An ACP-Independent Fatty Acid Synthesis Pathway in Archaea: Implications for the Origin of Phospholipids

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Abstract

Fatty acids (FAs) are major building blocks of membrane phospholipids in bacteria and eukaryotes. Their presumed absence in archaean led to propose a late origin in bacteria and eukaryotes and that the last common ancestor of living organisms (the cenancestor) was devoid of both FA and phospholipid membranes. However, small FA amounts and homologs of bacterial FA biosynthesis enzymes are found in archaean. We have investigated the origin of these archaean enzymes using phylogenomic analyses of all enzymes of the main bacterial FA biosynthesis pathway. Our results suggest that modern archaean and their last common ancestor possessed a complete pathway except for the acyl carrier protein (ACP) processing machinery, which evolved in the bacterial lineage. This has not only implications for archaean physiology but also opens the possibility for the presence of ACP-independent FA synthesis in the cenancestor, which may have been endowed with FA-phospholipid membranes.

Key words: fatty acid synthesis, acyl carrier protein, archaean, early evolution, cenancestor.

Although fatty acids (FAs) play major roles as storage molecules, secondary metabolites, and components of bacterial and eukaryotic membrane phospholipids, little is known about them in archaean. This reflects that, most often, archaean membrane phospholipids are considered to lack FA and to carry isoprenoids instead (Boucher 2007). In fact, although bacterial and eukaryotic phospholipids contain FA bound to sn-glycerol-3-phosphate with ester links, in archaean, isoprenoids are linked to sn-glycerol-1-phosphate through ether bounds (De Rosa et al. 1986; Boucher 2007). These striking differences figured among the main cellular characteristics that supported archaean as an independent domain of life (Woese et al. 1979).

However, experimental data show that FAs also exist in archaean. Biochemical evidence for FA synthase machineries (Pugh et al. 1971; Pugh and Kates 1994) and small amounts of free FA or their derivatives (Kates et al. 1968; Langworthy et al. 1974; Tornabene et al. 1978; Carballerera et al. 1997; Nishihara et al. 2000) have been reported in diverse archaean. Phospholipids bearing FA have been detected in very diverse euryarchaean (Gattinger et al. 2002), and, as in bacteria and eukaryotes, archaean FAs are sometimes involved in protein structure (Colella et al. 1998) and acylation (Pugh and Kates 1994). Finally, archaean homologs of bacterial FA biosynthesis enzymes have been reported in archaean genomes (Peretó et al. 2004; Iverson et al. 2012). Despite these arguments, the significance of FA in archaean (including the last archaean common ancestor [LACA]) remains poorly acknowledged in recent scientific literature (Boucher 2007; Dibrova et al. 2011). Similarly, the assumption that the last common ancestor of living organisms (the cenancestor) lacked FA has even served as a supplementary argument to propose that this organism could not synthesize phospholipids and, therefore, was devoid of lipid membranes (Martin and Russell 2003).

Determining the evolutionary origin of archaean FA has implications both to envisage early evolution scenarios and to better understand archaean physiology. FA biosynthesis occurs through repetitive condensation of acyl (malonyl-CoA) groups in a series of cyclic steps (fig. 1) catalyzed by enzymes that may be individual (FA synthase type II [FAS-II]) or fused in one unique polypeptide (FAS-I). FAS-II is the main FA biosynthesis pathway in most bacteria, mitochondria, and chloroplasts (White et al. 2005), whereas FAS-I is more rare in bacteria but predominant in the eukaryotic cytoplasm (Schweizer and Hofmann 2004). As we were particularly interested by the presence of FAS homologs in archaean and only individual FAS-II but not polypeptidic FAS-I homologs were detected in the available archaean genome sequences, we removed the FAS-I sequences from our analyses and restricted our systematic phylogenomic analyses to the FAS-II individual peptides. Our results support that the actual distinctive feature between archaean and bacteria is not the presence of the FAS system itself, but the lack of the FAS-related acyl carrier protein (ACP) processing machinery in archaean.

FAS-II Homologs in Archaea

In bacteria, several reactions supply the malonyl-ACP building blocks necessary to FAS-II: 1) acetyl-coenzyme A (CoA) is converted into malonyl-CoA by the acetyl-CoA carboxylase (ACC), 2) a phosphopantetheine group from CoA is attached by an ACP synthase (ACPS) to the apo-ACP, and 3) the malonyl-CoA:ACP transacylase (MCAT) charges malonyl-CoA to holo-ACP (fig. 1). A wide diversity of archaean bear ACC homologs of possible monophyletic origin, suggesting their vertical inheritance from the cenancestor (Lombard and Moreira 2011a). ACP is a peptide cofactor that covalently binds all FA intermediates, channeling them among the FAS-II enzymes. Except for a few unrelated species that
probably acquired ACP by independent horizontal gene transfers (HGTs), we did not retrieve homologs of the ACP-processing machinery (ACP, ACPS, and MCAT) in archaea. This contrasted with the presence of homologs of all the other FAS genes in a large diversity of archaea (supplementary table S1, Supplementary Material online).

Once malonyl-ACP is produced, it is condensed with acetyl-CoA (beta-ketoacyl synthase III [KAS III], FabH) to yield ketoacyl-ACP, an intermediate that is sequentially reduced (beta-ketoacyl reductase [KR], FabG), dehydrated (beta-hydroxyacyl dehydratase [DH], FabZ or FabA), and reduced again (enoyl reductase [ER], FabI). A subsequent extension with malonyl-ACP (beta-ketoacyl synthases KAS I or II, respectively, FabB and FabF) conducts a new elongation cycle (fig. 1). Although nonhomologous replacements exist in some bacteria (Marrakchi et al. 2003), we have focused our search on the most common archetypal FAS-II enzymes.

A phylogenetic analysis of the thiolase superfamily, to which KAS I, KAS II, and KAS III belong, has recently been published (Jiang et al. 2008). It separated this superfamily into five clusters based on phylogenetic trees and active site architecture: archaeal thiolases, bacterial + eukaryotic thiolases, bacterial + eukaryotic KAS I–KAS II, archaeal and eukaryotic hydroxymethylglutaryl-CoA synthase (HMGS), and bacterial + eukaryotic KAS III-chalcone synthases (CHS). However, this can be questioned as only one very divergent archaeal sequence was used as an outgroup, and HMGS was recently shown to be universal and probably present in the cenancestor (Lombard and Moreira 2011b). Thus, we carried out a more complete analysis of the thiolase superfamily (supplementary fig. S1, Supplementary Material online). Although these enzymes preserve strong structural similarity, primary sequence conservation is low. However, similarities within each group of sequences point to four different clusters with distinctive enzymatic functions: 1) universally distributed thiolases, 2) bacterial KAS I–KAS II, 3) bacterial KAS III–CHS, and 4) universally distributed HMGS. Thiolase and HMGS sequences supported the separation of most bacterial and archaeal sequences into two monophyletic groups, strongly suggesting that the cenancestor was endowed with these two enzymes. In contrast, KAS is widespread in bacteria and absent in archaea. Thiolases and HMGS catalyze the removal of an acetyl-CoA moiety from an acyl-CoA chain or the opposite condensation reaction, so the major difference between KAS and these other enzymes is the use of substrates linked to ACP instead of CoA. Thus, our results support that the cenancestor was able to (de)acetylate acyl-CoA chains, whereas the use of acyl-ACP seems to be a more recent bacterial derived character.

We obtained similar results on KR sequences that, together with ER, belong to the short-chain dehydrogenases/reductases superfamily (Kallberg et al. 2002). As this family is very diverse, we reconstructed independent phylogenies for KR and ER. The KR phylogeny incorporated the closely related PhaB acetoacetyl-CoA reductases, which are involved in polyhydroxyalkanoate (PHA) synthesis in bacteria and haloarchaea. The main enzymatic difference between PhaB and KR is the use of CoA- instead of ACP-linked substrates, although some enzymes are known to use both (Taguchi et al. 1999; Ren et al. 2000). Although PhaB sequences were scattered among KR homologs, the bacterial KR-PhaB sequences clustered according to main bacterial phyla (fig. 2). Some archaeal sequences were dispersed among the bacterial KR-PhaB, indicating independent HGTs from bacteria. Nevertheless, a wide diversity of uncharacterized archaeal sequences branched at the base of the bacterial KR-PhaB cluster. Maximum likelihood (ML) phylogenetic reconstruction of these archaeal sequences showed at least two paralogs in crenarchaeota (with some HGTs from them to other groups), sisters of a basal group of euryarchaeota (supplementary fig. S2, Supplementary Material online). This result indicates the ancient presence of these genes not only in the last
common ancestor of the crenarchaeota but likely also in LACA. The proximity to the KR-PhaB group suggests that these archaeal sequences may catalyze similar reactions (probably on CoA-linked substrates, ACP being absent in archaea). As for KR, the ER phylogeny supported the monophyly of most bacterial sequences and revealed two basal-branching ER-like paralogs in crenarchaeota, indicating an ancient duplication in this group (supplementary fig. S4, Supplementary Material online). HGTs from crenarchaeota to some euryarchaeota and bacteria could be noticed, but the presence of several basal-branching euryarchaeotal sequences supported an ancient origin also in Euryarchaeota. With the exception of some sequences (Halobacteriales) probably acquired by HGT from bacteria, most archaeal DH homologs were closely related to mitochondrial DH and MaoC/PhaJ enzymes (supplementary fig. S3, Supplementary Material online). Mitochondrial DH acts on acyl-ACP chains, whereas MaoC/PhaJ uses acyl-CoA substrates in PHA synthesis (Tsuge et al. 2003; Autio et al. 2008). Paralogs could be detected in crenarchaeota, but their monophyly was difficult to assess because of the high sequence divergence and small number of conserved alignment positions (112). Although the function of most archaeal enzymes remains unknown, they can reasonably be assumed to carry out DH-type reactions on CoA-linked substrates. In summary, our analyses suggest that LACA bore KR, ER, and possibly DH enzymes.

**Conclusion**

Except for some rare cases of HGT of ACP-processing machinery enzymes from bacteria, these enzymes are completely absent from archaea, in contrast with the presence of all other FAS-II enzymes. Moreover, all phylogenies of FAS enzymes support that archaeal sequences are more closely related to bacterial enzymes acting on FAS-like substrates linked to CoA than to enzymes using ACP-linked substrates. Thus, we propose that archaea should not be considered as completely devoid of the FAS machinery but only of the ACP-processing system, which seems to have specifically evolved in the bacterial lineage. Although this still requires biochemical verification, it seems likely that these enzymes account for the FA detected in archaea (Gattinger et al. 2002). From a biochemical perspective, recent work showing that the channeling of FA intermediates from one FAS-II enzyme to the next in the cycle is permitted by unique ACP conformations (Ploskon et al. 2010) raises the question of the biochemical mechanism of this putative archaeal FAS. The potential lower efficiency of an ACP-independent FAS could account for the small amounts of FA detected in archaea. From an evolutionary perspective, the presence of acyl-CoA FAS-like machineries in both bacteria and archaea argues for the existence of a similar pathway in the common ancestor, contrary to previous claims that the common ancestor lacked FA (Martin and Russell 2003; Dibrova
et al. 2011) or that it only bore abiotically synthesized FA (Koonin and Martin 2005). In this context, the preemience of FA in present-day bacterial membranes could be explained by the evolution of the very efficient ACP-processing machinery in this lineage, whereas archaea would have recruited other ancestral membrane-synthesizing mechanisms (e.g., isoprenoids [Lombard and Moreira 2011b]) and relegated FA to different cellular functions.

Materials and Methods

Similarity searches were done with basic local alignment search tool-p (Altschul et al. 1990) or psi-BLAST for DH analyses (Altschul et al. 1997) against complete genomes available in GenBank (supplementary table S2, Supplementary Material online) using seed sequences retrieved from KEGG (http://www.genome.jp/kegg). For each seed, homologs were aligned with Muscle 3.6 (Edgar 2004). Redundant sequences and sequences out of the size range known from the literature were removed. Preliminary trees were constructed by approximate ML with FastTree 2.1.3 (Price et al. 2010) and refined by iterative steps to remove the sequences the most divergent from the archaeal ones. These divergent sequences and ambiguously aligned regions were detected with Guidance (Penn et al. 2010) and discarded using NET from the MUST package (Philippe 1993). More accurate phylogenies were inferred by ML on the refined sequence data sets using TREEFINDER (Jobb et al. 2004) with the LG+I model (Le and Gascuel 2008) and four rate categories. Statistical support was assessed by approximate bootstrap (100 replicates).

Supplementary Material

Supplementary tables S1 and S2 and figures S1–S4 are available at Molecular Biology and Evolution online (http://www.mbe.oxfordjournals.org/).

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References


