Vitamin Formation from Fatty Acid Precursors

14

Michael F. Dunn

Contents

Abstract

Enzymes that require biotin or lipoic acid cofactors for their activity occur in all domains of life and play essential roles in metabolism. The de novo synthesis of these vitamins depends on the production of fatty acid precursors and has been most extensively characterized in *Escherichia coli* and *Bacillus* species. The octanoyl-acyl carrier protein precursor for lipoic acid is synthesized in reactions catalyzed by the fatty acid biosynthesis (Fab) enzymes. The octanoyl moiety is linked to the lipoyl domains of lipoic acid-dependent enzymes and then converted to lipoate by lipoyl synthase. For biotin biosynthesis, both the BioC-BioH pathway in *E. coli* and the BioI pathway in *Bacillus* species rely on Fab enzymes

M. F. Dunn (✉)
Programa de Genómica Funcional de Procariontes, Centro de Ciencias Genómicas, Universidad Nacional Autonoma de México, Av. Universidad s/n, Col. Chamilpa, Cuernavaca, Morelos, Mexico
e-mail: mike@ccg.unam.mx

O. Geiger (ed.), *Biogenesis of Fatty Acids, Lipids and Membranes*, Handbook of Hydrocarbon and Lipid Microbiology, https://doi.org/10.1007/978-3-319-50430-8_24

to produce the pimeloyl-acyl carrier protein required for biotin production. This review presents an overview of the biosynthetic pathways for biotin and lipoic acid, with an emphasis on the role of fatty acid metabolism in their synthesis.

1 Introduction

Fatty acids (FAs) are aliphatic hydrocarbons with a carboxylic acid group on carbon number 1 and a methyl group at the other end of the chain (see octanoic acid in Fig. 1a as an example). FAs are synthesized by organisms in all domains of life and play vital roles in membrane structure, energy storage, and as metabolic precursors (Cronan 2014; Dibrova et al. 2014; López-Lara and Geiger 2010; Sohlenkamp and Geiger 2016). This review presents a brief overview of how bacterial FA synthesis (FAS) participates in the production of the vitamins biotin and lipoic acid (LA) (Fig. 1), with emphasis on *Escherichia coli* and *Bacillus* species. Bacterial FAS is covered in detail in two chapters in this handbook: ▶ Chap. 3, "Formation of Fatty Acids" by López-Lara and Geiger and ▶ Chap. 21, "Fatty Acid Synthesis and Regulation" by López-Lara. Details of biotin and LA pathway enzyme mechanisms, structures, and regulation are described in recent reviews (Cronan 2014, 2016; Cronan and Lin 2011; Lin and Cronan 2011; Rock 2009).

Biotin and LA are prosthetic groups for selected metabolic enzymes, where they are covalently attached to a specific domain or subunit of the enzymes requiring them. Both cofactors act as "swinging arms" to shuttle reaction intermediates between different active sites of their cognate enzymes (Perham 2000). Examples of biotin- and LA-dependent enzymes and the de novo synthesis of these cofactors are described in the following sections.

2 Lipoic Acid Synthesis

The structure of LA is that of the eight-carbon saturated FA octanoic acid into which sulfur atoms are inserted at carbons 6 and 8 (Fig. 1A). Pyruvate dehydrogenase (PDH) is a well-characterized example of a LA-dependent enzyme. It catalyzes the oxidative decarboxylation of pyruvate to produce acetyl coenzyme A (acetyl-CoA), which serves as the acetate donor for FAS (Fig. 2) and in many other vital metabolic functions. PDH is a complex consisting of multiple copies of subunits, designated E1 (pyruvate dehydrogenase), E2 (dihydrolipoyl transacetylase), and E3 (dihydrolipoyl dehydrogenase). E2 contains the bound LA cofactor and forms the structural core of the enzyme by binding noncovalently to E1 and E3. The E2 lipoyl group (Fig. 1a) acts as a "swinging arm" to transfer acetate between the E1 and E3 active sites of the enzyme. First, subunit E1 decarboxylates pyruvate, and the resulting hydroxyethyl group is bound to the thiamine pyrophosphate (TPP) cofactor of the E1 subunit, forming hydroxyethyl TPP (E1-TPP-CHOH-CH_3). The

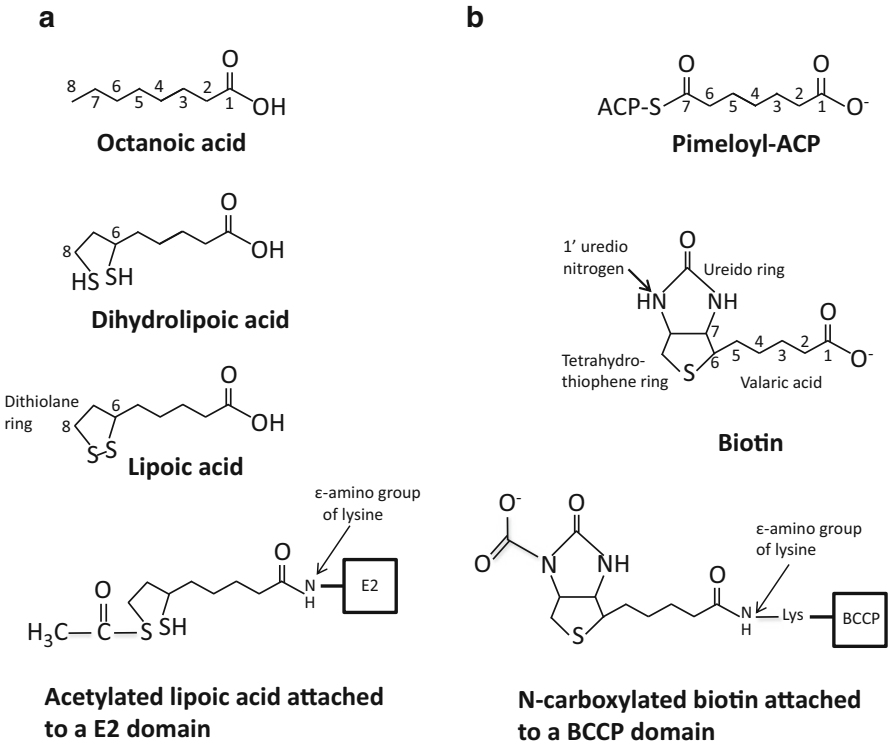

Fig. 1 Structural features of biotin and lipoic acid. (**a**) Lipoic acid (*LA*) and related structures. The C6 and C8 carbon atoms indicate those that are bound to sulfur in the dithiolane ring of LA. The structure at the bottom of the figure is the specific example of acetyl-modified LA attached to the E2 domain of pyruvate dehydrogenase. (**b**) Biotin and related structures. Pimeloyl-ACP donates the majority of carbon atoms (numbered) to biotin. The 1′ uredio nitrogen of biotin is the site of carboxylation, as shown in the structure of carboxylated biotin linked to the BCCP domain of a biotin-dependent enzyme

hydroxyethyl group is oxidized to an acetyl group that is then transferred to one of the LA sulfur atoms, forming acetyl-LA (Fig. 1a). The acetyl group is then transferred to CoA to produce acetyl-CoA. Finally, the reduced LA is reoxidized to reform the dithiolane ring (Fig. 1a) in an FAD-dependent reaction catalyzed by the E3 subunit (Perham 2000; Rock 2009).

2.1 Synthesis of Octanoyl-ACP

LA biosynthesis requires octanoic acid, a C8:0 FA formed in the normal course of bacterial FAS (Fig. 1a). Bacterial FAS systems are comprised of Fab (fatty acid biosynthesis) enzymes. Acyl carrier protein (ACP) shuttles FAs from one Fab enzyme to another and also prevents them from being degraded during their

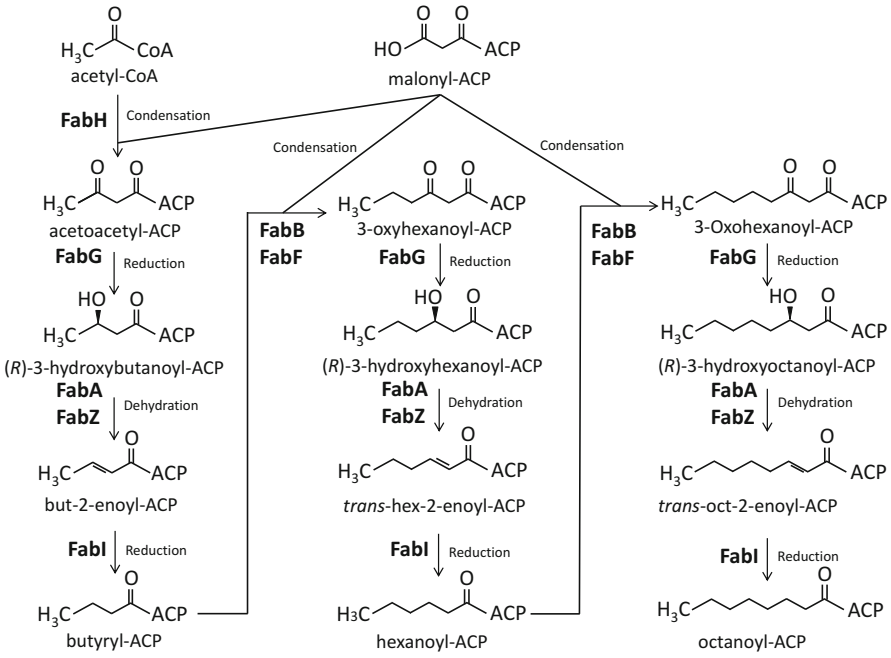

Fig. 2 Reactions of FAS producing the octanoyl-ACP precursor of lipoic acid in *E. coli* K-12 MG1655, based on the KEGG database (Kanehisa et al. 2016). See Sect. 2.1 for details. Abbreviations: *ACP* acyl carrier protein, *FabH* 3-oxoacyl-ACP synthase III, *FabG* 3-oxoacyl-ACP reductase, *FabA and FabZ* 3-hydroxyacyl-ACP dehydratases, *FabI* enoyl-ACP reductase I

synthesis (Chan and Vogel 2010; Janßen and Steinbüchel 2014; López-Lara and Geiger 2010). ACP contains a covalently bound 4′-phosphopantetheine prosthetic group to which fatty acyl groups are linked by a thioester bond between the carboxyl group of the FA and the sulfhydryl group of the 4′-phosphopantetheine (Janßen and Steinbüchel 2014).

The first reaction of FAS is catalyzed by the biotin-dependent enzyme acetyl-CoA carboxylase (ACC; see Sect. 3), which adds CO_2 to acetyl-CoA to form malonyl-CoA. Next, the CoA on malonyl-CoA is exchanged with ACP to form malonyl-ACP in a reversible reaction catalyzed by malonyl-CoA/ACP transacylase (FabD). A condensation reaction catalyzed by 3-oxoacyl-ACP synthase III (FabH) combines malonyl-ACP with acetyl-CoA to yield acetoacetyl-ACP (Fig. 2). The four-carbon acetoacetyl-CoA produced by FabH is subjected to a reduction, a dehydration, and a second reduction by Fab enzymes to yield butyryl-ACP (Fig. 2). Two more rounds of FAS comprised of condensation-reduction-dehydration-reduction reactions each add two carbon units to the acyl chain, resulting in the production of octanoyl-ACP (Janßen and Steinbüchel 2014; Qui et al. 2001; Rock and Jackowski 2002) (Fig. 2).

2.2 Lipoylation of LA-Dependent Enzymes Occurs by Two Pathways

Two different pathways have evolved in *E. coli* for the lipoylation of LA-dependent enzymes (Morris et al. 1995) (Fig. 3). The scavenging pathway starts with the passive uptake of LA from the environment (Cronan 2016), after which lipoate-protein ligase (LplA) attaches it to lipoyl domains (LDs) in a two-step reaction that involves an activated lipoyl-AMP intermediate. The activated lipoyl moiety is then transferred to a specific lysine residue of the LD and AMP is released (Cronan 2014; Rock 2009) (Fig. 3). In *Bacillus subtilis*, the LplA ortholog is designated LplJ (Martin et al. 2011).

In the *E. coli* pathway for the de novo synthesis of LA, octanoyl-ACP/protein *N*-octanoyltransferase (LipB) cleaves the ACP from the octanoyl-ACP produced by FAS (Fig. 2) and binds the octonate moiety (Fig. 3). The octanoyl-LipB intermediate then donates the octanoyl moiety to the LD to form octanoyl-LD. Finally, lipoyl synthase (LipA) catalyzes the SAM-dependent insertion of two sulfur atoms, derived from the LipA [Fe-S] cluster, into the LD-bound octanoyl moiety, converting it to a lipoyl-LD. In *B. subtilis*, LipM performs the same function as the *E. coli* LipB (Cronan 2014; Jordan and Cronan 1997; Martin et al. 2011; Morris et al. 1995).

LA synthesis is unusual in that it requires the octanoyl moiety precursor to be covalently linked to a LD. This in situ synthesis of the lipoyl moiety contrasts with the production of biotin (Sect. 3) and numerous other enzyme cofactors, which are

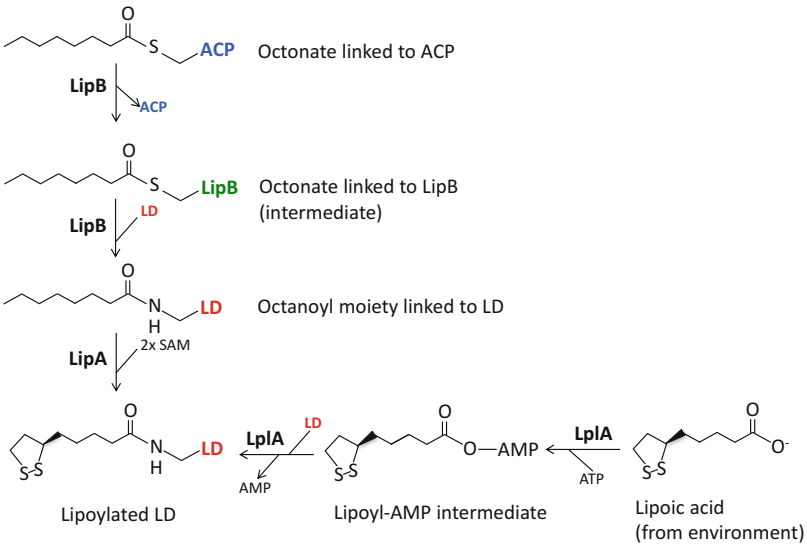

Fig. 3 The de novo synthesis of lipoic acid (*LA*) from octanoyl-ACP (*left* hand of figure) and the scavenging pathway utilizing exogenous LA (*bottom* of figure). See Sect. 2.2 for details. Abbreviations: *ACP* acyl carrier protein, *LD* lipoyl domain (The figure is modified from Cronan (2016))

fully synthesized prior to their attachment to their cognant enzymes (Sect. 3) (Cronan 2014; Jordan and Cronan 1997; Morris et al. 1995).

In *E. coli*, neither *lipA* nor *lipB* appears to be transcriptionally regulated. This may be because the sulfur insertions catalyzed by LipA occur only when the octanoyl moiety is linked to a LD, and so the supply of subunits requiring lipoylation determines the quantity of LA synthesized (Feng and Cronan 2014). In contrast, the expression of *lipA* and *lipB* in *Shewanella oneidensis*, which form an operon, is regulated by glucose in a cyclic adenosine monophosphate-dependent manner (Zhang et al. 2015).

3 Synthesis of Biotin

E. coli contains a single biotin-dependent enzyme, acetyl-CoA carboxylase (ACC), which is essential for FAS and thus viability (Beckett 2007; Cronan 2014). ACCs in prokaryotes are comprised of three subunits: biotin carboxylase (BC), biotin carboxyl carrier protein (BCCP), and carboxyltransferase (CT) (Tong 2013). ACC catalyzes a two-step reaction that adds CO_2 (from bicarbonate, HCO_3^-) to acetyl-CoA to produce malonyl-CoA:

1. ACC-biotin + CO_2 + MgATP – >ACC-biotin-CO_2^- + MgADP + Pi + H^+
2. ACC-biotin-CO_2^- + CH_3-CO-SCoA – >ACC-biotin + ^-O_2C-CH_2-CO-S-CoA

In step 1, the BC domain catalyzes the ATP-dependent carboxylation of the biotin attached to a specific lysine residue in the BCCP domain (Fig. 1b). In step 2, the activated CO_2 is transferred from biotin to acetyl-CoA in a reaction catalyzed by the CT domain. The valeryl side chain of biotin (Fig. 1b) is important in its ability to interact with the BC and CT domains (Tong 2013).

To satisfy their biotin requirements, bacteria can use specific transporters to obtain the vitamin from the environment or synthesize it de novo. Some bacteria lack complete biotin synthesis pathways and are able to obtain biotin only from external sources, while others are capable of its synthesis and lack transporters. Some bacteria can use both options (Feng et al. 2015; Guillén-Navarro et al. 2005; Rodionov et al. 2002; Satiaputra et al. 2016).

The four final steps of biotin biosynthesis (Sect. 3.2) have been extensively studied and are well conserved in different organisms (Beckett 2007; Lin 2012). In contrast, knowledge of how the pimelate moiety precursor for these final steps is produced is much more recent, as described below.

3.1 Production of the Pimelate Moiety

The pimelate moiety (pimeoyl-ACP) is the source of carbons 1 through 7 of biotin (Fig. 1b). The BioI and BioC-BioH pathways are distinct routes for producing the pimelate moiety that occur in different bacteria, and both are dependent on FAS (Cronan and Lin 2011) (Fig. 4). The BioI pathway occurs principally in *B. subtilis* and related bacteria. BioI is an oxygen-dependent cytochrome P450 family protein

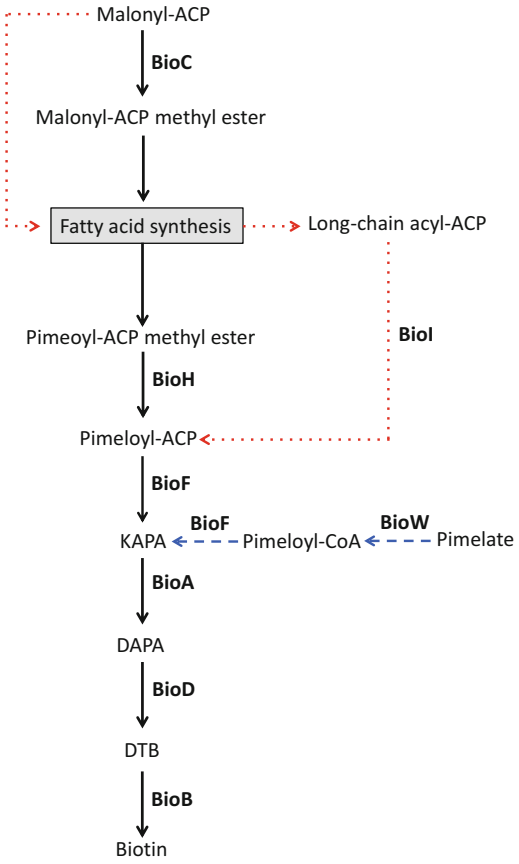

Fig. 4 A schematic view of showing enzymatic alternatives for biotin production. Fatty acid synthesis (*shaded box*) plays a central role in the BioI (*dotted arrows*) and BioC-BioH pathways (*solid arrows*), but not in the BioW-BioF pathway (*dashed arrows*). See Sects. 3.1 and 3.2 for details

that produces pimeloyl-ACP by the cleavage of saturated and unsaturated long-chain fatty acyl-ACPs produced by the FAS system (Stok and De Voss 2000) (Fig. 4). The FA chain of these substrates and the 4′-phosphopantetheine prosthetic group of ACP fit into the hydrophobic substrate-binding cavity of BioI. A sharp bend is induced in the acyl chain that places its C7 and C8 atoms in close proximity to the heme iron of the active site. Catalysis is thought to occur by the successive hydroxylation of C7 and C8, followed by the oxidative cleavage of the carbon-carbon bond between them (Cronan and Lin 2011; Cryle and Schlichting 2008). The pimeloyl-ACP thus produced is converted to biotin in four steps described in the next section. The heterologous overexpression of *Bacillus* BioI in *E. coli bioC* or *bioH* mutants (Figs. 4 and 5) permits their growth in biotin-free medium (Bower et al. 1996).

Despite decades of biochemical and genetic characterization of biotin biosynthesis in *E. coli*, the roles of BioC and BioH (Fig. 4) in producing the pimelate moiety were only recently defined. BioC is a *O*-methyltransferase that methylates the carboxyl group of malonyl-ACP to form malonyl-ACP methyl ester, using *S*-adenosyl-L-methionine (SAM) as a methyl group donor (Fig. 5). Replacement of

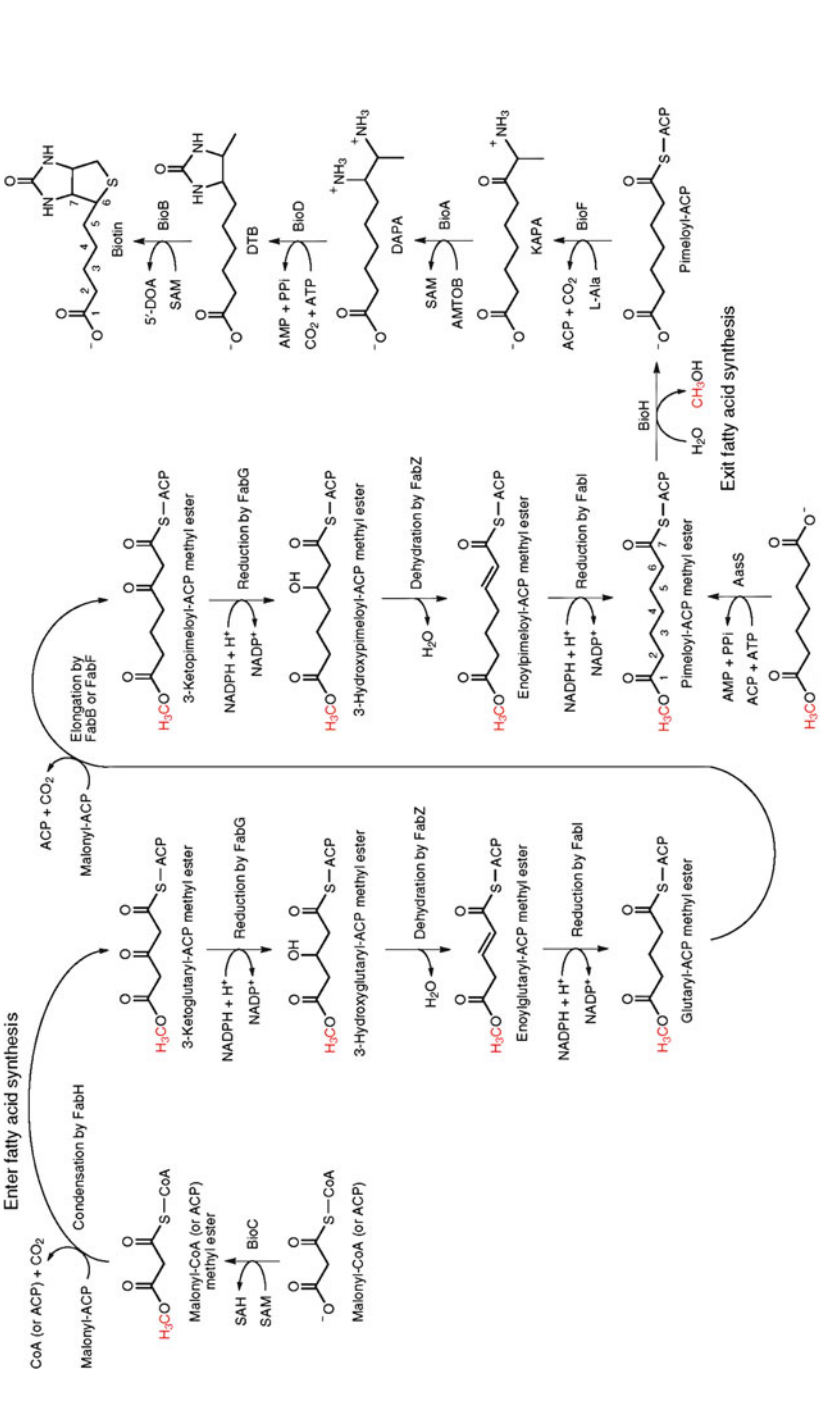

Fig. 5 Biotin biosynthesis by the BioC-BioH pathway showing the FAS reactions using methyl esterified FA intermediates and the formation of the bicyclic rings. See Sect. 3.2 for details (The figure is reproduced from Lin et al. 2010 and used by permission of the Nature Publishing Group)

the carboxyl group with a methyl ester group removes the molecule's negative charge and mimics the methyl groups normally present on FA chains. The methyl ester modification channels the BioC reaction product into biotin biosynthesis and allows it to enter the hydrophobic active sites of the FAS enzymes: three rounds of FAS result in the production of pimeloyl-ACP methyl ester (Bi et al. 2016; Lin 2012; Lin and Cronan 2011; Lin et al. 2010) (Fig. 5).

Much of what we know about BioC comes from the characterization of the *Bacillus cereus* enzyme, overexpressed in *E. coli* and obtained in purified form. This is because the BioC from *E. coli* and several other bacteria has proved intractable to efficient purification. The *B. cereus* BioC is able to functionally complement an *E. coli bioC* mutant's biotin auxotrophy, indicating that the *Bacillus* BioC is able to utilize FA substrates linked to the *E. coli* ACP. Malonyl-ACP, rather than malonyl-CoA, is by far the preferred methyl accepting substrate for the *Bacillus* BioC. Interestingly, the overexpression of the *Bacillus* BioC in *E. coli* leads to a shutdown of FA production and abolished growth. This is because the high-level expression of BioC converts too much of the available malonyl-ACP to its methyl ester, which is not a substrate for the normal FAS pathway (Lin 2012; Lin and Cronan 2012). In wild-type *B. cereus* and *E. coli*, BioC catalytic activity appears to be very low. This and the regulation of *bioC* transcriptional expression by BirA (Sect. 3.3) in *E. coli* (and possibly *B. cereus*) probably act to allow the enzyme to compete against FabH for malonyl-CoA without overly depleting the malonyl-CoA pool and reducing the synthesis of normal FAs (Lin 2012; Lin et al. 2010; Lin and Cronan 2012; Rodionov et al. 2002). It is reasonable to suspect that BioI in *Bacillus* is controlled in a similar manner to prevent the excess conversion of long-chain acyl-ACPs to pimeloyl-ACP (Stok and De Voss 2000).

The *E. coli* carboxylesterase BioH then removes the methyl group from pimeloyl-ACP methyl ester, allowing it to exit FAS and providing the substrate for the later stages of biotin biosynthesis (Fig. 5). BioH is much more catalytically active than BioC and the enzymes involved in biotin ring synthesis (Sect. 3.2). Possibly, BioH has not yet been completely integrated with the other enzymes of biotin synthesis, as evidenced by its location outside of the biotin gene operon and its lack of regulation by BirA (Sect. 3.3) (Lin 2012).

Some bacteria contain BioC but lack BioH, which is replaced by any one of several nonorthologous esterases (Bi et al. 2016; Feng et al. 2014; Rodionov et al. 2002; Shapiro et al. 2012). A possible reason that this disparate group of esterases can replace BioH in different bacteria is that the ester hydrolysis producing pimeloyl-ACP is a relatively unchallenging enzymatic reaction, in comparison to the chemically complex ring formation reactions catalyzed by the more evolutionary conserved BioFADB enzymes described in the next section (Lin 2012; Shapiro et al. 2012).

As a sole or additional (along with BioI) route for pimelate moiety synthesis, *B. subtilis* and a few other bacterial species produce pimeloyl-CoA synthetase (BioW) (Fig. 4). This enzyme converts pimelic acid, probably obtained from the environment, to pimeloyl-CoA, which is used by BioF to produce 7-keto-8-aminopelargonic acid (KAPA), an early intermediate in the ring closure reactions of biotin biosynthesis (Ploux et al. 1992; Rodionov et al. 2002) (Fig. 4).

3.2 Assembly of the Biotin Ring Structures

The second phase of biotin biosynthesis requires four enzymes to assemble the vitamin's bicyclic rings (Figs. 1 and 5). In the first reaction, KAPA synthase (BioF) decarboxylatively condenses L-alanine and pimeloyl-ACP to form KAPA, resulting in the cleavage of the pimelate thioester bond and release of CO_2. This results in the seven-carbon pimeloyl-ACP (Fig. 1) being extended with two carbons and a nitrogen derived from L-alanine, which constitute the C8, C9, and N8 atoms of biotin (Lin 2012) (Fig. 5).

KAPA is transaminated at C7 to produce 7,8-diaminopelargonic acid (DAPA) in the reaction catalyzed by DAPA synthase (BioA), which is unique among characterized enzymes in using SAM, the common methyl donor in enzyme reactions, as the amino donor. BioA and BioF are both pyridoxal 5′-phosphate (PLP)-dependent enzymes that belong to the same aminotransferase family and probably evolved from a common ancestor. The BioA reaction introduces the N7 amino group required for ureido ring formation. Dethiobiotin (DTB) synthetase (BioD) converts DAPA to DTB by the ATP-dependent insertion of CO_2 between N7 and N8 of DAPA to form the biotin ureido ring (Figs. 1 and 5). The final reaction of biotin synthesis is catalyzed by biotin synthase (BioB), an iron-sulfur enzyme. BioB inserts sulfur, obtained from its own $[2Fe\text{-}2S]^{2+}$ cluster, between the C6 methylene and C9 methyl groups of DTB to form the tetrathiophane ring of biotin (Lin 2012) (Figs. 1 and 5).

3.3 Attachment of Biotin to Biotin-Dependent Enzymes and Regulation of Biotin Biosynthesis

BirA (biotin retention protein A) is a bifunctional protein acting as a transcriptional repressor of most of the biotin genes and a biotin-protein ligase (BPL) that attaches biotin to the BCCP subunit or domain of biotin-dependent enzymes. BirAs are also referred to as class II BPLs. Class I BPLs contain only the domains required for ligating biotin to apo-BCCPs and lack the N-terminal DNA-binding domain present in the class II proteins, so they do not function as transcriptional regulators (Satiaputra et al. 2016). BPLs are ubiquitous in bacteria, although only a small portion of these are of the class II (bifunctional) type (Rodionov et al. 2002).

The ability of BirA to both ligate biotin to apo-BCCPs and regulate transcription are functionally linked and have been extensively studied in *E. coli*. In the first reaction of biotin ligation, biotinyl-5′-AMP is formed from biotin and ATP. This compound, rather than biotin itself, acts as the BirA corepressor of transcription. BirA::biotinyl-5′-AMP is able to dimerize and interact with the biotin operator regions to repress biotin gene transcription. Rapidly growing *E. coli* cells require a relatively high level of ACC activity for membrane FA synthesis. In these cells, biotin is in great demand because apo-BCCP levels are high. BirA::bio-5′-AMP functions as a biotinylating enzyme and not as a transcriptional repressor. In slowly dividing cells, BirA::bio-5′-AMP accumulates since there are few apo-BCCP

domains to biotinylate and thus acts as a transcriptional repressor (Beckett 2007; Cronan 2014; Satiaputra et al. 2016).

Alternative modes of biotin gene regulation occur in some bacteria having class I BPLs. BioR is a GntR family transcriptional regulator that represses the expression of biotin biosynthesis and/or transport genes in *Paracoccus denitrificans*, *Brucella melitensis*, and *Agrobacterium tumefaciens* (Feng et al. 2015; Saitputra et al. 2016). BioQ is a TetR family transcriptional repressor of biotin synthesis and transport in *Corynebacterium glutamicum* and of biotin biosynthesis in *Mycobacterium* species. However, the biotin metabolite ligand with which BioR or BioQ DNA binding is modulated has not been identified (Feng et al. 2015; Satiaputra et al. 2016). However, *Mycobacterium smegmatis* wild-type cultures grown with increasing concentrations of exogenous biotin showed reduced expression of their *bioF*, *bioD*, and *bioB* genes. This biotin-mediated repression does not occur in a *bioQ* deletion strain, suggesting that BioQ is able to directly or indirectly sense biotin. Similarly, the repression of biotin biosynthesis by exogenous biotin has been reported in the *A. tumefaciens* wild type, but does not occur in a *bioR* mutant strain (Satiaputra et al. 2016). A general two-protein "cross-talk" model in which class I BPLs and BioQ or BioR act together to coordinate biotin biosynthesis, apo-BCCP biotinylation, and biotin transport has been proposed, but many details are lacking (Beckett 2007; Satiaputra et al. 2016).

4 Research Needs

Unanswered questions on the reaction mechanisms, regulation, and structural characteristics of several enzymes involved in biotin and LA synthesis have been raised in several reviews (Cronan 2014, 2016; Cronan and Lin 2011; Lin and Cronan 2011; Rock 2009). For example, one long-standing area of doubt centers on whether the BioB is inactivated following each catalysis, since it donates sulfur from its own $[2Fe\text{-}2S]^{2+}$ cluster. Various proposals have been made that might allow reconstitution of the cluster to permit multiple turnovers (Choi-Rhee and Cronan 2005). Because very few molecules of biotin are required for the growth of *E. coli*, catalytic efficiency of BioB and other enzymes of the pathway may not be a physiological necessity (Choi-Rhee and Cronan 2005; Cronan 2014).

BioC acts as a "gatekeeper" that directs the entry of carbon skeletons into the FAS system for biotin biosynthesis. In *E. coli*, BioC's catalytic inefficiency and the transcriptional control by *bioC* by BirA probably act to limit its consumption of malonyl-ACP, which is also required for normal FAS. Are the BioCs in bacteria that lack dual function BPLs subject to a distinct genetic or biochemical regulation? Another area to explore is how biotin biosynthesis and the partitioning of biotin to different enzymes is controlled in bacteria that produce multiple biotin-dependent enzymes, especially those having only class I BPLs (Guillén-Navarro et al. 2005).

During LA synthesis in *E. coli*, *lipB* is not transcriptionally regulated. What controls the enzyme's production or activity to prevent it from withdrawing too

much of the octanoyl-ACP that is needed in further elongation reactions for the synthesis of membrane FAs?

An interesting and unanswered evolutionary conundrum is how did biotin synthesis evolve? Because FAS is required for making biotin, and the biotin-dependent ACC is required for FAS, biotin is required for its own synthesis. One suggestion is that early in evolution, malonyl-CoA was produced by an ancestral enzyme (Cronan and Lin 2011).

Given the diversity of many of the enzymes involved in biotin and LA synthesis in different bacteria, additional bioinformatic analysis of genome sequence data, including that obtained in metagenomic studies, would significantly advance our knowledge of the taxonomic distribution of the known pathways for the synthesis of these cofactors and might uncover completely novel pathways.

References

Beckett D (2007) Biotin sensing: universal influence of biotin status on transcription. Annu Rev Genet 41:443–464

Bi H, Zhu L, Jia J, Cronan JE (2016) A biotin biosynthesis gene restricted to *Helicobacter*. Sci Rep 6:21162. https://doi.org/10.1038/srep21162

Bower S, Perkins JB, Yocum RR, Howitt CL, Rahaim P, Pero J (1996) Cloning, sequencing, and characterization of the *Bacillus subtilis* biotin biosynthetic operon. J Bacteriol 178:4122–4130

Chan DI, Vogel HJ (2010) Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochem J 430:1–19

Choi-Rhee E, Cronan JE (2005) Biotin synthase is catalytic *in vivo*, but catalysis engenders destruction of the protein. Chem Biol 12:461–468

Cronan JE (2014) Biotin and lipoic acid: synthesis, attachment, and regulation. EcoSal Plus. https://doi.org/10.1128/ecosalplus ESP-0001-2012

Cronan JE (2016) Assembly of lipoic acid on its cognate enzymes: an extraordinary and essential biosynthetic pathway. Microbiol Mol Biol Rev 80:429–450

Cronan JE, Lin S (2011) Synthesis of the α, ω-dicarboxylic acid precursor of biotin by the canonical fatty acid biosynthetic pathway. Curr Opinion Chem Biol 15:407–413

Cryle MJ, Schlichting I (2008) Structural insights from a P450 carrier protein complex reveal how specificity is achieved in the $P450_{BioI}$ ACP complex. Proc Natl Acad Sci U S A 105:15696–15701

Dibrova DV, Galperin MY, Mulkidjanian AY (2014) Phylogenomic reconstruction of archeal fatty acid metabolism. Environ Microbiol 16:907–918

Feng Y, Cronan JE (2014) PdhR, the pyruvate dehydrogenase repressor, does not regulate lipoic acid synthesis. Res Microbiol 165:429–438

Feng Y, Napier BA, Manandhar M, Henke SK, Weiss DS, Cronan JE (2014) A *Francisella* virulence factor catalyzes an essential reaction of biotin biosynthesis. Mol Microbiol 91:300–314

Feng Y, Kumar R, Ravcheev DA, Zhang H (2015) *Paracoccus denitrificans* possesses two BioR homologs having a role in the regulation of biotin metabolism. Microbiol Open 4:644–659

Guillén-Navarro K, Encarnación S, Dunn MF (2005) Biotin biosynthesis, transport and utilization in rhizobia. FEMS Microbiol Lett 246:159–165

Janßen HJ, Steinbüchel A (2014) Fatty acid synthesis in *Escherichia coli* and its application towards the production of fatty acid based biofuels. Biotechnol Biofuels 7:7

Jordan SW, Cronan JE (1997) A new metabolic link. The acyl carrier protein of lipid synthesis donates lipoic acid to the pyruvate dehydrogenase complex in *Escherichia coli* and mitochondria. J Biol Chem 272:17903–17906

Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K (2016) KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 45(D1):D353–D361
Lin S (2012) Biotin synthesis in *Escherichia coli*. PhD thesis, University of Illinois at Urbana-Champaign, 140 pp
Lin S, Cronan JE (2011) Closing in on complete pathways of biotin biosynthesis. Mol BioSyst 7:1811–1821
Lin S, Cronan JE (2012) The BioC *O*-methyltransferase catalyzes methyl esterification of malonyl-acyl carrier protein, an essential step in biotin synthesis. J Biol Chem 287:37010–37020
Lin S, Hanson RE, Cronan JE (2010) Biotin synthesis begins by hijacking the fatty acid synthetic pathway. Nat Chem Biol 6:682–688
López-Lara IM, Geiger O (2010) Formation of fatty acids. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 385–393
Martin N, Christensen QH, Mansilla MC, Cronan JE, de Mendoza D (2011) A novel two-gene requirement for the octanoyltransfer reaction of *Bacillus subtilis* lipoic acid biosynthesis. Mol Microbiol 80:335–349
Morris TW, Reed KE, Cronan JE Jr (1995) Lipoic acid metabolism in *Escherichia coli*: the *lplA* and *lipB* genes define redundant pathways for ligation of lipoyl groups to apoprotein. J Bacteriol 177:1–10
Perham RN (2000) Swinging arms and swinging domains in multifunctional enzymes: catalytic machines for multistep reactions. Annu Rev Biochem 69:961–1004
Ploux O, Soularue P, Marquet A, Gloeckler R, Lemoine Y (1992) Investigation of the first step of biotin biosynthesis in *Bacillus sphaericus*. Biochem J 287:685–690
Qui X, Janson CA, Smith WW, Head M, Lonsdale J, Konstantinidis AK (2001) Refined structures of ß-ketoacyl-acyl carrier protein synthase III. J Mol Biol 307:341–356
Rock CO (2009) Opening a new path to lipoic acid. J Bacteriol 191:6782–6784
Rock CO, Jackowski S (2002) Forty years of bacterial fatty acid synthesis. Biochem Biophys Res Commun 292:1155–1166
Rodionov DA, Mironov AA, Gelfand MS (2002) Conservation of the biotin regulon and the BirA regulatory signal in eubacteria and Archaea. Genome Res 12:1507–1516
Satiaputra J, Shearwin KE, Booker GW, Polyak SW (2016) Mechanisms of biotin-regulated gene expression in microbes. Synth Syst Biotechnol 1:17–24
Shapiro MM, Chakravartty V, Cronan JE (2012) Remarkable diversity in the enzymes catalyzing the last step in synthesis of the pimelate moiety of biotin. PLoS One 7(11):e49440. https://doi.org/10.1371/journal.pone.0049440
Sohlenkamp C, Geiger O (2016) Bacterial membrane lipids: diversity in structures and pathways. FEMS Microbiol Rev 40:139–159
Stok JE, De Voss J (2000) Expression, purification, and characterization of BioI: a carbon-carbon bond cleaving cytochrome P450 involved in biotin biosynthesis in *Bacillus subtilis*. Arch Biochem Biophys 384:351–360
Tong L (2013) Structure and function of biotin-dependent carboxylases. Cell Mol Life Sci 70:863–891
Zhang H, Luo Q, Gao H, Feng Y (2015) A new regulatory mechanism for bacterial lipoic acid synthesis. Microbiol Open 4:282–300