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Biosynthesis of Polyketide Synthase Extender Units

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1. Introduction

Polyketides are a large class of structurally diverse natural products exhibiting a vast array of biological and pharmacological activities such as antibacterial, antifungal, anticholesterol, antiparasitic, anticancer, and immunosuppressive properties. While structurally diverse, all polyketides are assembled by successive rounds of decarboxylative Claisen condensations between a thioesterified malonate derivative and an acyl thioester (Fig. 1). The enzymes that catalyze these condensations are referred to as polyketide synthases (PKSs). Because of the mechanistic similarities between fatty acid and polyketide biosynthesis, PKSs are classified using nomenclature for types of fatty acid synthases, with some modifications added as needed. There have been an extensive number of reviews on the enzymes associated with each type of PKS.¹⁻⁵ Here we briefly summarize each type to set the stage for a more detailed discussion of the different types of PKS extender units, their biosynthesis, and their occurrence in different types of PKSs with an emphasis on bacterial systems.

Type I PKSs can generally be divided into two groups, modular and iterative. The modular system is the classic bacterial type I PKS best exemplified by the PKS responsible for assembling the 6-deoxyerythronolide B (6-DEB) scaffold of erythromycin A (Fig. 2).⁶ This PKS assembles seven precursors consisting of one propionyl-CoA starter unit and six (2*S*)-methylmalonyl-Coenzyme A (CoA) extender units into 6-DEB. The role each of these types of precursors plays in polyketide assembly is readily apparent from the unit names: the starter unit is the initiating precursor for polyketide synthesis, while the extender units elongate the polyketide backbone to completion. A set of catalytic domains, grouped together as a “module,” controls the incorporation of each precursor into the polyketide backbone. For modular type I PKSs, the number of modules is equivalent to the number of precursors incorporated into the polyketide. While the modules that incorporate the starter unit can have variable catalytic domains, the modules that incorporate extender units typically consist of three core domains for polyketide extension and up to three auxiliary domains involved in β -keto processing. The core domains are the ketosynthase (KS), acyltransferase (AT), and acyl carrier protein (ACP). The AT domain is the “gate keeper” of the module and recognizes the specific extender unit to be incorporated into the growing polyketide chain and covalently tethers the malonyl derivative of the extender unit onto the sulfhydryl group of the ACP prosthetic group. This prosthetic group is the 4'-phosphopantetheinyl (4'-Ppant) moiety of CoA. The KS domain catalyzes the decarboxylative Claisen condensation between a neighboring ACP-linked malonate derivative and an ACP-linked acyl thioester to extend the polyketide chain. The remaining optional domains (ketoreductase [KR], dehydratase [DH], and enoylreductase [ER]) alter the oxidation state of the β -keto group formed after the KS-catalyzed condensation.

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This review covers the biosynthesis of extender units that are utilized for the assembly of polyketides by polyketide synthases. The metabolic origins of each of the currently known polyketide synthase extender units are covered.

Because its structure is co-linear with the module and domain architecture, the assembly of 6-DEB from one starter unit and six extender units is readily apparent from an analysis of the erythromycin PKS (Fig. 2).

Iterative type I PKSs utilize the same core catalytic domains as modular type I PKSs, but these domains occur on a single polypeptide that is used repetitively to generate the entire polyketide backbone. An example of this type of system is the lovastatin PKS, in which an iteratively acting multidomain polypeptide condenses one starter unit with eight extender units and S-adenosylmethionine (SAM) to generate the dihydromonacolin L intermediate of lovastatin (Fig. 3).^{7, 8} While initially thought to be limited to fungal systems, iterative type I PKSs have now been found in many bacteria as well. Additionally, single modules within modular PKSs have been found to function iteratively to incorporate multiple extender units per polyketide synthesized. Thus, the definition of iterative type I PKSs is expanding, as discussed recently in reviews by Shen,² Müller,⁹ and Wilkinson.¹⁰

Type II PKSs contain similar core catalytic domains seen in type I PKSs, with the exception that there are typically two KS domains, KS_α and KS_β. The former is equivalent to the KS seen in type I PKSs, while the latter controls polyketide length, and the enzymatic activities are typically present on individual proteins. No reductive processing of the β-keto groups occurs until after the polyketide is fully synthesized. Classic examples of type II PKS systems are the two “minimal” PKSs involved in generating the anticancer drugs daunorubicin and doxorubicin (Fig. 4).^{11, 12}

Like type I and type II PKSs, type III PKSs condense a starter unit with a series of extender units to generate a poly-β-keto chain. Type III PKSs differ from the above types in that they typically lack multiple catalytic domains and utilize an ACP-independent mechanism. Instead, a single enzyme harnesses acyl-CoA thioesters and catalyzes acyl group transfers between CoA and an active site cysteine. For example, the type III PKS DpgA generates the hydrated precursor of the dihydroxyphenylacetyl thioester during 3,5-dihydroxyphenylglycine biosynthesis for vancomycin production (Fig. 5).¹³

The enormous structural diversity seen in the polyketide backbones of natural products comes predominantly from the diversity of starter and extender units, variations in the number of extender units incorporated, alterations in the oxidation state of the β-keto groups, and cyclization versus hydrolysis for chain termination. A review by Moore and Hertweck provides an excellent summary of the extensive diversity of starter units utilized by type I and type II PKSs.¹⁴ There have also been thorough reviews on the alterations of the oxidation state of the β-keto groups and termination reactions involving cyclization or hydrolysis by thioesterases.^{3, 15} The focus of this review is to provide an update on the biosynthetic pathways that produce the extender units utilized by PKSs, with an emphasis on those extender units found in bacterial systems. This is an appropriate time to review these components of polyketide biosynthesis due to the recent identification of a number of new extender units and the identification of a new metabolic pathway for acetate utilization, the ethylmalonyl-CoA pathway,¹⁶ which has implications on the metabolic origins of (2S)-methylmalonyl-CoA and (2S)-ethylmalonyl-CoA extender units. This review is divided into two sections based on the two types of extender units that have been identified. The first section focuses on the biosynthesis of the CoA-linked extender units: malonyl-CoA, (2S)-methylmalonyl-CoA, (2S)-ethylmalonyl-CoA, and chloroethylmalonyl-CoA. The second section focuses on the ACP-linked extender units: (2R)-methoxymalonyl-ACP, (2R)-hydroxymalonyl-ACP, and (2S)-aminomalonyl-ACP (Fig. 6). This second section will also include a brief discussion of the role glyceryl-ACP plays in tetronate ring formation for some polyketides due to the similarities between its biosynthesis and that of (2R)-methoxymalonyl-ACP and (2R)-hydroxymalonyl-ACP.

2 Coenzyme A-Linked Extender Units

CoA is essential in both primary and secondary metabolism as an acyl carrier. In polyketide biosynthesis, CoA can play two distinct roles as an acyl carrier. One role is to serve as a substrate for the post-translational modification of ACPs. This modification, catalyzed by phosphopantetheinyltransferases, is the addition of the 4'-Ppant moiety of CoA onto a conserved seryl residue of the ACP to generate a holo-ACP (Fig. 6), the active form of the ACP that functions during polyketide assembly. A second role for CoA in polyketide biosynthesis is as a carrier of carboxylic acid precursors, formally referred to as CoA-linked PKS extender units (Fig. 6). As stated above, the focus of this review is on the biosynthesis of PKS extender units with an emphasis on those observed in bacterial systems. This section focuses on the biosynthesis and occurrence of the four known CoA-linked PKS extender units malonyl-CoA, (2*S*)-methylmalonyl-CoA, (2*S*)-ethylmalonyl-CoA, and chloroethylmalonyl-CoA.

2.1 Malonyl-CoA

The formation of malonyl-CoA is the first committed step in fatty acid biosynthesis for many organisms; thus, malonyl-CoA is a readily available primary metabolite. Based on the evolutionary relationship of fatty acid and polyketide biosynthesis, it is not surprising that malonyl-CoA is a common extender unit used by all types of PKSs. There are two generally accepted pathways for the formation of malonyl-CoA. The more common pathway involves the carboxylation of acetyl-CoA to generate malonyl-CoA by acetyl-CoA carboxylases (ACCs), while the other involves the direct conversion of malonate to malonyl-CoA by malonyl-CoA synthetase.

The ACCs catalyze the carboxylation of acetyl-CoA by a biotin- and ATP-dependent mechanism.¹⁷ These enzymes require three components: 1) biotin carboxylase (BC), 2) biotin carboxyl carrier protein (BCCP), and 3) carboxyltransferase (CT). The BC component catalyzes the ATP-dependent activation of bicarbonate and subsequent carboxylation of the N1 atom of the biotin prosthetic group of BCCP (Fig. 7A). Once the carboxyl-BCCP intermediate is formed, the CT activates the methyl group of acetyl-CoA for nucleophilic attack of the activated carboxyl group tethered to BCCP, resulting in the transfer of the carboxyl group onto acetyl-CoA, forming malonyl-CoA (Fig. 7B).

While the mechanism of malonyl-CoA biosynthesis by an ACC is conserved throughout the three domains of life, four different forms of the enzyme have been identified. In *Escherichia coli*, as well as many other bacteria, the three components of ACC are found on four separate proteins.¹⁸ The BC and BCCP components are contained on individual polypeptides, AccB and AccC, respectively. The BC forms a homodimer in solution when isolated, while the BCCP is a tetramer.¹⁹ The CT component consists of α and β subunits, AccA and AccD in *E. coli*, which form an $\alpha_2\beta_2$ complex. In contrast to the enzyme architecture seen in *E. coli*, *Streptomyces coelicolor*^{20, 21} and other closely related bacteria such as *Mycobacterium tuberculosis*²² and *Saccharopolyspora erythraea*²³ have BC and BCCP components contained on a single polypeptide (α subunit, AccA2), and the α and β subunits of the CT component are also a single polypeptide (β subunit, AccB). The AccA2 and AccB polypeptides are likely to form higher oligomeric states with an $\alpha_6\beta_6$ quaternary structure based on the structure of the homolog propionyl-CoA carboxylase.²⁴ Khosla, Gramajo, and colleagues have also identified a third subunit (ϵ subunit, AccE) in *S. coelicolor* that forms a complex with the β subunit and enhances the ACC activity.²⁵ Although the precise mechanism for how the ϵ subunit enhances ACC activity has yet to be elucidated, the prevailing model is that the ϵ subunit facilitates the formation of the quaternary structure of ACC.²⁰

The importance of ACC to the viability of *S. coelicolor* is exemplified by the inability to genetically inactivate the gene coding for AccA2 and the finding that an *accB* mutant strain could only be constructed when long-chain fatty acids were added to the growth medium.²⁰ Importantly, while the addition of long-chain fatty acids to the growth medium enabled the *accB* mutant to grow, it did not restore the ability of the mutant strain to biosynthesize actinorhodin or the prodiginines. The production of these natural products requires the incorporation of malonyl-CoA extender units by PKS enzymology;^{26, 27} thus, while feeding long-chain fatty acids enables fatty acid biosynthesis for growth, the absence of a source of malonyl-CoA prohibits the biosynthesis of some polyketide natural products. Conversely, the overexpression of *accA2*, *accB*, and *accE* in *S. coelicolor* results in a significant increase in actinorhodin production.²⁸ These data strongly suggest that ACC plays a direct role in producing the malonyl-CoA extender units needed for the production of actinorhodin, prodiginines, and likely other polyketides in *S. coelicolor*; however, its most fundamental role is the production of malonyl-CoA extender units for fatty acid biosynthesis.

In contrast to the component organization seen with ACCs from bacteria, a third type of ACC is observed in animals, plants, and yeast.¹⁷ These ACCs are single polypeptides divided into three regions. The N-terminal region contains the BC and BCCP components of the ACC, while the C-terminal region contains the α and β portions of the CT component. A central region covering approximately a third of the protein is located between the BC-BCCP and CT components and is only seen in the single polypeptide versions of ACC. The role this region plays in malonyl-CoA formation is currently unknown. Finally, the fourth form of ACC is from the archaeon *Metallosphaera sedula* appears to be a fusion of the two types of bacterial ACCs. The BC and BCCP components are contained on separate proteins while the α and β portions of the CT component are fused as a single protein.²⁹

The second mechanism for malonyl-CoA formation is the direct condensation of malonate and CoA by a malonyl-CoA synthetase (Fig. 7C). While ACC appears to be the dominant enzyme for malonyl-CoA formation during fatty acid and polyketide biosynthesis, malonyl-CoA synthetase appears to be involved in the catabolism of malonate in select bacteria³⁰. Malonate is generated during the degradation of pyrimidines, and it has been proposed that this malonate is converted to malonyl-CoA and subsequently decarboxylated to generate either acetyl-CoA and CO₂ or acetate and CO₂.^{31, 32} These reactions are likely to be biologically relevant for certain plant-associated bacteria, based on the finding that leguminous plants produce malonate in high levels when they are associated with nitrogen-fixing symbionts, potentially providing a carbon source for the bacteria.³³ Consistent with this hypothesis, An and Kim³¹ identified a gene cluster in *Rhizobium trifolii* that codes for a dicarboxylate transporter, a malonyl-CoA synthetase, and a malonyl-CoA decarboxylase. These enzymes are proposed to coordinate the transport of malonate into the cell, convert it to malonyl-CoA, and decarboxylate the malonyl-CoA to generate acetyl-CoA and CO₂.

While it is not clear whether the malonyl-CoA synthetase makes significant contributions to the malonyl-CoA pool used for polyketide biosynthesis, the malonyl-CoA synthetase is of interest for metabolic engineering purposes. The natural substrate for the enzyme is malonate, but Khosla and colleagues have determined that the enzyme from *R. trifolii* also recognizes methylmalonate, thereby generating methylmalonyl-CoA.^{34,35} This provides a simple mechanism for enabling a strain to produce methylmalonyl-CoA from exogenously added methylmalonate. The importance of methylmalonyl-CoA as a PKS extender unit is discussed in the next section.

As stated above, malonyl-CoA is used as an extender unit by all types of PKSs. In all cases, the incorporation of malonyl-CoA into the growing polyketide proceeds via a decarboxylative Claisen condensation between the carboxyl group of a thioester and the enolate ion generated

by the decarboxylation of malonyl-CoA. This results in the addition of a two-carbon unit into the polyketide backbone. This can be easily detected by including ^{13}C - or ^{14}C -labeled acetate in the growth medium and analyzing the ^{13}C - or ^{14}C -enrichment pattern. For example, the modular type I PKS that generates the immunosuppressant rapamycin incorporates 14 extender units into the macrocyclic polyketide. Inclusion of [$^{13}\text{C}_2$]acetate in the growth medium resulted in the ^{13}C enrichment of C8-C9, C12-C13, C14-C15, C18-C19, C20-C21, C26-C27, and C32-C33, consistent with the incorporation of seven malonyl-CoA extender units into the rapamycin polyketide core (Fig. 8).³⁶ Analysis of the rapamycin biosynthetic gene cluster led to the identification of seven AT domains within the type I PKS showing signature sequences for malonyl-CoA recognition.³⁷ The positions of these malonyl-CoA-dependent AT domains within the PKS are consistent with the location of the acetate units in the rapamycin polyketide backbone identified through the precursor labeling studies.

Iterative type I PKSs have also harnessed malonyl-CoA extender units for the assembly of polyketides. For example, labeling studies on the esperamicins, produced by the bacterium *Actinomadura verrucosospora*, revealed that all 14 carbons of the enediyne ring portion of the natural product are derived from seven malonyl-CoA extender units.³⁸ When the biosynthetic gene clusters of the structurally related enediynes calicheamicin³⁹ and C-1027⁴⁰ were identified, the PKS involved in the assembly of the 14-membered ring was found to be an iterative type I PKS contained on a single polypeptide with six distinct domains (Fig. 9). Based on the structural similarities of esperamicin A1 with calicheamicin and C-1027, it has been proposed that the biosynthesis of all of these molecules use malonyl-CoA extender units for the carbons found in the enediyne portion of these natural products.³⁹⁻⁴¹

In addition to the modular and iterative type I PKSs, type II and type III PKSs also use malonyl-CoA extender units for assembling the polyketide backbones. The biosynthesis of the anticancer drugs doxorubicin and daunorubicin are assembled by type II PKSs that condense a propionyl-CoA starter unit with nine malonyl-CoA extender units to generate the polyketide backbone of these natural products as discussed previously (Fig. 4). Additionally, the assembly of the nonproteinogenic amino acid 3,5-dihydroxyphenylglycine used in the biosynthesis of the vancomycin-family of antibiotics involves a type III PKS as discussed above (Fig. 5).

2.2 (2S)-Methylmalonyl-CoA

(2S)-Methylmalonyl-CoA is the second most commonly used extender unit for polyketide biosynthesis. As with malonyl-CoA, (2S)-methylmalonyl-CoA incorporation into polyketides is not surprising based on the widespread use of this primary metabolite as an extender unit in fatty acid biosynthesis. For example, *Mycobacterium tuberculosis* uses (2S)-methylmalonyl-CoA for the biosynthesis of mycolic acids and complex glycolipids for its unusual cell membrane.⁴² While (2S)-methylmalonyl-CoA is a common primary metabolite, its use as a PKS extender unit is almost exclusively limited to iterative and modular type I PKSs in bacteria. One exemption is the type III PKS from the pine *Pinus strobus*, PstrCHS2, which has been observed to use a single (2S)-methylmalonyl-CoA extender unit to generate a C-methylated chalcone.⁴³ The incorporation of (2S)-methylmalonyl-CoA extends the polyketide backbone by two carbons, with one carbon bearing a methyl branch, thereby generating a propionyl moiety in the polyketide. While malonyl-CoA incorporated into a polyketide backbone comes predominantly, if not exclusively, from the carboxylation of acetyl-CoA by ACC, (2S)-methylmalonyl-CoA can be generated by a number of mechanisms depending on the growth conditions and the metabolic capability of the producing organism. (2S)-methylmalonyl-CoA utilized for polyketide biosynthesis can arise from any of the following processes: 1) the carboxylation of propionyl-CoA, 2) the rearrangement and epimerization of succinyl-CoA, 3) the catabolism of valine, or 4) the multistep conversion of acetoacetyl-CoA to (2S)-methylmalonyl-CoA through a crotonyl-CoA-dependent pathway (Fig. 10). Integrated into

some of these pathways is the potential incorporation of intermediates from the β -oxidation pathway of fatty acid catabolism. While these are not the only mechanisms by which (2*S*)-methylmalonyl-CoA can be biosynthesized, the pathways listed above have been linked to the production of polyketide natural products through extensive labeling studies and will be the only pathways discussed here. The metabolic capabilities of the producing organism as well as the carbon source the organism is growing on determine the pathway(s) used for the production of (2*S*)-methylmalonyl-CoA for polyketide biosynthesis. This variability has made it difficult to fully understand the metabolic origin of the (2*S*)-methylmalonyl-CoA in some polyketides.

Propionyl-CoA is frequently the metabolic precursor of (2*S*)-methylmalonyl-CoA, and it is formed as a product of the β -oxidation of odd- and branched-chain fatty acids; the catabolism of amino acids isoleucine, methionine, and valine; or the breakdown of cholesterol.^{44, 45} Propionyl-CoA can also be formed by the incorporation of propionate from the environment followed by thioesterification with CoA by an acyl-CoA ligase.⁴⁶ Once formed, propionyl-CoA enters into cellular metabolism through a variety of pathways, one of which is the carboxylation of propionyl-CoA to (2*S*)-methylmalonyl-CoA. The predominant enzyme that catalyzes this carboxylation is mechanistically similar to the ACC involved in malonyl-CoA formation discussed in the previous section. The propionyl-CoA carboxylase (PCC) is an ATP- and biotin-dependent enzyme complex that catalyzes the activation of carbonate and the decarboxylative condensation between the activated carbonyl moiety and the C2 methyl group of propionyl-CoA. The mechanistic similarity of PCC with ACC is best exemplified by the finding that the α subunit of PCC is the same protein as the α subunit of ACC in *S. coelicolor*.²⁴ The β subunits of ACC and PCC are distinct and control which acyl-CoA substrate is carboxylated. A second mechanism for propionyl-CoA carboxylation involves methylmalonyl-CoA transcarboxylase.⁴⁷ This enzyme catalyzes the carboxylation of propionyl-CoA using oxaloacetate as the carboxyl donor to generate (2*S*)-methylmalonyl-CoA and pyruvate (Fig. 10).

While growth conditions can alter whether propionyl-CoA carboxylation is essential for (2*S*)-methylmalonyl-CoA formation in the natural producer of a specific polyketide, the PCC enzymes have been exploited in heterologous systems to make this extender unit available in organisms that naturally lack this metabolic capability. For example, the genes coding the components of the *S. coelicolor* PCC, along with a propionyl-CoA synthetase, have been introduced into *E. coli* to enable this bacterium to synthesize (2*S*)-methylmalonyl-CoA for the heterologous production of erythromycin.⁴⁸ These same genes have also been introduced into *Saccharomyces cerevisiae* to enable this model eukaryote to assemble a (2*S*)-methylmalonyl-CoA-dependent triketide.⁴⁹ These heterologous systems benefit from the ability to introduce propionate into the culture medium for incorporation into the polyketide, thus circumventing the need to divert important precursors away from primary metabolism for production of the non-native polyketide pathway.

Another precursor to the (2*S*)-methylmalonyl-CoA extender unit is the primary metabolite succinyl-CoA. One of the most common sources of succinyl-CoA is the tricarboxylic acid cycle (TCA) cycle where succinyl-CoA is an intermediate that can be diverted away for a variety of metabolic purposes including the generation of (2*S*)-methylmalonyl-CoA. The conversion of succinyl-CoA to (2*S*)-methylmalonyl-CoA is a two-step process (Fig. 10). First, methylmalonyl-CoA mutase rearranges succinyl-CoA to (2*R*)-methylmalonyl-CoA in a coenzyme B₁₂-dependent reaction. (2*R*)-methylmalonyl-CoA has the incorrect stereochemistry for incorporation into a polyketide, but this problem is corrected by a methylmalonyl-CoA racemase that converts (2*R*)-methylmalonyl-CoA to (2*S*)-methylmalonyl-CoA. The importance of succinyl-CoA conversion to (2*S*)-methylmalonyl-CoA in some organisms is best exemplified by recent work by Leadlay and colleagues which

found the gene coding for the methylmalonyl-CoA mutase and genes involved in the biosynthesis of coenzyme B₁₂ are immediately adjacent to the putative elaiophyllin biosynthetic gene cluster in *Streptomyces* sp. DSM4137.⁵⁰ Elaiophyllin biosynthesis requires six (2*S*)-methylmalonyl-CoA extender units (Fig. 11).

Determining whether producing organisms use propionyl-CoA or succinyl-CoA as the precursor to (2*S*)-methylmalonyl-CoA is complicated by the interconnection of these pathways. One pathway for an organism to catabolize propionate is to convert propionate to succinyl-CoA. This pathway involves the carboxylation of propionyl-CoA by PCC and racemization and rearrangement via methylmalonyl-CoA intermediates to succinyl-CoA for entry into the TCA cycle. Thus, in many cases the producing organism can adjust the metabolic source of (2*S*)-methylmalonyl-CoA according to the growth conditions. For example, extensive labeling studies of the source of (2*S*)-methylmalonyl-CoA for erythromycin biosynthesis found that either propionate or succinate was incorporated into the macrolide.^{51, 52} Complicating this was the finding that when the gene coding for either the β subunit of PCC⁵³ or the methylmalonyl-CoA mutase⁵⁴ was independently inactivated, erythromycin was still produced by *Saccharopolyspora erythraea*. In fact, when a strain of *S. erythraea* that was lacking a functional methylmalonyl-CoA mutase was grown in a carbohydrate-based medium, erythromycin production was increased by 126% relative to the wild-type strain grown under identical conditions.⁵⁴ In contrast, when the same mutant was grown in oil-based medium, the amount of erythromycin produced was decreased by 66% relative to the wild-type strain.⁵⁴ These data highlight the variability in the metabolic origin of (2*S*)-methylmalonyl-CoA, especially when the growth medium is varied. These data also demonstrate that propionyl-CoA and succinyl-CoA are not the only sources of precursors for (2*S*)-methylmalonyl-CoA in *S. erythraea*, an observation that is true in other polyketide-producing organisms as well.

The identification of additional sources of (2*S*)-methylmalonyl-CoA has come from the extensive studies of polyether and macrolide biosynthesis. One of these sources is the catabolism of the amino acid L-valine.⁵⁵⁻⁶¹ The initial model for the catabolism of L-valine to (2*S*)-methylmalonyl-CoA was based on the known pathway in for L-valine degradation to isobutyryl-CoA, which is subsequently converted to propionyl-CoA via a (2*S*)- β -hydroxyisobutyryl-CoA intermediate. Propionyl-CoA is subsequently carboxylated to (2*S*)-methylmalonyl-CoA by PCC (Fig. 10, Path A). Labeling studies alone or in combination with mutant analysis have shown this is not the metabolic route utilized for polyether or macrolide assembly in a variety of actinomycetes. For example, labeling studies on monensin A and B, lasalocid A, and tylosin⁵⁹⁻⁶¹ (Fig. 11) using [1-¹³C]isobutyrate resulted in ¹³C enrichment of the C1 of each propionate moiety in the polyketides. If the pathway to (2*S*)-methylmalonyl-CoA proceeded through propionyl-CoA as initially hypothesized, the label would have been lost as CO₂. Furthermore, the addition of [3,3'-¹³C₂]isobutyrate to the culture medium of *Streptomyces cinnamonensis* resulted in the ¹³C enrichment of the C27, C29, C30, C31, C34, C35 and C36 carbons of propionyl units of monensin A.⁶⁰ These data are inconsistent with the initially proposed pathway, which predicted the ¹³C enrichment of C1, C3, C5, C11, C17, C21, and C23 of the propionyl units (Fig. 11). These data led to the proposal that the isobutyryl-CoA formed from L-valine is converted to (2*S*)-methylmalonyl-CoA via a methylmalonyl-CoA semialdehyde intermediate (Fig. 10, Path B).⁶²

An additional source of (2*S*)-methylmalonyl-CoA has been identified in the monensin A and B-producing bacterium *S. cinnamonensis*. Reynolds and colleagues have performed an extensive analysis of the metabolic origin of the carbons incorporated into monensin A and B when the producing bacterium is grown on distinct carbon sources, providing one of the most thorough analyses of precursor flux into a polyketide.⁶³⁻⁶⁹ In these studies, they determined that crotonyl-CoA carboxylase/reductase (CCR) is essential for providing the majority of the (2*S*)-methylmalonyl-CoA extender units for monensin A and B production when *S.*

cinnamomensis is grown on an oil-based medium.⁶⁷ CCR is involved in the incorporation of acetyl-CoA generated from the degradation of the fatty acids found in the oil-based medium. In this pathway, two acetyl-CoA units are condensed into acetoacetyl-CoA, followed by the two-step conversion to crotonyl-CoA. Models for how crotonyl-CoA is converted to (2*S*)-methylmalonyl-CoA were based on the results from the initial *in vitro* characterization of CCR.⁷⁰ This work found that CCR catalyzed the reduction of crotonyl-CoA to butyryl-CoA. With butyryl-CoA as an intermediate, the generation of (2*S*)-methylmalonyl-CoA was proposed to proceed to isobutyryl-CoA, which can enter the same pathway as discussed above for L-valine-derived (2*S*)-methylmalonyl-CoA (Fig. 10).

Recent data by Alber and colleagues suggest this model needs to be reevaluated.¹⁶ Alber and colleagues were investigating an alternative to the glyoxylate cycle for acetyl-CoA assimilation by phototrophic bacteria. They detected a carboxylation reaction (using [¹⁴C]carbonate) in extracts of *Rhodobacter sphaeroides* that were incubated with acetoacetyl-CoA. This carboxylation reaction required the addition of NADPH, and when the carboxylated molecule was purified, it was identified as ethylmalonyl-CoA. Importantly, the carboxylation reaction did not require the addition of ATP, eliminating either ACC or PCC as the possible source of the carboxylation activity. The enzyme catalyzing this activity was determined to be CCR, the enzyme that was previously thought to function solely as a crotonyl-CoA reductase. Alber and colleagues showed that CCR can catalyze the conversion of crotonyl-CoA to butyryl-CoA, but it is more efficient at catalyzing the conversion of crotonyl-CoA to ethylmalonyl-CoA. Thus, CCR is a crotonyl-CoA carboxylase/reductase, not solely a crotonyl-CoA reductase. Once ethylmalonyl-CoA is formed, the pathway would involve the rearrangement to form methylsuccinyl-CoA, which is reduced to mesaconyl-CoA, followed by its hydration to β -methylmalyl-CoA. Finally, a β -methylmalyl-CoA/L-malyl-CoA lyase would generate glyoxylate and propionyl-CoA, with the carboxylation of propionyl-CoA by PCC resulting in the generation of (2*S*)-methylmalonyl-CoA (Fig. 10). Importantly, Alber and colleagues also analyzed *S. coelicolor* for CCR activity and showed this organism carries an enzyme with the same enzymatic properties as that seen for the CCR from *R. sphaeroides*. Therefore, the pathway is likely to function in a similar manner in both *R. sphaeroides* and *S. coelicolor*.

In further support of this proposed pathway, Alber and colleagues also identified a potential function for MeaA in (2*S*)-methylmalonyl-CoA formation. Reynolds and colleagues originally identified MeaA as a putative coenzyme-B₁₂-dependent mutase based on its sequence similarity to methylmalonyl-CoA mutase and isobutyryl-CoA mutase.⁶⁶ The potential role of MeaA in (2*S*)-methylmalonyl-CoA formation came from the finding that overexpression of *meaA* in *S. cinnamomensis* resulted in a significant increase in monensin B production over that of monensin A. Additionally, inactivation of *meaA* resulted in higher production levels of monensin A compared to monensin B. Monensin A and B differ in whether an ethylmalonyl-CoA or (2*S*)-methylmalonyl-CoA extender unit is used for C-15 and C-16 (Fig. 11). Clearly, MeaA is not the only source of (2*S*)-methylmalonyl-CoA, but it does influence the cellular pool of the precursor that is available for polyketide biosynthesis. At the time these studies were done, the identity of the MeaA substrate was unknown. However, it was noted that *meaA* was located immediately downstream of the gene coding for CCR in *S. collinus*.⁷¹ Alber and colleagues report that MeaA catalyzes the rearrangement of ethylmalonyl-CoA to methylsuccinyl-CoA. This is the metabolic step immediately following the formation of ethylmalonyl-CoA by the CCR (Fig. 10). Thus, CCR and MeaA (ethylmalonyl-CoA mutase) are components of the same pathway that has now been called the ethylmalonyl-CoA pathway.¹⁶ This proposal is consistent with the genetic data discussed above. Inactivating *meaA* will result in an increase in (2*S*)-ethylmalonyl-CoA concentrations with the expected increase in monensin A production over monensin B. Overexpression of *meaA* would likely result in a depletion of (2*S*)-ethylmalonyl-CoA pools since the increased levels of MeaA will

convert the available (2*S*)-ethylmalonyl-CoA to methylsuccinyl-CoA and then subsequently to (2*S*)-methylmalonyl-CoA. Thus, the ratio of monensin A and B change accordingly.

While there are a variety of potential sources of (2*S*)-methylmalonyl-CoA, labeling studies have focused on the use of [¹³C]propionate to investigate whether the extender unit incorporated into the polyketide backbone is (2*S*)-methylmalonyl-CoA. The use of [¹³C]propionate depends on the presence of PCC or alternative carboxylases that are capable of catalyzing propionyl-CoA carboxylation to (2*S*)-methylmalonyl-CoA. For example, investigations into the precursors used for rapamycin biosynthesis identified seven sites for (2*S*)-methylmalonyl-CoA incorporation based on the intact incorporation of [¹³C]propionate into these sites. Consistent with this, the modular type I PKS contains seven modules specific for (2*S*)-methylmalonyl-CoA (Fig. 8).⁷² Similarly, labeling studies on erythromycin using [¹³C]propionate established that one propionyl-CoA starter unit and six (2*S*)-methylmalonyl-CoA extender units are used by the modular PKS to assemble the macrolide polyketide core (Fig. 2).⁷³

The use of (2*S*)-methylmalonyl-CoA extender units is also found in iterative type I PKS systems as well. For example, labeling studies on aureothin biosynthesis found that the assembly requires one nitrobenzoate starter unit along with one malonyl-CoA and four (2*S*)-methylmalonyl-CoA extender units.⁷⁴ The evidence for the (2*S*)-methylmalonyl-CoA extender units was based on the observation that the intact [¹³C]propionate was incorporated into the corresponding sites. Interestingly, sequencing of the aureothin biosynthetic gene cluster suggested the complete type I PKS was “missing” one module.⁷⁴ Further investigation into the first module of the PKS determined this module works iteratively to incorporate two (2*S*)-methylmalonyl-CoA extender units. Thus, aureothin is assembled by a combination of an iterative type I PKS module, and three modular type I PKS modules to complete the synthesis of the polyketide backbone of aureothin (Fig. 12).

2.3 (2*S*)-Ethylmalonyl-CoA

A number of polyketides synthesized by type I PKSs, including monensin A,⁷⁵ elaiophyllin,⁷⁶ concanamycin A,⁷⁷ tylosin,⁷³ leptomycin B,⁷⁸ spiramycin,⁷⁹ and indanomycin⁸⁰ contain a butyryl group within the polyketide core (Fig. 11). More recently, such a moiety was also found to be a component of the germicidin polyketides synthesized by a type III PKS (Fig. 13).⁸¹ The butyryl moiety in these, and many other polyketides, can be attributed to the use of the extender unit (2*S*)-ethylmalonyl-CoA based on labeling studies and biosynthetic gene cluster analyses. The direct source of (2*S*)-ethylmalonyl-CoA was thought for many years to be solely butyryl-CoA that was carboxylated to form (2*S*)-ethylmalonyl-CoA. The source of this butyryl-CoA was believed to be through the catabolism of L-valine, the conversion of acetoacetyl-CoA, or the β -oxidation of even-chain fatty acids. As discussed in the prior section, the determination that crotonyl-CoA is directly converted to (2*S*)-ethylmalonyl-CoA without first going through butyryl-CoA makes a subtle adjustment to one of the proposed pathways leading to (2*S*)-ethylmalonyl-CoA.

One pathway that does proceed through butyryl-CoA has its metabolic origin with L-valine (Fig. 10). As discussed for (2*S*)-methylmalonyl-CoA formation, L-valine is catabolized to isobutyryl-CoA. Instead of proceeding towards (2*S*)-methylmalonyl-CoA as discussed previously, isobutyryl-CoA is converted to butyryl-CoA by a coenzyme-B₁₂-dependent isobutyryl-CoA mutase.⁶² The proposed pathway involves the subsequent carboxylation of butyryl-CoA to (2*S*)-ethylmalonyl-CoA by an as yet to be identified carboxylase. An additional source of butyryl-CoA is likely to be derived from the β -oxidation of even-chain fatty acids. As with the catabolism of L-valine, a carboxylating enzyme is needed for the conversion of butyryl-CoA to (2*S*)-ethylmalonyl-CoA. For both the L-valine and β -oxidation pathways, a possible enzyme for this activity is PCC. This hypothesis comes from the finding that Alber

and colleagues investigated the source of butyryl-CoA carboxylation in *R. sphaeroides* and subsequently isolated PCC.¹⁶ Thus, the carboxylation of butyryl-CoA was a side activity of PCC. This carboxylation is the most likely reason labeled butyrate added to the culture of a polyketide-producing bacterium results in the intact incorporation of butyrate into the butyryl moieties of the associated polyketides. It is also reasonable to hypothesize that some organisms will code for a butyryl-CoA carboxylase to enhance the levels of (2*S*)-ethylmalonyl-CoA. One possible example of this occurs in the biosynthesis of the insecticide indanomycin (Fig. 11). Recent work by Kelly and colleagues determined that the indanomycin biosynthetic gene cluster codes for a homolog of the β -subunit of ACC and PCC from *Streptomyces* species.⁸⁰ The β -subunit of these enzymes controls substrate specificity; therefore, it is reasonable to speculate that this may code for the CT portion of a butyryl-CoA carboxylase to increase the availability of (2*S*)-ethylmalonyl-CoA for indanomycin biosynthesis. Further genetic and biochemical studies are needed to test this hypothesis and the alternative hypothesis that this enzyme might be present to increase the levels of (2*S*)-methylmalonyl-CoA by functioning as part of a PCC.

The two other pathways for (2*S*)-ethylmalonyl-CoA biosynthesis have their metabolic origin with acetoacetyl-CoA or the β -oxidation of even-chain fatty acids, with both pathways leading to the formation of crotonyl-CoA. This intermediate is converted directly to (2*S*)-ethylmalonyl-CoA by CCR as discussed for methylmalonyl-CoA (Fig. 10). While the newly identified (2*S*)-ethylmalonyl-CoA pathway has been proposed as an alternative to the glyoxylate cycle for utilization of acetate as a carbon source, not all polyketide-producing organisms have this pathway. For example, no CCR activity or *ccr* gene has been detected in the erythromycin-producing bacterium *S. erythraea*.⁸² Thus, when the erythromycin pathway was engineered to generate 6-desmethyl-6-ethylerythromycin A, a copy of the gene coding for the CCR from *S. collinus* had to be introduced into *S. erythraea* before it would produce the engineered macrolide.⁸² In contrast, the introduction of the leptomycin gene cluster into *S. lividans* did not require the addition of a CCR-coding gene for production of the butyryl-containing natural product leptomycin B (Fig. 11), suggesting *S. lividans* already has the ability to produce (2*S*)-ethylmalonyl-CoA at sufficient levels for polyketide production.⁸³

Finally, sequencing of the biosynthetic gene cluster for the immunosuppressant FK520 produced by *Streptomyces hygroscopicus* var. *ascomyceticus* suggested that this organism uses the storage compound polyhydroxybutyrate as the metabolic origin of (2*S*)-ethylmalonyl-CoA.⁸⁴ This hypothesis is based on the FK520 biosynthetic gene cluster coding for homologs of a polyhydroxybutyrate depolymerase and a 3-hydroxyacyl-CoA dehydratase, along with a CCR (Fig. 10). Labeling studies suggest the producing organism also retains the ability to catalyze the carboxylation of butyryl-CoA for (2*S*)-ethylmalonyl-CoA as well.⁸⁵ *S. hygroscopicus* var. *ascomyceticus* is therefore likely to be capable of generating (2*S*)-ethylmalonyl-CoA from acetoacetyl-CoA, β -oxidation pathway intermediates, or polyhydroxybutyrate (Fig. 10).

While there is more than one pathway to (2*S*)-ethylmalonyl-CoA, the CCR-dependent pathways may predominate for polyketide production. This proposal is based on the prevalence of genes coding for CCRs within the biosynthetic gene clusters for butyryl-containing polyketides. For example, the elaiophyllin,⁵⁰ tylosin,⁸⁶ spiramycin,⁸⁷ concanamycin A,⁸⁸ and indanomycin⁸⁰ biosynthetic gene clusters all code for CCR homologs. This may reflect the need to generate excess (2*S*)-ethylmalonyl-CoA from the catabolism of acetoacetyl-CoA; alternatively the organism may be siphoning crotonyl-CoA away from the β -oxidation of fatty acids. Consistent with either of these proposals, the concanamycin A, elaiophyllin, and indanomycin biosynthetic gene clusters code for not only a CCR, but also a 3-hydroxybutyryl-CoA reductase that potentially reduces acetoacetyl-CoA to 3-hydroxybutyryl-CoA. Most likely the dehydratase for the conversion of 3-hydroxybutyryl-CoA to crotonyl-CoA comes from primary metabolism resulting in an increase in metabolic flux through crotonyl-CoA to

provide sufficient quantities of (2*S*)-ethylmalonyl-CoA extender units for optimal polyketide production.

The biosynthetic pathways that utilize (2*S*)-ethylmalonyl-CoA but do not code for a CCR will likely rely on metabolic flux through the ethylmalonyl-CoA pathway for butyrate utilization as proposed by Alber and colleagues.¹⁶ Reynolds and colleagues have also shown that there is only one CCR coded by the *S. cinnamomensis* genome, and the associated gene is adjacent to other genes likely to be associated with the ethylmalonyl-CoA pathway.⁶⁴ This finding suggests that in organisms such as *S. cinnamomensis*, flux through the ethylmalonyl-CoA pathway is sufficient for primary metabolism and the formation of polyketides.

2.4 Chloroethylmalonyl-CoA

The extender units discussed up to this point can be involved in both primary metabolism and polyketide biosynthesis. However, chloroethylmalonyl-CoA discussed here and the ACP-linked extender units discussed below are used solely for the production of polyketides. This assertion is based on the failure to find these extender units as part of any specific primary metabolic pathway. Additionally, the biosynthetic enzymes needed for the production of these extender units are coded within the biosynthetic gene cluster for the specific polyketide that contains the extender unit. The utilization of these extender units is much less common than those discussed above; in fact, in some cases they have been found only in a single biosynthetic pathway. The first of these unusual extender units to be discussed is chloroethylmalonyl-CoA, an extender unit that was discovered while investigating the metabolic potential of marine actinomycetes.

Moore, Fenical and colleagues at the Scripps Institute for Oceanography have been leaders in the analysis of marine actinomycetes for unique natural products. They have isolated over 2500 members of a new actinomycete genus, *Salinospora*, which have proven to be prolific producers of natural products with antibiotic and anticancer activities.⁸⁹ Of particular interest is the production of salinosporamide A that is produced by *S. tropica* CNB-440 (Fig. 14A).⁸⁹ This metabolite was originally isolated because of its potent activity against HCT-116 human colon carcinoma, exerting its cytotoxic activity by inactivating the S20 subunit of the proteasome.^{90, 91}

At the core of salinosporamide A is a fused γ -lactam- β -lactone ring structure that is decorated with a cyclohexanyl carbinol at the C4 ring junction, a chloroethyl substituent at C2, and a methyl group at C3. It is the source of the chloroethyl substituent that is of interest for this review. Initial insights into the metabolic origin of this substituent came from comparisons of the biosynthesis of salinosporamide A and a related molecule produced by *S. tropica* CNB-440, salinosporamide B (Fig. 14A). This related molecule lacks the chlorination on the ethyl substituent on C2, and the initial hypothesis was that salinosporamide B was converted to salinosporamide A based on a recently identified mechanism for halogenations in other natural products.⁹² The biosynthetic mechanism would involve the formation and incorporation of (2*S*)-ethylmalonyl-CoA by a polyketide synthase, followed by the chlorination of the ethyl substituent at some later step in the assembly of salinosporamide A. Surprisingly, it has now been determined that the chloroethyl substituent in salinosporamide A and the analogous ethyl substituent in salinosporamide B have different metabolic origins; thus, salinosporamide B is not an intermediate on the pathway to salinosporamide A. Two groups based this conclusion on a series of feeding and labeling studies. While investigating fermentation conditions to enhance the production of salinosporamide B, Lam and colleagues found that the addition of excess butyric acid to the growth medium enhanced production of salinosporamide B by 319% but reduced production of salinosporamide A by 24% relative to wild-type levels.⁹³ Furthermore, addition of [U-¹³C₄]butyrate to the culture medium resulted in an enrichment

of ^{13}C in the four-carbon unit C1, C2, C12, and C13 of salinosporamide B, but not in the corresponding carbons of salinosporamide A.

At approximately the same time, Beer and Moore performed an extensive set of feeding experiments with stable isotopes to investigate the metabolic origin of each component of salinosporamide A and salinosporamide B.⁹⁴ They also observed that the addition of ^{13}C -labeled butyrate to the culture medium did not result in the enrichment of ^{13}C into the chloroethyl substituent of salinosporamide A, but enrichment was observed in the corresponding carbons of salinosporamide B. Furthermore, feeding of $[1,2\text{-}^{13}\text{C}_2]\text{acetate}$ resulted in the ^{13}C -enrichment of the carbons in the ethyl substituent of salinosporamide B, but not the chloroethyl substituent of salinosporamide A. Thus, formation of the chloroethyl substituent does not involve the formation and incorporation of ethylmalonyl-CoA followed by chlorination. Beer and Moore went on to investigate the enrichment of the C1, C2, C12, and C13 carbons in salinosporamide A using $[\text{U-}^{13}\text{C}_6]\text{glucose}$ and $[1,3\text{-}^{13}\text{C}_2]\text{glycerol}$. From these studies it was hypothesized that the metabolic source of these carbons in salinosporamide A is a sugar. The intact incorporation of the four carbons from a single $[\text{U-}^{13}\text{C}_6]\text{glucose}$ to form the C1, C2, C12, and C13 carbons suggests the involvement of the pentose phosphate pathway in generating the sugar precursor. Based on these data, Beer and Moore proposed the existence of a previously unknown precursor pathway that formed the PKS extender unit chloroethylmalonyl-CoA. This precursor would be incorporated into salinosporamide A, while ethylmalonyl-CoA would replace the chloroethylmalonyl-CoA to generate salinosporamide B.

While these labeling studies were essential in providing insights into how the cell assembles salinosporamide A and salinosporamide B, it was not until the biosynthetic gene cluster associated with the production of these natural products was uncovered that a clear picture appeared for how the chloroethyl substituent is likely to be assembled. Moore and colleagues sequenced the genome of *S. tropica* CNB-440 and discovered a 41-kb gene cluster coding for 29 ORFs they proposed to be involved in the production of salinosporamide A and salinosporamide B.^{95, 96} One of the key findings was that while the cluster does not code for a homolog of the recently characterized oxidative chlorinating enzymes⁹², it does code for a homolog of the fluorinase FIA involved in fluoroacetate production. FIA is an unusual enzyme that catalyzes the conversion of SAM to 5'-fluoro-5'-deoxyadenosine (5'-FDA) (Fig. 14B).⁹⁷ Processing of 5'-FDA eventually leads to the formation of fluoroacetate production.⁹⁸ Moore and colleagues hypothesized that the initial steps in chloroethylmalonyl-CoA formation follow a similar route based on a FIA homolog being coded by the salinosporamide A and salinosporamide B biosynthetic gene cluster. Indeed, Moore, Noel, and colleagues showed that SalL, the FIA homolog, catalyzes the conversion of SAM to 5'-chloro-5'-deoxyadenosine (5'-CDA), analogous to the formation of 5'-FDA (Fig. 14B).⁹⁶ In support of these *in vitro* data, a *S. tropica* CNB-440 strain that is deleted for *salL* continues to produce salinosporamide B but fails to produce salinosporamide A.⁹⁹

The conversion of 5'-CDA to chloroethylmalonyl-CoA remains to be fully elucidated, but it is clear the next step will likely follow that seen for fluoroacetate formation. Briefly, FIB has been shown to catalyze the conversion of 5'-FDA to 5-fluoro-5-deoxy-D-ribose-1-phosphate (5'-FDRP) (Fig. 14B).⁹⁸ A homolog of FIB, SalT, is coded by the biosynthetic gene cluster for salinosporamide A and salinosporamide B. Thus, it was proposed that SalT enzyme will catalyze the conversion of 5'-CDA to 5-chloro-5-deoxy-D-ribose-1-phosphate (5'-FDRP).⁹⁹ Based on this proposal, Moore and colleagues recently used mutasynthesis to show that feeding 5'-FDA or 5'-FDRP to a *salL* mutant results in the formation of fluorosalinosporamide (Fig. 14A), consistent with the mutasynthesis of an unnatural extender unit fluoroethylmalonyl-CoA. These data are consistent with the current hypothesis that chloroethylmalonyl-CoA is derived from SAM and proceeds through both 5'-CDA and 5'-CDRP. On-going studies by the Moore group continue to investigate this fascinating metabolic pathway.

The incorporation of chloroethylmalonyl-CoA into salinosporamide A is controlled by a mixed PKS/NRPS megasynthase with unusual domain architecture. Briefly, *salA* codes for a six-domain megasynthase that is proposed to incorporate acetyl-CoA and chloroethylmalonyl-CoA (or ethylmalonyl-CoA and fluoroethylmalonyl-CoA) (Fig. 14C). Once the diketide is formed, it is likely condensed with the cyclohexenyl alanine moiety tethered to SalB, a didomain nonribosomal peptide synthetase. What makes the SalA domain architecture unusual is that it does not follow the standard KS-AT-ACP module organization commonly seen with modular type I PKSs. Instead, the megasynthase initiates with an ACP domain that is likely acetylated by the AT domain located at the center of the protein adjacent to the AT domain that incorporates chloroethylmalonyl-CoA or ethylmalonyl-CoA. A single KS domain likely condenses the acetyl and chloroethylmalonyl groups and leaves the diketide tethered to the second AT domain. The C-terminal condensation domain of SalA could then transfer the diketide to SalB for further processing to salinosporamide A or B. At this time, the salinosporamide system is the only PKS system known to use chloroethylmalonyl-CoA as an extender unit.

3 Acyl Carrier Protein-Linked Extender Units

The next class of PKS extender units differs from the CoA-linked extender units discussed above in that they are covalently tethered to the 4'-Pant groups of holo-ACPs (Fig. 6). Also, unlike malonyl-CoA, (2*S*)-methylmalonyl-CoA, and (2*S*)-ethylmalonyl-CoA, these precursors are unique to polyketide biosynthesis, with the genes coding for their biosynthesis found within or flanking the associated biosynthetic gene clusters. Although they are not nearly as common as malonyl-CoA or (2*S*)-methylmalonyl-CoA extender units, they are becoming more prevalent as more natural product biosynthetic pathways are elucidated. Currently, they are all found in pathways involving modular type I PKSs, although future natural product discoveries may reveal their presence in other types of PKSs as well.

(2*R*)-Methoxymalonyl-ACP, (2*R*)-hydroxymalonyl-ACP, and (2*S*)-aminomalonyl-ACP elongate a polyketide chain with methoxyacetyl, glycolyl, or glycyl units, respectively, with the substituents at the α -carbons (methoxyl, hydroxyl, and amino groups) conferring functionalities and hydrogen-bonding potential not available through the use of the CoA-linked extender units.^{84, 100} While it has not been described as a PKS extender unit, another ACP-linked PKS precursor worthy of discussion is glyceryl-ACP, which incorporates a glycerol-derived three-carbon unit into a tetronate ring of polyketides known as acyltetronic acids.¹⁰¹ The occurrence and biosynthesis of these four precursors is discussed below.

3.1 (2*R*)-Methoxymalonyl-ACP

The first ACP-linked PKS extender unit to be reported was (2*R*)-methoxymalonyl-ACP, which results in the incorporation of a methoxyacetyl group into a polyketide.⁸⁴ First proposed as an extender unit in the biosynthesis of FK520 by *Streptomyces hygroscopicus*, (2*R*)-methoxymalonyl-ACP has since been proposed as a precursor in a number of other polyketides including ansamitocin P-3,¹⁰² bafilomycin A1,¹⁰³ concanamycin A,⁸⁸ geldanamycin,¹⁰⁴ herbimycin,¹⁰⁵ leucomycin,¹⁰⁶ midecamycin,¹⁰⁷ niddamycin,¹⁰⁸ oxazolomycin,¹⁰⁹ soraphen A,¹¹⁰ and tautomycin,¹¹¹ all of which have α -carbons bearing methoxyl groups (Figs. 11-15). Feeding experiments performed with the FK520-producer and ¹³C-labeled precursors demonstrated that this unusual moiety is derived not from acetate, methoxyacetate, or methoxymalonnate but rather from an intermediate of glycolysis.^{112, 113} Feeding experiments performed with a number of other polyketides containing this unusual moiety are consistent, suggesting the involvement of a "glycolate" extender unit.^{103, 106, 114-119}

To investigate the possibility that this "glycolate" extender unit is derived from a CoA-linked thioester such as hydroxymalonyl-CoA or methoxymalonyl-CoA, Floss and colleagues fed the

ansamitocin P-3 producer ^{13}C -labelled *N*-acetyl-cysteamine (SNAC) thioesters,¹²⁰ which have been shown to be cell-permeable and effective as *in vivo* and *in vitro* mimics of CoA thioesters.^{35, 121, 122} After feeding the producer $[1-^{13}\text{C}](2R)$ -hydroxymalonyl-SNAC and $[1-^{13}\text{C}](2R)$ -methoxymalonyl-SNAC thioesters, they observed no ^{13}C enrichment. Similar experiments performed by Grond and Schuhmann¹⁰³ with the bafilomycin A₁ and concanamycin A producers also resulted in no ^{13}C enrichment. These data suggested that hydroxymalonyl-CoA and methoxymalonyl-CoA are unlikely precursors of the methoxyacetyl moiety.

Significant insight into the identity of this precursor was gained from the sequencing and analysis of the biosynthetic gene cluster for FK520. Reeves and colleagues proposed that a subset of five genes coding the enzymes FkbG, FkbH, FkbI, FkbJ, and FkbK were involved in the formation of the extender unit (2*R*)-methoxymalonyl-ACP.⁸⁴ All of the enzymes except FkbH were homologous to proteins of known function: FkbG was homologous to plant caffeoyl-CoA *O*-methyltransferases; FkbI, acyl-CoA dehydrogenases; FkbJ, ACPs; and FkbK, 3-hydroxyacyl-CoA dehydrogenases. In their proposal, FkbH covalently tethers a glycolytic pathway intermediate to the 4'-Ppant group of holo-FkbJ, forming glyceryl-FkbJ. Next, FkbK oxidizes glyceryl-FkbJ to an aldehyde intermediate, and FkbI further oxidizes this intermediate to (2*R*)-hydroxymalonyl-FkbJ. As a final step, FkbG catalyzes the *O*-methylation of (2*R*)-hydroxymalonyl-FkbJ to form (2*R*)-methoxymalonyl-FkbJ (Fig. 16A).

In support of this proposal, genes coding homologs to these five enzymes have been found in the biosynthetic pathways for a number of other methoxyacetyl-containing natural products, including ansamitocin P-3, concanamycin A1, geldanamycin, herbimycin, oxazolomycin, spiramycin, and tautomycin.^{87, 88, 104, 105, 109, 111} Interestingly, the biosynthetic gene cluster for soraphen A codes for homologs to FkbK and FkbI but lacks a gene coding an FkbH homolog; instead, *sorC* codes a three-domain protein containing an AT, ACP, and methyltransferase (MT) domain (Fig. 16B).¹¹⁰ The AT domain is predicted to covalently tether the glycolytic substrate to the ACP domain of SorC, forming glyceryl-SorC. The predicted dehydrogenases SorD and SorE may oxidize this intermediate to (2*R*)-hydroxymalonyl-SorC, which is then *O*-methylated by the MT domain of SorC. The presence of the MT domain on the same polypeptide as the ACP led Floss and colleagues to propose that glyceryl-SorC is first *O*-methylated to form 2-*O*-methyl-glyceryl-SorC, which is then oxidized to (2*R*)-methoxymalonyl-SorC. By extension, Floss *et al.* argue that *O*-methylation likely precedes the oxidation steps during ansamitocin biosynthesis as well.¹¹⁴ Further work is necessary to establish the order of the oxidation/methylation events, which may differ from pathway to pathway.

Work by Floss and colleagues has provided genetic evidence supporting the proposal that this subset of five genes is involved in (2*R*)-methoxymalonyl-ACP formation. In the ansamitocin biosynthetic pathway, the genes *asm13-17* code homologs to FkbK, J, I, H, and G, respectively.¹²³ A mutation in *asm15* eliminated ansamitocin production, though small amounts of the derivative 10-desmethoxy-ansamitocin were produced as a result of the misincorporation of a malonyl-CoA extender unit in the absence of the “glycolate” extender unit.¹²⁰ Supplementing the *asm15* mutant with (2*R*)-hydroxymalonyl-SNAC or (2*R*)-methoxymalonyl-SNAC thioesters did not restore ansamitocin production, consistent with previous experiments in which labelled SNAC thioesters were not incorporated by the wild-type producer. The lack of incorporation of SNAC thioesters is also consistent with the notion of an ACP-linked versus CoA-linked precursor. Further evidence for the involvement of an ACP came from an *asm14* insertional mutant that was abolished for the production of ansamitocin and any ansamitocin-related compounds; this suggests that the ACP is an essential component for not only the incorporation of the methoxymalonyl unit but also the misincorporation of malonate units. These mutagenesis experiments show that at least *asm14* and *asm15* are necessary for the incorporation of a methoxymalonyl unit. In another set of experiments, a cassette containing

asm13-17 was introduced into a vector co-expressing an *eryAI, II, III* construct coding the 6-DEB PKS with its (2*S*)-methylmalonate-specific AT6 domain replaced with the putative hydroxymalonyl-/methoxymalonyl-ACP-specific AT8 domain from *fkBA*.^{113, 124} Heterologous expression of the modified 6-DEB vector alone resulted in the production of 6-DEB and 2-desmethyl-6-DEB. Co-expression of the *asm13-17* cassette abolished production of these compounds but resulted in the production of the novel compound 2-desmethyl-2-methoxy-6-DEB, in which a (2*R*)-methoxymalonyl extender unit was incorporated.^{113, 124} To probe whether *O*-methylation occurs before or after incorporation of the “glycolate” extender unit, a significant portion of the MT-coding gene *asm17* was deleted. Co-expression of the modified 6-DEB vector with a cassette containing the mutated *asm17* gene resulted in the production of 6-DEB and 2-desmethyl-6-DEB only. The lack of 2-desmethyl-2-hydroxy-6-DEB suggested that *O*-methylation occurs prior to incorporation by the PKS and that the unmethylated precursor is not competent for incorporation by the PKS. Taken together, these data demonstrate that a cassette containing *asm13-17* is sufficient for forming the “glycolate” extender unit, which is likely (2*R*)-methoxymalonyl-ACP.

Additional genetic evidence that this subset of five genes is involved in (2*R*)-methoxymalonyl-ACP biosynthesis came from Katz *et al.* and their work on the heterologous production of midecamycin in *Streptomyces fradiae*.¹⁰⁷ Midecamycin, an antibiotic made by *Streptomyces mycarofaciens*, contains a methoxyacetyl moiety proposed to be derived from (2*R*)-methoxymalonyl-ACP. When the midecamycin PKS genes are expressed in the *S. fradiae* host, no production of midecamycin or midecamycin analogs occurs; however, the integration of *fkBGHIJK* into the *S. fradiae* chromosome confers the host strain the ability to produce a midecamycin analog bearing a methoxyacetyl unit in its polyketide backbone.

These genetic data have been supported by biochemical investigations involving enzymes from the FK520 biosynthetic pathway. In 2003, Tsai and colleagues solved the crystal structure for FkbI and proposed that its structure supports its role as an acyl-ACP versus acyl-CoA dehydrogenase.¹²⁵ This proposal was based upon docking simulations of FkbI with a homology model of FkbJ as well as sequence comparisons of FkbI and its homologs to acyl-CoA dehydrogenases. In this work, FkbG, FkbH, FkbJ, and FkbK, were also heterologously overproduced and purified, although no biochemical characterization of these four enzymes was reported at the time. In more recent work, FkbGHIJK were heterologously co-overproduced and purified in an *E. coli* strain containing *sfp*, which codes for a PPTase that post-translationally modifies apo-ACPs to their active holo forms.¹²⁶ To determine if *E. coli* expressing the *fkBGHIJK* cassette could produce (2*R*)-methoxymalonyl-FkbJ *in vivo*, FkbJ was further purified from the other Fkb enzymes, digested with trypsin, and analyzed by LC/MS. In addition to peaks with masses corresponding to FkbJ + 2H⁺ and FkbJ + H⁺ + Na⁺, a peak with a mass corresponding to (2*R*)-methoxymalonyl-FkbJ + 2H⁺ was observed, and the presence of this peak was dependent upon the co-expression of *fkBGHIK*. Based on a comparison of the peak area ratios, it was estimated that 20% of FkbJ was converted to (2*R*)-methoxymalonyl-FkbJ. Similar experiments were performed in which FkbGHIK were heterologously co-overproduced with GdmJ, the FkbJ homolog from geldanamycin biosynthesis. LC/MS analysis of trypsin-digested purified GdmJ revealed that FkbGHIK were capable of forming (2*R*)-methoxymalonyl-GdmJ *in vivo*. Interestingly, about 60% of GdmJ was converted to (2*R*)-methoxymalonyl-GdmJ by the Fkb enzymes.

While these data provided biochemical evidence of (2*R*)-methoxymalonyl-ACP formation *in vivo*, they did not identify the glycolytic substrate of FkbH. Insights into the identity of this substrate were gained from a sequence analysis of FkbH and its homologs.¹²⁷ The N-terminal domain contains the motif DXDX(T/V) as well as other conserved residues found in phosphatases belonging to the haloacid dehalogenase (HAD) superfamily of hydrolases; the C-terminal domain contains the conserved SCR motif, consistent with acyltransferases. The

AT-like domain was proposed to transfer the glycolytic substrate, an activated carboxylic acid, to the 4'-Ppant group of a holo-ACP. This substrate is likely already activated at its carboxyl group, due to the absence of any obvious carboxyl-activating domains or NTP-binding motifs in the C-terminal domain of FkbH or in any other enzymes associated with the methoxymalonyl-ACP subcluster. Using this metabolic logic, the common glycolytic intermediate 1,3-bisphospho-D-glycerate (1,3-bPG) was proposed to be the substrate for FkbH, with its N-terminal domain responsible for removing the phosphate from the phosphoester group of C3.¹²⁷

In their investigations with OzmB, the FkbH homolog from the oxazolomycin biosynthetic pathway, Shen and colleagues provided biochemical evidence supporting the proposal that 1,3-bPG is in fact the glycolytic precursor to (2R)-methoxymalonyl-ACP¹⁰⁹. Using HPLC-based assays and MS, they showed that a glyceryl moiety becomes tethered to OzmB in the presence of 1,3-bPG. MS/MS analysis revealed that the addition of this moiety is localized to the CRVV amino acid sequence of OzmB and that glycerate is presumably covalently bound as a thioester to cysteine. To determine if OzmB could transfer this glyceryl moiety to an ACP, they incubated glyceryl-OzmB with holo-TtmD (an ACP from tautomycin biosynthesis). HPLC and MS analysis confirmed the formation of glyceryl-TtmD, suggesting that OzmB was competent for acylating a non-cognate ACP. To investigate the timing of the phosphatase activity of OzmB, they mutated the active site aspartate of the N-terminal HAD domain to a valine and performed the same loading experiments with 1,3-bPG. MS analysis of the resulting product yielded a mass that was consistent with 3-phosphoglyceryl-OzmB. These experiments clearly demonstrate that 1,3-bPG is loaded onto OzmB, forming 3-phosphoglyceryl-OzmB, which is then dephosphorylated to form glyceryl-OzmB, an important first step in forming (2R)-methoxymalonyl-ACP.

The biochemical evidence that 1,3-bPG is the metabolic precursor of the (2R)-methoxymalonyl group is supported most recently by a series of *in vivo* labeling experiments by Floss, Müller, and colleagues on ansamitocin and soraphen A biosynthesis.¹¹⁴ The ansamitocin P-3 producer, *Actinosynnema pretiosum*, was fed D-[1,2-¹³C₂]glucose, and the ansamitocin was purified and analyzed by ¹³C-NMR. This analysis determined that there was no C-C coupling between C9-C10 carbons of the methoxyacetyl group, in contrast to the coupling seen for the carbons originating from malonyl-CoA (Fig. 15). These data are consistent with the hypothesis that the methoxyacetyl group is derived from a triose phosphate or phosphoglycerate precursor originating from the glycolytic pathway.

In further investigations, feeding *A. pretiosum* [1,2-¹³C₂]glycerate failed to result in any ¹³C-enrichment, but the addition of [1,2-¹³C₂]glycerate to the culture of *Sorangium cellulosum* resulted in the expected enrichment at C3-C4 and C11-C12 of the methoxyacetyl groups of soraphen A (Fig. 15). In *S. cellulosum* D-[1,2-¹³C₂]glycerate was preferentially incorporated over L-[1,2-¹³C₂]glycerate for incorporation into soraphen A, consistent with the involvement of the glycolytic pathway in generating the metabolic precursor to (2R)-methoxymalonyl-ACP since only phospho-D-glycerates are intermediates in the glycolytic pathway. The failure to detect any ¹³C-enrichment in any carbons of ansamitocin likely reflects the inability of *A. pretiosum* to phosphorylate exogenously added glycerate for incorporation into the glycolytic pathway.

Finally, using R-[1,2-¹³C₂]glycerol and S-[1,2-¹³C₂]glycerol Floss, Müller, and colleagues investigated whether one stereoisomer was preferentially incorporated over the other in ansamitocin P-3 and soraphen A. The incorporation of S-[1,2-¹³C₂]glycerol over R-[1,2-¹³C₂]glycerol it would provide strong evidence that a glycolytic intermediate such as 1,3-bPG is the metabolic origin of (2R)-methoxymalonyl-ACP. As predicted, ¹³C-enrichment in both *A. pretiosum* and *S. cellulosum* required S-[1,2-¹³C₂]glycerol, supporting the role of the

glycolytic pathway. These *in vivo* data, combined with the *in vitro* biochemical data support the conclusion that 1,3-bPG is the precursor for (2*R*)-methoxymalonyl-ACP. Importantly, due to the stereospecificity of the glycolytic pathway, the C2 carbon of the (2*R*)-methoxymalonyl-ACP has the opposite stereochemistry as seen in (2*S*)-methylmalonyl-CoA and (2*S*)-ethylmalonyl-CoA. Thus, the incorporation of (2*R*)-methoxymalonyl-ACP by the associated PKSs requires an AT domain capable of recognizing a protein-linked extender unit that is structurally and stereochemically distinct from the CoA-linked extender units.

3.2 (2*R*)-Hydroxymalonyl-ACP

Another ACP-linked extender unit, (2*R*)-hydroxymalonyl-ACP (Figs. 16C, 17), is a proposed precursor in the biosynthesis of (2*R*)-methoxymalonyl-ACP and is thus formed in a similar way. Reeves and colleagues, in their paper describing their proposals for (2*R*)-methoxymalonyl-ACP formation in the FK520 biosynthetic pathway, first proposed that (2*R*)-hydroxymalonyl-ACP could be an additional extender unit in the biosynthesis of aflastatin A, an inhibitor of aflatoxin production.^{84, 128} Previously, labeling studies with ¹³C-labelled precursors and the streptomycete aflastatin A producer were performed to gain insight into the biosynthetic origin of this unusual polyketide, which has five hydroxyl groups in its long alkyl chain in positions that are atypical for polyketides.¹²⁸ Feeding the producer ¹³C-labelled acetate, propionate, glucose, and glycolate, Ono and colleagues determined that while most of the alkyl chain is derived from acetate and propionate, there are five two-carbon units derived from glycolate. The finding that these unusual moieties are derived from a glycolytic intermediate rather than acetate or propionate is consistent with the proposal for a novel enzymatic mechanism involving (2*R*)-hydroxymalonyl-ACP as an extender unit. It is expected that the sequencing and analysis of the biosynthetic gene clusters for aflastatin A and blasticidin A,¹²⁹ a structurally related antibiotic and inhibitor of aflatoxin production, will reveal genes coding homologs of four of the five enzymes involved in (2*R*)-methoxymalonyl-ACP formation. The presence of hydroxyl versus methoxyl groups at the α -carbons suggests that a gene coding a methyltransferase will be absent.

An example of a biosynthetic gene cluster containing genes coding four of the five enzymes involved in (2*R*)-methoxymalonyl-ACP formation is the cluster for zwittermicin A (Fig. 17), an antibiotic first identified in *Bacillus cereus*.¹³⁰ The presence of the genes *zmaN*, *zmaD*, *zmaG*, and *zmaE*, coding homologs of FkbH, FkbJ, FkbK, and FkbI, respectively, as well as an unusual glycolyl moiety in the chemical structure led to the proposal that (2*R*)-hydroxymalonyl-ACP is used as a PKS extender unit in the biosynthesis of zwittermicin A (Fig. 16).^{100, 131} While no labelling studies with ¹³C-labelled glycolytic precursors have been performed, the pathway for (2*R*)-hydroxymalonyl-ACP formation has been reconstituted *in vitro* using heterologously purified zwittermicin A enzymes.¹⁰⁰ Prior to these experiments, which were concurrent with work in the Shen lab on (2*R*)-methoxymalonyl-ACP formation, the substrate for the FkbH homolog was unknown, although 1,3-bPG was the most likely candidate.^{84, 127} This proposed substrate was shown to be the correct one, and HPLC-based assays and mass spectrometry were used to show that ZmaN dephosphorylates 1,3-bPG while covalently tethering it to the 4'-Ppant group of holo-ZmaD, forming glyceryl-ZmaD. The NAD⁺-dependent enzyme ZmaG converts glyceryl-ZmaD to 2-hydroxy-3-oxopropionyl-ACP, the substrate for the FAD-dependent dehydrogenase ZmaE, which then converts this intermediate to (2*R*)-hydroxymalonyl-ZmaD. All of the components of the pathway are required for (2*R*)-hydroxymalonyl-ZmaD formation. Importantly, MS/MS analysis showed that the tethering and modification of the glycolytic substrate was localized to the amino acid sequence GYVNS of ZmaD, with the serine residue being the site of 4'-Ppant modification. Additional 4'-Ppant elimination experiments confirmed the identity of the covalently-linked extender unit.

Other natural products that have glycolyl units include the linear aminopolyol antibiotic galantin and the pseudopeptide herbicide amicoumacin B, both produced by *Bacillus* species (Fig. 17).^{132, 133} While these glycolyl units may be derived from a post-assembly tailoring enzyme such as a P450 monooxygenase, labelling studies and/or sequencing efforts may reveal the involvement of (2*R*)-hydroxymalonyl--ACP in forming these unusual moieties.

3.3 (2*S*)-Aminomalonyl-ACP

A second ACP-linked PKS extender unit also found in zwittermicin A biosynthesis is (2*S*)-aminomalonyl-ACP (Figs. 16D,17). The unusual ethanolamine moiety in zwittermicin A suggested that an atypical extender unit might be involved.¹³¹ An examination of the biosynthetic gene cluster revealed the presence of genes coding additional homologs to (2*R*)-methoxymalonyl-ACP enzymes, including a carrier protein (ZmaH) and a dehydrogenase (ZmaI). The presence of a gene coding an adenylation domain with a specificity code for L-serine suggested that instead of a glycolytic precursor, L-serine is covalently tethered to the ACP to form seryl-ZmaH. The FkbK and FkbI homologs ZmaG and ZmaI were proposed to catalyze the two-step oxidation of seryl-ZmaH to (2*S*)-aminomalonyl-ZmaH in reactions analogous to the oxidation of glyceryl-ZmaD to (2*R*)-hydroxymalonyl-ZmaD (Fig. 16C).

Although no labelling studies with ¹³C-labelled serine have been performed, (2*S*)-aminomalonyl-ACP has been reconstituted *in vitro* using heterologously purified biosynthetic enzymes.¹⁰⁰ ATP/PP_i-exchange assays demonstrated the specificity of the A domain ZmaJ for L-serine, and HPLC-based assays coupled with MS provided evidence for the formation of (2*S*)-aminomalonyl-ACP. ZmaJ was shown to activate and covalently tether L-serine to the ACP ZmaH, forming seryl-ZmaH. The dehydrogenases ZmaG and ZmaI were also shown to oxidize seryl-ZmaH in two-steps to form (2*S*)-aminomalonyl-ACP, an unstable product that spontaneously decarboxylates under the reaction conditions used to form glycyl-ZmaH. Nevertheless, the formation of glycyl-ZmaH is indicative of the formation of (2*S*)-aminomalonyl-ZmaH. Furthermore, all the components of the pathway (L-serine, ATP, ZmaH, ZmaJ, ZmaG, and ZmaI) were required for (2*S*)-aminomalonyl-ZmaH formation. Importantly, MS/MS analysis showed that the tethering and modification of the seryl substrate was localized to the sequence GLVNS, with the final serine being the site of 4'-Ppant addition. Analysis of the substituent tethered to the 4'-Ppant prosthetic group through 4'-Ppant elimination experiments confirmed the mass changes.

Interestingly, the zwittermicin A cluster only codes one FkbK homolog (ZmaG), and this enzyme functions in the biosynthetic pathways for both (2*R*)-hydroxymalonyl-ACP and (2*S*)-aminomalonyl-ACP to catalyze the first oxidation step of the glyceryl- or seryl-tethered moieties. When the specificities of the FkbI homologs were tested in each pathway, it was discovered that ZmaE functions in the (2*R*)-hydroxymalonyl-ACP pathway, whereas ZmaI could function in both the (2*R*)-hydroxymalonyl-ACP and (2*S*)-aminomalonyl-ACP pathways. Substrate selectivity was more stringent with ZmaN and ZmaJ, however, as each could only acylate/aminoacylate its cognate ACP.

To date, zwittermicin A represents the only known example in which aminomalonyl-ACP is found as a PKS extender unit. It also represents the only known instance in which NRPS components (an A domain and a carrier protein) are used to form a type I PKS extender unit. We propose that (2*S*)-aminomalonyl-ACP may also act as an NRPS extender unit in the biosynthesis of other natural products. One such example is the cyclic heptapeptide antibiotic GE23077, which contains an aminomalonic moiety with its amino group in peptidic linkage with an iso-serine residue and one of its carboxyl groups in peptidic linkage with an acylated L-2,3-diaminopropionate residue (Fig. 18).¹³⁴ Although the biosynthetic gene cluster has not yet been reported, it is possible that a (2*S*)-aminomalonyl-ACP NRPS extender unit is involved.

3.4 Glyceryl-ACP

Glyceryl-ACP is not technically considered to be an extender unit because it does not elongate the main linear chain of polyketides but rather adds three-carbon units that branch from the main chain. However, because its biosynthesis is so similar to that of (2*R*)-methoxymalonyl-ACP and (2*R*)-hydroxymalonyl-ACP and it occurs as an intermediate in the biosynthesis of these extender units, a discussion of glyceryl-ACP is included in this review. Glyceryl-ACP is found as a precursor in the biosynthesis of certain acyltetronic acids, polyketides containing an unusual five-membered tetronate (4-hydroxy-[5*H*]furan-2-one) ring, though not all acyltetronic acids are believed to be derived from this precursor.¹²⁷ Labelling studies performed with the antitumor antibiotic chlorothricin show that while most of the structure is derived from acetate and propionate, the three-carbon unit consisting of C22, C23, and C24 is derived from glycerol (Fig. 19).¹³⁵ Feeding experiments involving other acyltetronic acids such as tetrocarcin A and tetronasin (Fig. 19) are also consistent with a glycolytic precursor.¹³⁶⁻¹³⁸

The sequencing and analysis of the chlorothricin biosynthetic gene cluster in 2006 revealed the presence of genes coding for homologs of FkbH and FkbJ, suggesting the involvement of an ACP-linked precursor.¹³⁹ In addition, genes coding a putative pyruvate dehydrogenase/dehydratase and a putative hydrolase/AT were also found and were proposed to modify and incorporate the ACP-linked precursor into the tetronate group. Parallel work with the kijanimicin and tetronomycin biosynthetic gene clusters also revealed subclusters coding FkbH homologs and other enzymes proposed to form and incorporate the tetronate groups of these polyketides (Fig. 19).^{140, 141} The proposals for tetronate-ring formation in these three natural products are similar in that they all involve the formation of glyceryl-ACP as a precursor: the FkbH homolog covalently tethers the glycolytic intermediate (1,3-bPG) to an ACP, forming glyceryl-ACP.

In 2007, Leadlay and colleagues reconstituted the pathway for glyceryl-ACP formation *in vitro* using heterologously purified enzymes from the tetronomycin biosynthetic pathway.¹⁰¹ HPLC-based assays and MS were used to show that the FkbH homolog Tmn16 covalently tethers the substrate 1,3-bPG to the holo-ACP Tmn7a and dephosphorylates it, forming glyceryl-Tmn7a. The cross-reactivity of the Tmn proteins with enzymes from the chlorothricin and concanamycin pathways was also examined. Interestingly, Tmn16 was capable of acylating the ACP homolog ChlD2 from the chlorothricin pathway but not Con2 from the concanamycin pathway. Con2 could be acylated by its cognate FkbH homolog Con4, but Con4 was not capable of acylating either Tmn7a or ChlD2. These results indicate that substrate flexibility may be more likely to occur for enzymes of pathways that are more related (i.e. tetronate polyketides) versus those that are less related (i.e. methoxyacetyl-containing polyketides). Another intriguing observation is that the FkbH homologs Tmn16 and ChlD1 have highly conserved, long N-terminal extensions not present in the other FkbH homologs involved in (2*R*)-methoxymalonyl-ACP formation (i.e. Con4), and this N-terminal extension was found to be critical for proper function of Tmn7a. It is possible that this feature may contribute to substrate selectivity.

For chlorothricin, tetronomycin, and kijanimicin (Fig. 19) biosynthesis, the pathway for glyceryl-ACP formation is predicted to occur similarly; the proposals for how this precursor is incorporated into the tetronate ring, however, vary from pathway to pathway. For example, in chlorothricin biosynthesis, it has been proposed that glyceryl-ACP is converted to enoylpyruvoyl-ACP, the substrate for a putative hydrolase/AT that incorporates it into the chlorothricin aglycone.¹³⁹ Meanwhile, in kijanimicin biosynthesis, an AT domain of a putative bifunctional enzyme is predicted to convert glyceryl-ACP to glyceryl-CoA prior to incorporation into the ACP-linked kijanimicin precursor.¹⁴⁰ The proposal for tetronomycin formation is that glyceryl-ACP is directly incorporated without prior modification.¹⁴¹ Further

investigations are necessary to determine the mechanism of incorporation of this unusual ACP-linked precursor.

4. Other Potential Extender Units

Clearly, the number of PKS extender units has expanded recently from the initial model of the use of only malonyl-CoA and (2*S*)-methylmalonyl-CoA. While the identification of ACP-linked extender units has expanded the number of extender units considerably, the real expansion of the available extender units may come from those biosynthesized by a mechanism similar to that seen for crotonyl-CoA conversion to (2*S*)-ethylmalonyl-CoA. The finding by Alber and colleagues that CCR catalyzes not only the reduction of crotonyl-CoA but also its carboxylation raises the possibility that previously unknown extender units could be generated by an analogous reaction on any 2,3-desaturated acyl-CoA thioester. Since these types of intermediates are found during each cycle of the β -oxidation pathway for fatty acid degradation, any of these intermediates could be converted to a PKS extender unit. For example, the structure of FK506 contains an allyl side chain at position C21 (Fig. 20). It has been proposed that a five-carbon PKS extender unit, potentially propylmalonyl-CoA, would function to introduce the carbon unit into the growing polyketide chain.¹⁴² Such an extender unit could be formed by the reductive carboxylation of 2-pentenyl-CoA, an intermediate of the β -oxidation of odd-chain fatty acids.

There are possibilities not only with straight-chain fatty acid degradation but also with branched-chain fatty acids. For example, polyoxypeptin A contains a methylbutyl group associated with its alkyl side chain (Fig. 20). This side chain may potentially come from a 2-(2-methyl-butyl)-malonyl-CoA extender unit. The metabolic origin of this extender unit would be the β -oxidation of anteiso-fatty acids that are commonly found in Gram-positive organisms. Feeding the producing *Streptomyces* strain with L-[¹³C]isoleucine resulted in the ¹³C-enrichment of the C7, C8, C8-Me, C9, and C10 carbons of polyoxypeptin A.¹⁴³ This result is consistent with anteiso-fatty acids as the source of this portion of the polyketide since isoleucine is the source of the branched portion of these fatty acids. The presence of a CCR homolog that recognizes the 4-methyl-2-hexanoyl-CoA would provide a source of this extender unit.

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6 References

1. Staunton J, Weissman KJ. Nat. Prod. Rep 2001;18:380–416. [PubMed: 11548049]
2. Shen B. Curr. Opin. Chem. Biol 2003;7:285–295. [PubMed: 12714063]
3. Smith S, Tsai SC. Nat. Prod. Rep 2007;24:1041–1072. [PubMed: 17898897]
4. Austin MB, Noel JP. Nat. Prod. Rep 2003;20:79–110. [PubMed: 12636085]
5. Hertweck C, Luzhetskyy A, Rebets Y, Bechthold A. Nat. Prod. Rep 2007;24:162–190. [PubMed: 17268612]
6. Rawlings BJ. Nat. Prod. Rep 2001;18:190–227. [PubMed: 11336289]
7. Kennedy J, Auclair K, Kendrew SG, Park C, Vederas JC, Hutchinson CR. Science 1999;284:1368–1372. [PubMed: 10334994]
8. Ma SM, Tang Y. FEBS J 2007;274:2854–2864. [PubMed: 17466016]
9. Müller R. Chem Biol 2004;11:4–6. [PubMed: 15112984]
10. Moss SJ, Martin CJ, Wilkinson B. Nat. Prod. Rep 2004;21:575–593. [PubMed: 15459756]

11. Strohl, WR.; Dickens, ML.; Rajgarhia, VB.; Woo, AJ.; Priestley, ND. *Biotechnology of Antibiotics*. Vol. 2 edn. Strohl, editor. Vol. vol. 82. Marcel Dekker; New York: 1997. p. 577-657.
12. Otten SL, Stutzman-Engwall KJ, Hutchinson CR. *J. Bacteriol* 1990;172:3427–3434. [PubMed: 2345153]
13. Chen H, Tseng CC, Hubbard BK, Walsh CT. *Proc. Natl. Acad. Sci. USA* 2001;98:14901–14906. [PubMed: 11752437]
14. Moore BS, Hertweck C. *Nat. Prod. Rep* 2002;19:70–99. [PubMed: 11902441]
15. Grünewald J, Marahiel MA. *Microbiol. Mol. Biol. Rev* 2006;70:121–146. [PubMed: 16524919]
16. Erb TJ, Berg IA, Brecht V, Müller M, Fuchs G, Alber BE. *Proc. Natl. Acad. Sci. USA* 2007;104:10631–10636. [PubMed: 17548827]
17. Tong L. *Cell. Mol. Life Sci* 2005;62:1784–1803. [PubMed: 15968460]
18. Cronan JE Jr. Waldrop GL. *Prog. Lipid Res* 2002;41:407–435. [PubMed: 12121720]
19. Choi-Rhee E, Cronan JE. *J. Biol. Chem* 2003;278:30806–30812. [PubMed: 12794081]
20. Rodríguez E, Banchio C, Diacovich L, Bibb MJ, Gramajo H. *Appl. Environ. Microbiol* 2001;67:4166–4176. [PubMed: 11526020]
21. Gago G, Kurth D, Diacovich L, Tsai SC, Gramajo H. *J. Bacteriol* 2006;188:477–486. [PubMed: 16385038]
22. Rainwater DL, Kolattukudy PE. *J. Bacteriol* 1982;151:905–911. [PubMed: 6807964]
23. Hunaiti AR, Kolattukudy PE. *Arch. Biochem. Biophys* 1982;216:362–371. [PubMed: 7103514]
24. Diacovich L, Mitchell DL, Pham H, Gago G, Melgar MM, Khosla C, Gramajo H, Tsai SC. *Biochemistry* 2004;43:14027–14036. [PubMed: 15518551]
25. Diacovich L, Peirú S, Kurth D, Rodríguez E, Podestá F, Khosla C, Gramajo H. *J. Biol. Chem* 2002;277:31228–31236. [PubMed: 12048195]
26. Malpartida F, Hopwood DA. *Mol. Gen. Genet* 1986;205:66–73. [PubMed: 3025560]
27. Cerdeño AM, Bibb MJ, Challis GL. *Chem. Biol* 2001;8:817–829. [PubMed: 11514230]
28. Ryu YG, Butler MJ, Chater KF, Lee KJ. *Appl. Environ. Microbiol* 2006;72:7132–7139. [PubMed: 16950896]
29. Hügler M, Krieger RS, Jahn M, Fuchs G. *Eur. J. Biochem* 2003;270:736–744. [PubMed: 12581213]
30. Kim YS. *J. Biochem. Mol. Biol* 2002;35:443–451. [PubMed: 12359084]
31. An JH, Kim YS. *Eur. J. Biochem* 1998;257:395–402. [PubMed: 9826185]
32. An JH, Lee GY, Jung JW, Lee W, Kim YS. *Biochem. J* 1999;344(Pt 1):159–166. [PubMed: 10548546]
33. Kim YS, Bang SK. *J. Biol. Chem* 1985;260:5098–5104. [PubMed: 3921541]
34. Lombó F, Pfeifer B, Leaf T, Ou S, Kim YS, Cane DE, Licari P, Khosla C. *Biotechnol. Prog* 2001;17:612–617. [PubMed: 11485419]
35. Pohl NL, Gokhale RS, Cane DE, Khosla C. *J. Amer. Chem. Soc* 1998;120:11206–11207.
36. Paiva NL, Demain AL, Roberts MF. *J. Nat. Prod* 1991;54:167–177. [PubMed: 2045814]
37. Schwecke T, Aparicio JF, Molnár I, König A, Khaw LE, Haydock SF, Oliynyk M, Caffrey P, Cortés J, Lester JB, et al. *Proc. Natl. Acad. Sci USA* 1995;92:7839–7843. [PubMed: 7644502]
38. Lam KS, Veitch JA, Golik J, Krishnan B, Klohr SE, Volk KJ, Forenza S, Doyle TW. *J. Am. Chem. Soc* 1993;115:12340–12345.
39. Ahlert J, Shepard E, Lomovskaya N, Zazopoulos E, Staffa A, Bachmann BO, Huang K, Fonstein L, Czisny A, Whitwam RE, Farnet CM, Thorson JS. *Science* 2002;297:1173–1176. [PubMed: 12183629]
40. Liu W, Christenson SD, Standage S, Shen B. *Science* 2002;297:1170–1173. [PubMed: 12183628]
41. Liu W, Ahlert J, Gao Q, Wendt-Pienkowski E, Shen B, Thorson JS. *Proc. Natl. Acad. Sci. USA* 2003;100:11959–11963. [PubMed: 14528002]
42. Gokhale RS, Saxena P, Chopra T, Mohanty D. *Nat. Prod. Rep* 2007;24:267–277. [PubMed: 17389997]
43. Schröder J, Raiber S, Berger T, Schmidt A, Schmidt J, Soares-Sello AM, Bardshiri E, Strack D, Simpson TJ, Veit M, Schröder G. *Biochemistry* 1998;37:8417–8425. [PubMed: 9622493]
44. Gottschalk, G. *Bacterial Metabolism*. Vol. 2 edn. Springer-Verlag; New York: 1985.

45. Van der Geize R, Yam K, Heuser T, Wilbrink MH, Hara H, Anderton MC, Sim E, Dijkhuizen L, Davies JE, Mohn WW, Eltis LD. *Proc. Natl. Acad. Sci. USA* 2007;104:1947–1952. [PubMed: 17264217]
46. Horswill AR, Escalante-Semerena JC. *J. Bacteriol* 1999;181:5615–5623. [PubMed: 10482501]
47. Northrop DB. *J. Biol. Chem* 1969;244:5808–5819. [PubMed: 5350937]
48. Pfeifer BA, Admiraal SJ, Gramajo H, Cane DE, Khosla C. *Science* 2001;291:1790–1792. [PubMed: 11230695]
49. Mutka SC, Bondi SM, Carney JR, Da Silva NA, Kealey JT. *FEMS Yeast Res* 2006;6:40–47. [PubMed: 16423069]
50. Haydock SF, Mironenko T, Ghoorahoo HI, Leadlay PF. *J. Biotechnol* 2004;113:55–68. [PubMed: 15380647]
51. Friedman SM, Kaneda T, Corcoran JW. *J Biol Chem* 1964;239:2386–2391. [PubMed: 14209973]
52. Hunaiti AA, Kolattukudy PE. *Antimicrob Agents Chemother* 1984;25:173–178. [PubMed: 6143534]
53. Donadio S, Staver MJ, Katz L. *Mol. Microbiol* 1996;19:977–984. [PubMed: 8830278]
54. Reeves AR, Brikun IA, Cernota WH, Leach BI, Gonzalez MC, Weber JM. *J. Ind. Microbiol. Biotechnol* 2006;33:600–609. [PubMed: 16491356]
55. Pospíšil S, Královcová E, Stajner K, Tax J, Krumphanzl V, Vaněk Z. *Folia Microbiol* 1982;27:275–277. [PubMed: 7141331]
56. Ōmura S, Tsuzuki K, Tanaka Y, Sakakibara H, Aizawa M, Lukacs G. *J. Antibiot* 1983;36:614–616. [PubMed: 6874580]
57. Leiser A, Birch A, Robinson JA. *Gene* 1996;177:217–222. [PubMed: 8921870]
58. Tang L, Zhang YX, Hutchinson CR. *J. Bacteriol* 1994;176:6107–6119. [PubMed: 7928973]
59. Pospíšil S, Sedmera P, Havránek M, Krumphanzl V, Vaněk Z. *J. Antibiot* 1983;36:617–619. [PubMed: 6874581]
60. Reynolds KA, O'Hagan D, Gani D, Robinson JA. *J. Chem. Soc. Perkin Trans. I* 1988:3195–3207.
61. Sherman MM, Yue S, Hutchinson CR. *J. Antibiot* 1986;39:1135–1143. [PubMed: 3759663]
62. Vrijbloed JW, Zerbe-Burkhardt K, Ratnatilleke A, Grubelnik-Leiser A, Robinson JA. *J. Bacteriol* 1999;181:5600–5605. [PubMed: 10482499]
63. O'Hagan D, Rogers SV, Duffin GR, Reynolds KA. *J. Antibiot* 1995;48:1280–1287. [PubMed: 8557569]
64. Liu H, Reynolds KA. *J. Bacteriol* 1999;181:6806–6813. [PubMed: 10542184]
65. Liu H, Reynolds KA. *Metab. Eng* 2001;3:40–48. [PubMed: 11162231]
66. Zhang W, Reynolds KA. *J. Bacteriol* 2001;183:2071–2080. [PubMed: 11222607]
67. Li C, Florova G, Akopiants K, Reynolds KA. *Microbiology* 2004;150:3463–3472. [PubMed: 15470123]
68. Li C, Akopiants K, Reynolds KA. *J. Ind. Microbiol. Biotechnol* 2006;33:75–83. [PubMed: 16292556]
69. Akopiants K, Florova G, Li C, Reynolds KA. *J. Ind. Microbiol. Biotechnol* 2006;33:141–150. [PubMed: 16187095]
70. Wallace KK, Bao ZY, Dai H, Digate R, Schuler G, Speedie MK, Reynolds KA. *Eur. J. Biochem* 1995;233:954–962. [PubMed: 8521864]
71. Han L, Reynolds KA. *J. Bacteriol* 1997;179:5157–5164. [PubMed: 9260959]
72. Caffrey P, Bevirt DJ, Staunton J, Leadlay PF. *FEBS Lett* 1992;304:225–228. [PubMed: 1618327]
73. Ōmura S, Takeshima H, Nakagawa A, Miyazawa J, Piriou F, Lukacs G. *Biochemistry* 1977;16:2860–2866. [PubMed: 18162]
74. He J, Hertweck C. *Chem. Biol* 2003;10:1225–1232. [PubMed: 14700630]
75. Day LE, Chamberlin JW, Gordee EZ, Chen S, Gorman M, Hamill RL, Ness T, Weeks RE, Stroschane R. *Antimicrob. Agents Chemother* 1973;4:410–414. [PubMed: 4791301]
76. Gerlitz M, Hammann P, Thiericke R, Rohr J. *J. Org. Chem* 1992;57:4030–4033.
77. Bindseil KU, Zeeck A. *Liebigs Ann. Chem* 1994:305–312.
78. Hamamoto T, Uozumi T, Beppu T. *J. Antibiot* 1985;38:533–535. [PubMed: 4008348]
79. Inoue A, Deguchi T, Yoshida M, Shirahata K. *J. Antibiot* 1983;36:442–444. [PubMed: 6853373]

80. Kelly, WL. Personal communication
81. Song L, Barona-Gomez F, Corre C, Xiang L, Udvary DW, Austin MB, Noel JP, Moore BS, Challis GL. *J. Am. Chem. Soc* 2006;128:14754–14755. [PubMed: 17105255]
82. Stassi DL, Kakavas SJ, Reynolds KA, Gunawardana G, Swanson S, Zeidner D, Jackson M, Liu H, Buko A, Katz L. *Proc. Natl. Acad. Sci. USA* 1998;95:7305–7309. [PubMed: 9636144]
83. Hu Z, Reid R, Gramajo H. *J. Antibiot* 2005;58:625–633. [PubMed: 16392678]
84. Wu K, Chung L, Revill WP, Katz L, Reeves CD. *Gene* 2000;251:81–90. [PubMed: 10863099]
85. Byrne KM, Shafiee A, Nielsen JB, Arison B, Monaghan RL, Kaplan L. *Dev. Ind. Microbiol* 1993;32:29–45.
86. Gandeche AR, Large SL, Cundliffe E. *Gene* 1997;184:197–203. [PubMed: 9031628]
87. Karray F, Darbon E, Oestreicher N, Dominguez H, Tuphile K, Gagnat J, Blondelet-Rouault MH, Gerbaud C, Pernodet JL. *Microbiology* 2007;153:4111–4122. [PubMed: 18048924]
88. Haydock SF, Appleyard AN, Mironenko T, Lester J, Scott N, Leadlay PF. *Microbiology* 2005;151:3161–3169. [PubMed: 16207901]
89. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W. *Angew. Chem. Int. Ed. Engl* 2003;42:355–357. [PubMed: 12548698]
90. Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, Mitsiades C, Mitsiades N, Yasui H, Letai A, Ovaa H, Berkers C, Nicholson B, Chao TH, Neuteboom ST, Richardson P, Palladino MA, Anderson KC. *Cancer Cell* 2005;8:407–419. [PubMed: 16286248]
91. Groll M, Huber R, Potts BC. *J Am Chem Soc* 2006;128:5136–5141. [PubMed: 16608349]
92. Vaillancourt FH, Yeh E, Vosburg DA, Garneau-Tsodikova S, Walsh CT. *Chem. Rev* 2006;106:3364–3378. [PubMed: 16895332]
93. Tsueng G, McArthur KA, Potts BC, Lam KS. *Appl. Microbiol. Biotechnol* 2007;75:999–1005. [PubMed: 17340108]
94. Beer LL, Moore BS. *Org. Lett* 2007;9:845–848. [PubMed: 17274624]
95. Udvary DW, Zeigler L, Asolkar RN, Singan V, Lapidus A, Fenical W, Jensen PR, Moore BS. *Proc. Natl. Acad. Sci. USA* 2007;104:10376–10381. [PubMed: 17563368]
96. Eustáquio AS, Pojer F, Noel JP, Moore BS. *Nat. Chem. Biol* 2008;4:69–74. [PubMed: 18059261]
97. Dong C, Huang F, Deng H, Schaffrath C, Spencer JB, O'Hagan D, Naismith JH. *Nature* 2004;427:561–565. [PubMed: 14765200]
98. Huang F, Haydock SF, Spiteller D, Mironenko T, Li TL, O'Hagan D, Leadlay PF, Spencer JB. *Chem. Biol* 2006;13:475–484. [PubMed: 16720268]
99. Eustáquio AS, Moore BS. *Angew. Chem. Int. Ed. Engl* 2008;47:3936–3938. [PubMed: 18407559]
100. Chan YA, Boyne MT II, Podevels AM, Klimowicz AK, Handelsman J, Kelleher NL, Thomas MG. *Proc. Natl. Acad. Sci USA* 2006;103:14349–14354. [PubMed: 16983083]
101. Sun Y, Hong H, Gillies F, Spencer JB, Leadlay PF. *ChemBioChem* 2008;9:150–156. [PubMed: 18046685]
102. Yu T-W, Bai L, Clade D, Hoffmann D, Toelzer S, Trinh KQ, Xu J, Moss SJ, Leistner E, Floss HG. *Proc. Natl. Acad. Sci USA* 2002;99:7968–7973. [PubMed: 12060743]
103. Schuhmann T, Vollmar D, Grond S. *J. Antibiot* 2007;60:52–60. [PubMed: 17390589]
104. Rascher A, Hu Z, Viswanathan N, Schirmer A, Reid R, Nierman WC, Lewis M, Hutchinson CR. *FEMS Microbiol. Lett* 2003;218:223–300. [PubMed: 12586396]
105. Rascher A, Hu Z, Buchanan GO, Reid R, Hutchinson CR. *Appl. Environ. Microbiol* 2005;71:4862–4871. [PubMed: 16085885]
106. Ōmura S, Tsuzuki K, Nakagawa A, Lukacs G. *J. Antibiot* 1983;36:611–613. [PubMed: 6874579]
107. Rodriguez E, Ward S, Fu H, Revill WP, McDaniel R, Katz L. *Appl. Genet. Mol. Biotechnol* 2004;66:85–91.
108. Kakavas SJ, Katz L, Stassi D. *J. Bacteriol* 1997;179:7515–7522. [PubMed: 9393718]
109. Zhao C, Ju J, Christenson SD, Smith WC, Song D, Zhou X, Shen B, Deng Z. *J. Bacteriol* 2006;188:4142–4147. [PubMed: 16707707]
110. Ligon J, Hill S, Beck J, Zirkle R, Molnár I, Zawodny J, Money S, Schupp T. *Gene* 2002;285:257–267. [PubMed: 12039053]

111. Li W, Ju J, Osada H, Shen B. J. Bacteriol 2006;188:4148–4152. [PubMed: 16707708]
112. Byrne KM, Shafiee A, Nielsen JB, Arison B, Monaghan RL, Kaplan L. Dev. Indust. Microbiol 1993;32:29–45.
113. Reeves CD, Chung LM, Liu Y, Xue Q, Carney JR, Revill WP, Katz L. J. Biol. Chem 2002;277:9155–9159. [PubMed: 11786554]
114. Wenzel SC, Williamson RM, Grünanger C, Xu J, Gerth K, Martinez RA, Moss SJ, Carroll BJ, Grond S, Unkefer CJ, Müller R, Floss HG. J. Am. Chem. Soc 2006;128:14325–14336. [PubMed: 17076505]
115. Schuhmann T, Grond S. J. Antibiot 2004;57:655–661. [PubMed: 15638326]
116. Haber A, Johnson RD, Rinehart KL Jr. J. Am. Chem. Soc 1977;99:3541–3544. [PubMed: 853197]
117. Ōmura S, Nakagawa A, Takeshima H, Atsumi K, Miyazawa J. J. Am. Chem. Soc 1975;97:6600–6602. [PubMed: 1184876]
118. Hatano K, Mizuta E, Akiyama S, Higashide E, Nakao Y. Agricult. Biol. Chem 1985;49:327–333.
119. Hill AM, Harris JP, Siskos AP. Chemical Communications 1998;21:2361–2362.
120. Carroll BJ, Moss SJ, Bai L, Kato Y, Toelzer S, Yu T-W, Floss HG. J. Am. Chem. Soc 2002;124:4176–4177. [PubMed: 11960423]
121. Yue S, Duncan JS, Yamamoto Y, Hutchinson CR. J. Am. Chem. Soc 1987;109.
122. Cane DE, Yang C-C. J. Am. Chem. Soc 1987;109:1253–1255.
123. Yu T-W, Bai L, Clade D, Hoffman D, Toelzer S, Trinh KQ, Xu J, Moss SJ, Leistner E, Floss HG. Proc. Natl. Acad. Sci. USA 2002;99:7968–7973. [PubMed: 12060743]
124. Kato Y, Bai L, Xue Q, Revill WP, Yu T-W, Floss HG. J. Am. Chem. Soc 2002;124:5268–5269. [PubMed: 11996558]
125. Watanabe K, Khosla C, Stroud RM, Tsai S-C. J. Mol. Biol 2003;334:435–444. [PubMed: 14623185]
126. Rude MA, Khosla C. J. Antibiot 2006;59:464–470. [PubMed: 17080682]
127. Walton LJ, Corre C, Challis GL. J. Ind. Microbiol. Biotechnol 2006;33:105–120. [PubMed: 16187096]
128. Ono M, Sakuda S, Ikeda H, Furihata K, Nakayama J, Suzuki A, Isogai A. J. Antibiot 1998;51:1019–1028. [PubMed: 9918395]
129. Sakuda S, Ono M, Ikeda H, Inagaki Y, Nakayama J, Suzuki A, Isogai A. Tetrahedron Lett 1997;38:7399–7402.
130. Siloh-Suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, Handelsman J. Appl. Environ. Microbiol 1994;60:2023–2030. [PubMed: 8031096]
131. Emmert EAB, Klimowicz AK, Thomas MG, Handelsman J. Appl. Environ. Microbiol 2004;70:104–113. [PubMed: 14711631]
132. Shoji J, Sakazaki R, Wakisaka Y, Koizumi K, Mayama M, Matsuura S. J. Antibiot 1975;28:122–125. [PubMed: 1112763]
133. Gebhardt K, Schimana J, Müller J, Fiedler H-P, Kallenborn HG, Holzenkämpfer M, Krastel P, Zeeck A, Vater J, Hölzel A, Schmid DG, Rheinheimer J, Dettner K. FEMS Microbiol. Lett 2002;217:199–205. [PubMed: 12480104]
134. Sarubbi E, Monti F, Corti E, Miele A, Selva E. Eur. J. Biochem 2004;271:3146–3154. [PubMed: 15265034]
135. Lee JJ, Lee JP, Keller PJ, Cottrell CE, Chang C-J, Zühner H, Floss HG. J. Antibiot 1986;39:1123–1134. [PubMed: 3531133]
136. Park H-R, Furihata K, Hayakawa Y, Shin-ya K. Tetrahedron Lett 2002;43:6941–6945.
137. Bulsing JM, Laue ED, Leeper FJ, Staunton J, Davies DH, Ritchie GAF, Davies A, Davies AB, Mabelis RP. Chem. Comm 1984:1301–1302.
138. Tamaoki T, Tomita F. J. Antibiot 1983;36:595–598. [PubMed: 6874575]
139. Jia X-Y, Tian Z-H, Shao L, Qu X-D, Zhao Q-F, Tang J, Tang G-L, Liu W. Chem. Biol 2006;13:575–585. [PubMed: 16793515]
140. Zhang H, White-Phillip JA, Melançon CE 3rd, Kwon HJ, Yu WL, Liu HW. J. Am. Chem. Soc 2007;129:14670–14683. [PubMed: 17985890]

141. Demydchuk Y, Sun Y, Hong H, Staunton J, Spencer JB, Leadlay PF. *ChemBioChem* 2008;9:1136–1145. [PubMed: 18404760]
142. Motamedi H, Shafiee A. *Eur. J. Biochem* 1998;256:528–534. [PubMed: 9780228]
143. Umezawa K, Ikeda Y, Kawase O, Naganawa H, Kondo S. *J. Chem. Soc. Perkin Trans. I* 2001:1550–1553.

Figure 1.
 Basic mechanism of decarboxylative Claisen condensations for extender unit incorporation.

Figure 2.

Erythromycin A-associated modular type I PKS incorporating six (2S)-methylmalonyl-CoA extender units. Labeling above the PKS identifies the loading module (LM) and six modules (M1-M6). The abbreviations for domains are in the text. The carbons in bold represent the extender unit incorporation.

Figure 3. Lovastatin-associated iterative type I PKS incorporating eight malonyl-CoA extender units. The ER domain of LovB is inactive and replaced by the function of LovC.

Figure 4.

Daunorubicin and doxorubicin-associated type II PKS incorporating nine malonyl-CoA extender units. The KS_{α} enzyme is the KS domain that catalyzes the decarboxylative Claisen condensation of the precursors. The KS_{β} enzyme controls polyketide length.

Figure 5.
3,5-Dihydroxyphenylglycine-associated type III PKS incorporating three malonyl-CoA extender units.

Figure 6.

Schematic of CoA, holo-ACP, and the associated extender units. The brackets around glyceryl-ACP denote that it is not a classic extender unit involved in decarboxylative Claisen condensation reactions.

Figure 8. Schematic of the rapamycin modular type I PKS. The AT domains that recognize malonyl-CoA are in bold. The PKS domains that are inactive are italicized and underlined.

Figure 9. Schematic of the iterative type I PKSs involved in enediyne biosynthesis along with representative enediyne structures. The abbreviations of the enzymatic domains are as described in the text with the addition of PPT, phosphopantetheinyltransferase.

Figure 10. Metabolic pathways leading to the formation of (2*S*)-methylmalonyl-CoA and (2*S*)-ethylmalonyl-CoA. These two extender units are in solid boxes; starting metabolites that can lead to these extender units are in dashed boxes. Path A and B are pathways described in the text. Abbreviations: ICM, isobutyryl-CoA mutase; BCC, butyryl-CoA carboxylase; PCC, propionyl-CoA carboxylase; CCR, crotonyl-CoA carboxylase/reductase; PHB, polyhydroxybutyrate; MCR, methylmalonyl-CoA racemase; MCM, methylmalonyl-CoA mutase; MM-CoA TC, methylmalonyl-CoA transcarboxylase.

Figure 11. Chemical structures of natural products that incorporate (2S)-methylmalonyl-CoA, (2S)-ethylmalonyl-CoA, or (2R)-methoxymalonyl-ACP extender units.

Figure 12.

Schematic of the type I PKS involved in aureothin biosynthesis. The domain abbreviations are as in the text with the addition of AT representing an inactive AT domain.

Figure 13.
Chemical structures of the germicidins.

Figure 14.

A) Chemical structures of salinosporamide A, salinosporamide B, and fluorosalinosporamide; B) Schematic of chloroethylmalonyl-CoA biosynthesis by *S. tropica* and fluoroacetate biosynthesis by *Streptomyces cattleya*; C) Megasyntase involved in chloroethylmalonyl-CoA incorporation and salinosporimide A biosynthesis.

Figure 15.
Chemical structures of natural products that incorporate (2R)-methoxymalonyl-ACP.

Figure 16.

A) Proposed biosynthetic pathway for FkbH-dependent (2*R*)-methoxymalonyl-ACP formation; B) Proposed biosynthetic pathway for (2*R*)-methoxymalonyl-ACP formation during sorafen A biosynthesis; C) Biosynthetic pathway for (2*R*)-hydroxymalonyl-ACP formation during zwittermucin A biosynthesis; D) Biosynthetic pathway for (2*S*)-aminomalonyl-ACP formation during zwittermucin A biosynthesis. SAHC = *S*-adenosylhomocysteine.

Figure 17.
Chemical structures of natural products incorporating (2R)-hydroxymalonyl-ACP or (2S)-aminomalonyl-ACP extender units.

Figure 18.
Chemical structure of GE23077.

Figure 19. Chemical structures of natural products that incorporate glyceryl-ACP precursors and a proposed scheme for glyceryl-ACP incorporation into tetronomycin.

Figure 20.
Chemical structures of FK506 and polyoxypeptin A which potentially incorporate propylmalonyl-CoA and 2-(2-methyl-butyl)-malonyl-CoA extender units, respectively.