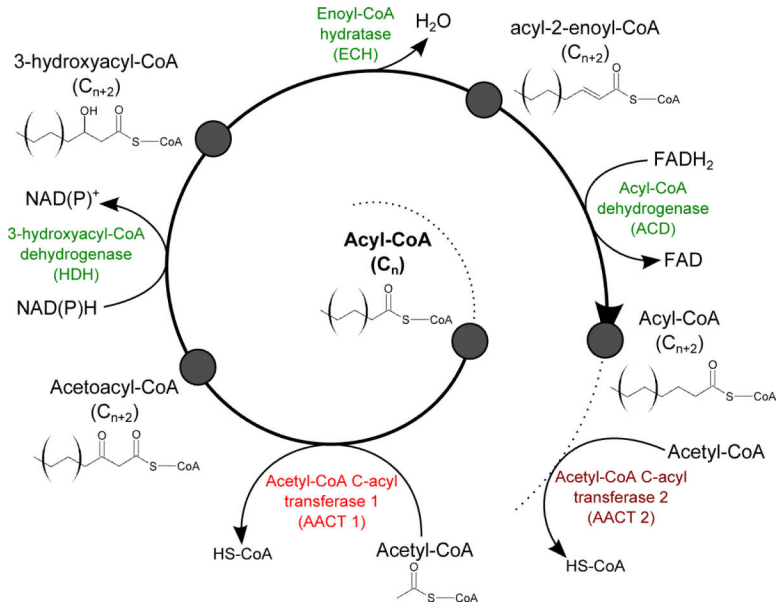

Figure 2. Proposed scheme of archaeal fatty acid biosynthesis

The synthesis of fatty acids in archaea is proposed to proceed through essentially the same steps as in FAS I or FAS II mechanisms (see Figure S3), but by using CoA instead of the acyl-carrier protein, as in the β-oxidation of fatty acids (see Figure S1). Enzymes that catalyze the reduction (HDH and ACD) and dehydratation (ECH) steps are proposed to be the same as those involved in the bacterial β-oxidation pathway, whereas initiation of the cycle and fatty acid chain elongation are proposed to be catalyzed by various paralogs of the archaeal acetyl-CoA C-acetyltransferase. As discussed in the text, the presence of several paralogs of the latter enzyme in many archaeal genomes (denoted here as AACT1 and AACT2) might reflect their specificity towards chains of particular lengths. As an example, enzymes of *Aeropyrum pernix* K1, proposed to catalyze the respective steps, are indicated by their genome locus tags (GenBank accession no. BA000002).

Table 1

Potential enzymes of fatty acid β-oxidation and membrane energy-converting complexes in archaea

Organism name	Steps of β-oxidation				Biosynthetic thiolases		Rhodopsin	Complex III subunits		Complex IV subunits		
	1a | ACDa HpaB	1 | ACD FadE	2 | ECH FadB1	3 | HDH FadB2	BKL FadA	BKL AcaB		CytB	Rieske	CoxA	CoxB	CoxC
Crenarchaeota	COG 2358	COG 1950	COG 1024	COG 1250	COG0183 Bact.	Arch.	COG 5524	COG 1290	COG 0723	COG 0843	COG 1522	COG 1845
Acidilobus saccharovorans 345 15	–	4	3	1	1	4	–	1	–	–	–	–
Aeropyrum pernix K1	–	5	3	1	1	4	–	1	2	2	2	1
Desulfurococcus kamchatkensis 1221 n	–	–	–	–	–	1	–	–	–	–	–	–
Desulfurococcus mucosus DSM 2162	–	–	–	–	–	1	–	–	–	–	–	–
Hyperthermus butylicus DSM 5456	–	–	–	–	–	1	–	–	–	–	–	–
Ignicoccus hospitalis KIN4 1	1	–	1	1	1	1						
Ignisphaera aggregans DSM 17230	–	–	–	–	–	1	–	–	–	–	–	–
Pyrolobus fumarii 1A	1	–	1	1	1	1						
Staphylothermus hellenicus DSM 12710	–	–	–	–	–	1	–	–	–	–	–	–
Staphylothermus marinus F1	–	–	–	–	–	1	–	–	–	–	–	–
Thermosphaera aggregans DSM 11486	–	–	–	–	–	1	–	–	–	–	–	–
Acidianus hospitalis W1	1	3	2	2	1	1	–	2	2	4	1	–
Metallosptiaera sedula DSM 5348	4	6	6	4	1	7	–	3	4	6	4	1
Sulfolobus solfataricus P2	3	9	8	4	2	8	–	3	3	3	2	1
Caldivirga maquilingensis IC 167	–	2	2		1	1	–	2	2	1	1	–
Pyrobaculum aerophiium IM2	1	4	3		2	5	–	1	1	2	2	1
Thermofilum pendens Hrk 5	–	–	–		–	1	–	–	–	–	–	–
Thermoproteus neutrophilus V24Sta	1	3	1		1	1						
Thermoproteus tenax Kra 1	1	4	3		2	5	–	1	1	–	–	–
Thermoproteus uzoniensis 768 20	1	4	3		2	5	–	2	2	1	1	1
Vulcanisaeta moutnovskia 768 28	2	7	6	2	2	5						
Euryarchaeota												
Archaeoglobus fulgidus DSM 4304*	3	14	9	10	3	12	–	–	–	–	2	–

Archaeoglobus profundus DSM 5631	–	1	–	–	–	2	–	–	–	–	–	–
Archaeoglobus veneficus SNP6	–	1	–	–	1	1						
Ferroglobus placidus DSM 10642	1	9	6	5	2	7	–	–	–	–	2	–
Halalkalicoccus jeotgali B3	–	12	12	5	2	3	–	2	2	2	1	1
Haloarcula marismortui ATCC 43049	–	10	7	5	3	4	6	3	3	4	4	3
Halobacterium salinarum R1	–	6	3	2	1	2	4	2	1	2	2	1
Haloferax volcanii DS2	–	7	7	4	1	3	–	4	3	3	3	2
Halogeometricum borinquense DSM 11551	–	8	7	4	1	4	–	3	2	4	3	2
Halomicrobium mukohataei DSM 12286	–	6	3	3	1	2	3	3	2	4	2	1
Halopiger xanaduensis SH 6	–	6	1	2	2	2	2	–	1	4	3	3
Haloquadratum walsbyi DSM 16790	–	1	1	1	–	3	3	2	1	1	1	1
Halorhabdus utahensis DSM 12940	–	–	2	1	–	1	2	3	2	1	1	1
Halorubrum lacusprofundi ATCC 49239	–	7	3	2	1	3	2	3	2	3	2	2
Haloterrigena turkmenica DSM 5511	–	18	16	10	4	12	1	–	1	4	3	3
Natrialba magadii ATCC 43099	–	14	9	7	3	5	3	–	1	3	3	1
Natronomonas pharaonis DSM 2160*	–	12	8	5	3	5	4	–	–	3	3	1
Methanothermus fervidus DSM 2088	–	–	–	–	–		–	–	–	–	–	–
Methanobrevibacter smithii ATCC 35061	–	–	–	1	–		–	–	–	–	–	–
Methanosphaera stadtmanae DSM 3091	–	–	–	1	–		–	–	–	–	–	–
Methanothermobacter thermautotrophicus Delta H												
Methanocaldococcus jannaschii DSM 2661	–	–	–	–	–		–	–	–	–	–	–
Methanococcus aeolicus Nankai 3	–	–	–	–	–		–	–	–	–	–	–
Methanothermococcus okinawensis IH1	–	–	–	–	–		–	–	–	–	–	–
Methanotorris igneus Kol 5	–	–	–	–	–		–	–	–	–	–	–
Methanocella paludicola SANAE	–	–	–	–	–		–	–	–	–	–	–
Methanocorpusculum labreanum Z	–	–	–	–	–		–	–	–	–	–	–
Methanoculleus marisnigri J R1	–	–	–	–	–		–	–	1	–	–	–
Methanoplanus petrolearius DSM 11571	–	–	–	–	–		–	–	–	–	–	–
Methanoregula boonei 6A8	–	–	–	–	–		–	–	–	–	–	–
Methanosphaerula palustris E1 9c	–	–	–	–	–		–	–	–	–	–	–
Methanospirillum hungatei JF 1	–	–	–	–	–		–	–	–	–	–	–

Methanococcoides burtonii DSM 6242	–	–	–	–	–		–	–	–	–	–	–
Methanohalobium evestigatum Z 7303	–	–	–	–	–		–	–	–	–	–	–
Methanohaiophilus mahii DSM 5219	–	–	–	–	–		–	–	–	–	–	–
Methanosaeta thermophila PT	–	–	–	–	–		–	–	–	–	–	–
Methanosalsum zhilinae DSM 4017	–	–	–	–	–		–	–	–	–	–	–
Methanosarcina barkeri Fusaro	–	–	–	–	–		–	–	–	–	–	1
Methanopyrus kandleri AV19	–	–	–	–	–		–	–	–	–	–	–
Pyrococcus furiosus DSM 3638	–	–	–	–	–		–	–	–	–	–	–
Thermococcus sibiricus MM 739	–	–	–	–	–		–	–	–	–	–	–
Picrophilus torridus DSM 9790	–	6	2	2	2	2	–	1	1	1	1	–
Thermoplasma acidophilum DSM 1728	–	7	5	3	2	3	–	2	2	–	–	–
Aciduliprofundum boonei T469	–	–	–	–	–	1	–	–	–	–	–	–
other archaea												
Korarchaeum cryptofilum	–	1	1	1	1	1	–	1	1	–	–	–
Nanoarchaeum equitans												
Cenarchaeum symbiosum	1	–	1	1	–	2	–	1	1	1	1	–
Nitrosopumilus maritimus	1	–	1	1	–	2	–	1	1	1	1	–

The organisms are ordered according to their taxonomic positions (see Table S1). Two organisms shown to metabolize fatty acids (Falb *et al.*, 2008; Khelifi *et al.*, 2010) are marked with asterisks. The columns represent the numbers of paralogous genes within each respective COG (Tatusov *et al.*, 2003; Wolf *et al.*, 2012), or their absence, indicated with a dash, in the corresponding genome. For COG0183 (3-ketothiolases, acetyl-CoA C-acetyltransferases, and related enzymes), the two columns show the numbers of genes in the “bacterial” and “archaeal” parts of the tree (Fig. 1). Grey shading indicates those genomes that encode only a single “archaeal” form member of COG0183. The enzyme names are abbreviated as follows: ACD, acyl-CoA-dehydrogenase; ECH, enoyl-CoA hydratase; HDH, 3-hydroxyacyl-CoA dehydrogenase; BKL, β-ketothiolase. The prediction that members of COG2368 function as alternative acyl-CoA-dehydrogenases, ACDa, has not been experimentally verified (see text for details).