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# Genes and enzymes involved in bacterial isoprenoid biosynthesis

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Isoprenoids produced by bacteria are of particular interest as they encompass huge structural and functional diversity. A rapidly increasing number of bacterial derived isoprenoids have been reported in recent years, as many of the genes and biosynthetic gene clusters are responsible for their biosynthesis. Polyprenyl chains, synthesized by the condensation of isopentenyl diphosphate units, serve as the substrates for cyclases and subsequent tailoring enzymes. It is these enzymes, particularly the cyclases, which are responsible for the structural diversity of this chemical class. Recent studies have revealed novel insights into isoprenoid biosynthesis, and in several cases enzymatic mechanisms have been redefined.

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**Current Opinion in Chemical Biology** 2009, **13**:180–188

This review comes from a themed issue on
Molecular Diversity
Edited by Christian Hertweck and Hiroyuki Osada

Available online 21st March 2009

1367-5931/$ – see front matter


**DOI** 10.1016/j.cbpa.2009.02.029

## Introduction

Isoprenoids comprise the largest single family of compounds found in nature with the most chemically diverse pool of structures [1,2]. Because of this structural diversity they exhibit many variable biological functions, for example, they are industrially useful as antibiotics, hormones, anticancer drugs, and insecticides. Historically, prokaryotes have not been a rich source of isoprenoids, but this situation has changed dramatically over the last decade as numerous organisms, particularly those belonging to the Actinomycetales, have been identified as producers of novel isoprenoids [2]. Isoprenoids are biosynthesized by the condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) units to yield a polyprenyl chain which is then most often processed by a cyclase to yield a much more complex structure. Typical bacterial cyclases contain an acid-rich motif and a downstream triad (NSE) [3] which coordinates $Mg^{2+}$. Together these enable either the direct protonation of a double bond of the linear isoprenoid chain, or the cleavage of the diphosphate to initiate the cyclization reaction. An alternative elaboration strategy involves epoxidation of the linear isoprenoid chain to activate it for cyclization [4]. The new terpenoid skeletons formed are then typically elaborated through the action of further modifying enzymes, resulting in many structurally and functionally diverse natural compounds. The IPP units required for isoprene biosynthesis are generated by two distinct pathways (Figure 1). While the mevalonate (MV) pathway is very common in eukaryotes, in prokaryotes the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (also known as 1-deoxy-D-xylulose-5-phosphate (DXP) pathway), [5] is most commonly used. In some cases both pathways contribute IPP units for the biosynthesis of a single compound [6]. Recent efforts have focused particularly upon gaining a deeper genetic understanding of biosynthetic pathways for newly discovered isoprenoids, and individual enzymes from these pathways have been studied in detail. For example, the biosynthesis of the volatile terpenoids geosmin and 2-methylisoborneol, and of albaflavenone and pentalenolactone has been the subject of intensive study. Herein, we provide a brief overview of the key research published between 2006 and 2008.

## Terpenoids from bacteria

Terpenoids are generally classified by the number of IPP units from which they derive, and by the way in which the ring systems are functionalized. The following chapter focuses on the most recently identified, and the most commonly observed terpenoids produced by bacteria; their structures are given in Figure 2. One of the largest terpenoids known as longestin (KS-505a) is produced by *Streptomyces argenteolus*. It has a unique nona-cyclic structure which includes 2-*O*-methylglucuronic acid and *o*-succinyl benzoate moieties. The two tricyclic antibiotics brasilicardin A from *Nocardia terpenica* (formerly known as *Nocardia brasiliensis*) and phenalinolactone A from *Streptomyces* species Tü6071 [7•] are diterpenoids. They derive from a perhydrophenanthrene skeleton which is first functionalized by oxidation and then elaborated through the attachment of several moieties deriving from different pathways. Terpentecin from *Streptomyces griseolosporeus* MF-730-N6 also consists of a highly oxidized isoprenoid structure but does not contain additional structural elements. Recently,

the diterpenoid oxaloterpins A–E were isolated from *Streptomyces* sp. KO-3988 and contain a viguiepinol skeleton [8].

The pentalenolactones are produced by many *Streptomyces* strains where the lactone formation and functionalization of the ring system are accomplished by redox enzymes [9•]. In 2009 the Laatsch group reported five sesquiterpenes from the marine strain *Streptomyces* species M491 comprising a muurolane skeleton that was originally identified in plants [10]. Additionally, they discovered a further isoprenoid in the marine strain *Streptomyces* species QD518 in 2006, selina-4(14),7(11)-diene-8,9-diol which belongs to the group of selinane/eudesmane sesquiterpenoids [11].

Meroterpenoids are hybrid compounds, derived from different pathways, in which several isoprene units are present in the basic structure. The furaquinocins from *Streptomyces* sp. KO-3988 were the first example involving a carbon–carbon bond between a nonterminal carbon atom of the isoprenoid chain and the polyketide nucleus [12]. Related prenylated naphthoquinones include furanonaphthoquinone I from *Streptomyces cinnamonensis* ATCC 15413, and the napyradiomycins from *Streptomyces aculeolatus* NRRL 18422. Recently, the biosynthesis of the endophenazines from *S. cinnamonensis* DSM 1042 has been elucidated [13]. These C-prenylated or N-prenylated phenazine derivatives are also produced by *Streptomyces anulatus*. Another fascinating hybrid molecule moenomycin is produced by *Streptomyces ghanaensis* (ATCC14672). Moenomycin is a phosphoglycolipid antibiotic with an unprecedented structure [14•] comprising a pentasaccharide scaffold attached by a phosphoglycerate-ether linkage to an irregular isoprenoid chain. Further meroterpenoids of note are the indole-isoprenoid-hybrid 5-dimethylallylindole-3-carboxylic acid, the phosphatoquinone A derivative A80915G-8″-acid both produced by *Streptomyces* sp. MS239 [15] and naphterpin B and C produced by *Streptomyces* sp. CL-190 [16].

## Isoprenoid biosynthetic gene clusters

Isoprenoid biosynthetic gene clusters have been identified in bacteria either by direct cloning or by genome mining, with the majority found in members of the order Actinomycetales. The organization of the biosynthetic gene clusters for the phenalinolactones [7•], brasilicardin A [17], terpentecin [18,19], pentalenolactone (from *Streptomyces avermitilis*) [9•], longestin [20], furaquinocin [21], and napyradiomycin [22] is presented in Figure 3.

So far, the largest cluster identified is that responsible for the biosynthesis of the phenalinolactones. This contains a geranylgeranyl diphosphate (GGPP) synthase gene (*plaT4*) that is responsible for the generation of the polyprenyl chain, a terpene cyclase gene (*plaT2*), and a prenyltransferase gene (*plaT3*). Surprisingly, a gene (*plaT1*) encoding for an enzyme that is similar to a eukaryotic squalene epoxidase is also located in the cluster. In contrast to many other terpene cyclization processes in bacteria, the cyclization process is putatively initiated by an epoxidation reaction of the linear terpene chain, as has been described for myxobacterial steroid formation [23]. This is corroborated by the presence of the oxygen function at C-3 of the phenalinolactones and the absence of an acid-rich motif in PlaT2. The structure of brasilicardin resembles phenalinolactone and both gene clusters exhibit similarity that is the brasilicardin cluster also contains a squalene epoxidase gene (*bra5*) and a cyclase gene (*bra4*). Hence, we can assume a common pathway for the two tricyclic diterpenoids. Additionally, feeding experiments with [1-$^{13}$C]D-glucose revealed that the IPP units derive from the MEP pathway in both compounds. This is supported by the presence of two genes in the phenalinolactone cluster, *plaT5* and *plaT6*, which encode a 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase and a DXP synthase, respectively. Interestingly, *plaT5* and *plaT6* analogs (*lon4* and *lon5*) were identified in the longestin biosynthetic gene cluster indicating the importance of both genes and gene products. Pentalenolactone is also synthesized by the MEP pathway as shown by feeding experiments but none of the MEP pathway genes are located in its biosynthetic gene cluster [9•]. Conversely, all of the genes encoding enzymes required for IPP biosynthesis via the MV pathway are found in the gene clusters of terpentecin, napyradiomycin, and furaquinocin. The gene clusters for furanonaphthoquinone I and endophenazine A contain only one gene of the MV pathway which encodes a putative MV kinase [24].

## Enzymes involved in bacterial isoprenoid biosynthesis

Many enzymes of the MEP pathway have been subjected to detailed biochemical and/or structural study. In 2006 the first crystal structure of a DXP synthase (DXS) in complex with thiamine pyrophosphate was solved [25]. More recently, the crystal structure of the DXP reductoisomerase from *Escherichia coli* was described [26]. Lauw *et al.* investigated in detail the reaction mechanism of the enzyme MEP synthase from different organisms [27], and the MEP cytidyltransferase from *Mycobacterium tuberculosis* has been functionally characterized [28].

The ever-increasing availability of genetic information for bacterial isoprenoids has driven rapid advances in our mechanistic understanding of the enzymes involved in their biosynthesis (Figure 4). For example, in 2005 an alternative mechanism for the conversion of farnesyl diphosphate (FPP) to the fragmented sesquiterpenoid alcohol geosmin in myxobacteria was described [29]; this was rather different to the mechanisms which have been previously suggested [30,31]. This prompted a range of

**Figure 1**

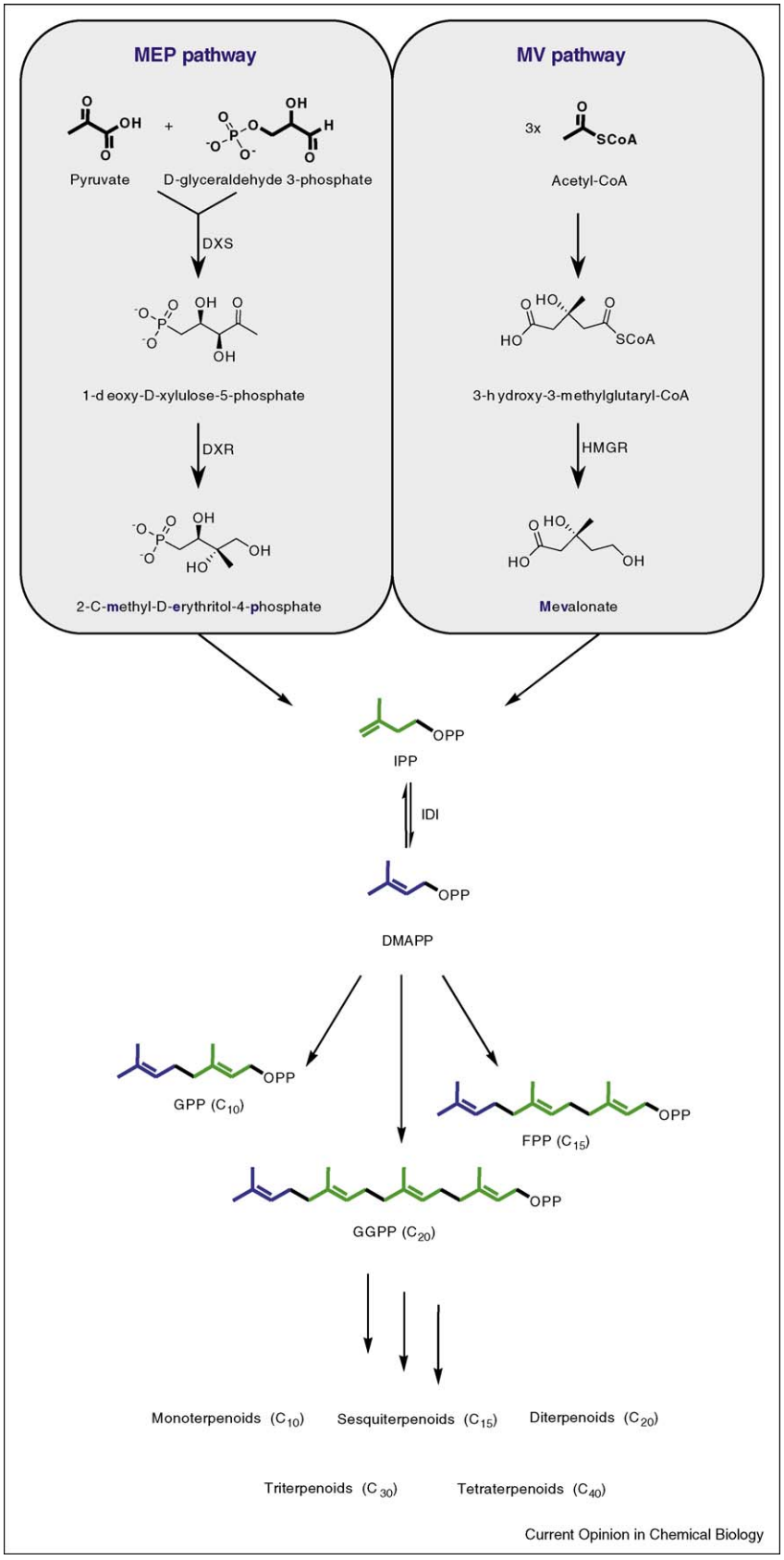
MEP pathway
MV pathway
Pyruvate
D-glyceraldehyde 3-phosphate
DXS
1-deoxy-D-xylulose-5-phosphate
DXR
2-C-methyl-D-erythritol-4-phosphate
3x
Acetyl-CoA
3-hydroxy-3-methylglutaryl-CoA
HMGR
Mevalonate
IPP
IDI
DMAPP
GPP (C10)
FPP (C15)
GGPP (C20)
Monoterpenoids (C10)
Sesquiterpenoids (C15)
Diterpenoids (C20)
Triterpenoids (C30)
Tetraterpenoids (C40)
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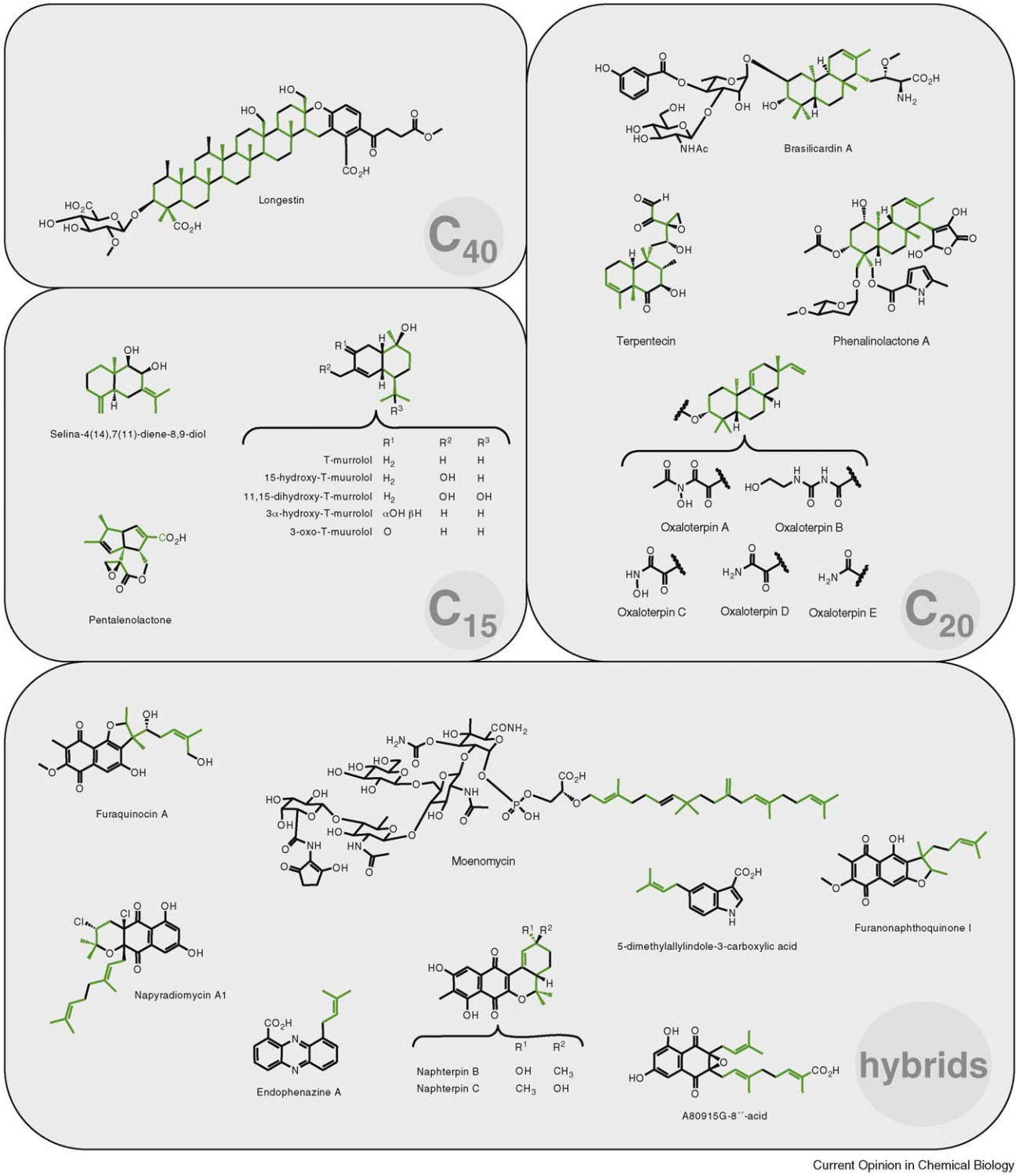
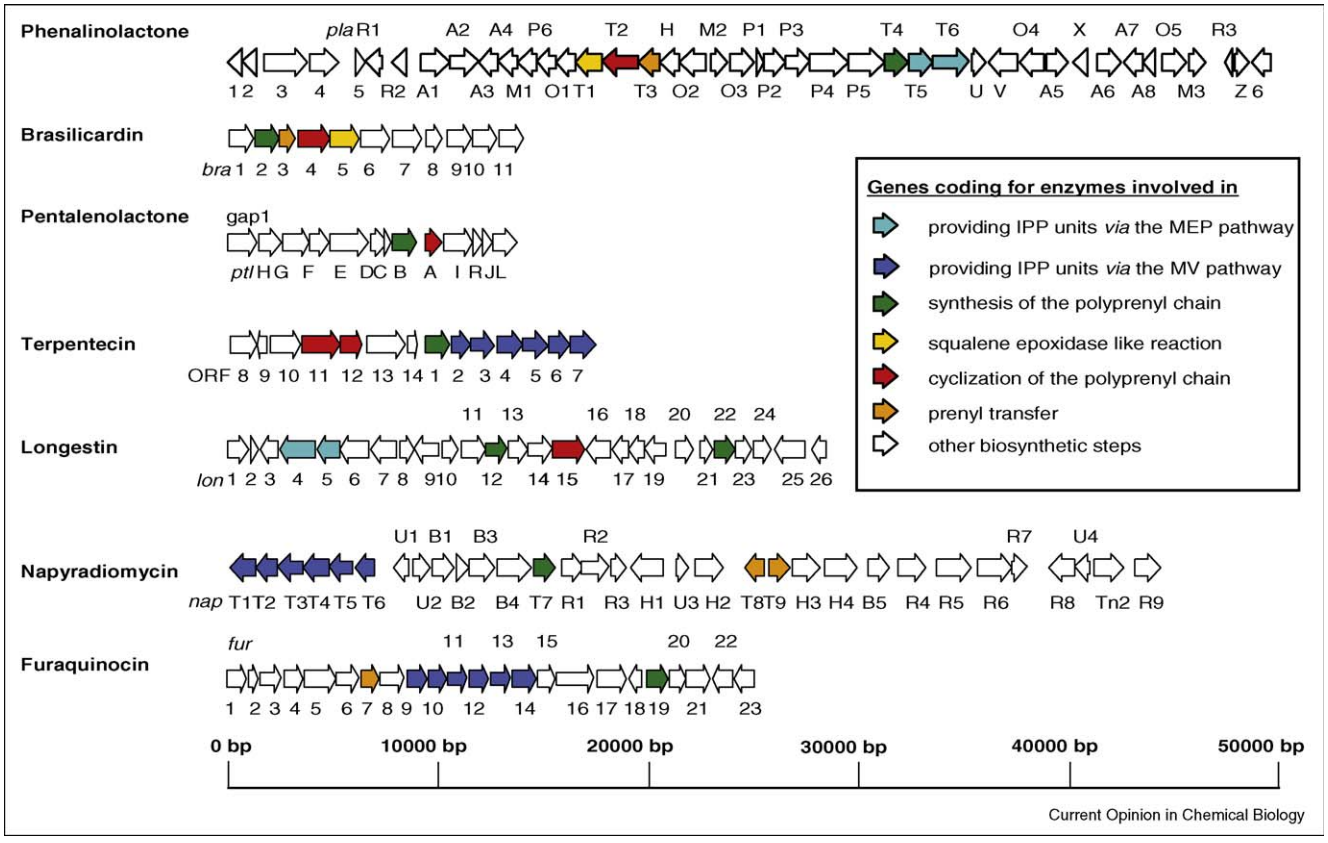
**Figure 2**




Diversity of isoprenoid structures. A selection of isoprenoid structures, with their constituent isoprenoid units highlighted in green. These belong to the tetraterpenoid ($C_{40}$), diterpenoid ($C_{20}$), sesquiterpenoid ($C_{15}$), and hybrid product families.

**(Figure 1 Legend)** A general overview of isoprenoid biosynthesis. IPP and DMAPP are produced by the MV pathway or by the MEP pathway. Acetyl-CoA acts as the sole precursor of the MV pathway, while pyruvate and D-glyceraldehyde-3-phosphate are the required precursors of the MEP pathway. Key enzymes of each pathway are shown. IPP and DMAPP are afterwards converted to the terpenoid precursors GPP for monoterpene ($C_{10}$) biosynthesis, FPP for sesquiterpene ($C_{15}$) and triterpene ($C_{30}$) biosynthesis, and GGPP for diterpene ($C_{20}$) and tetraterpene ($C_{40}$) biosynthesis. The DMAPP unit is highlighted in blue and the IPP unit in green. 1-Deoxy-D-xylulose-5-phosphate reductase (DXR); 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR); IPP-DMAPP isomerase (IDI).

**Figure 3**



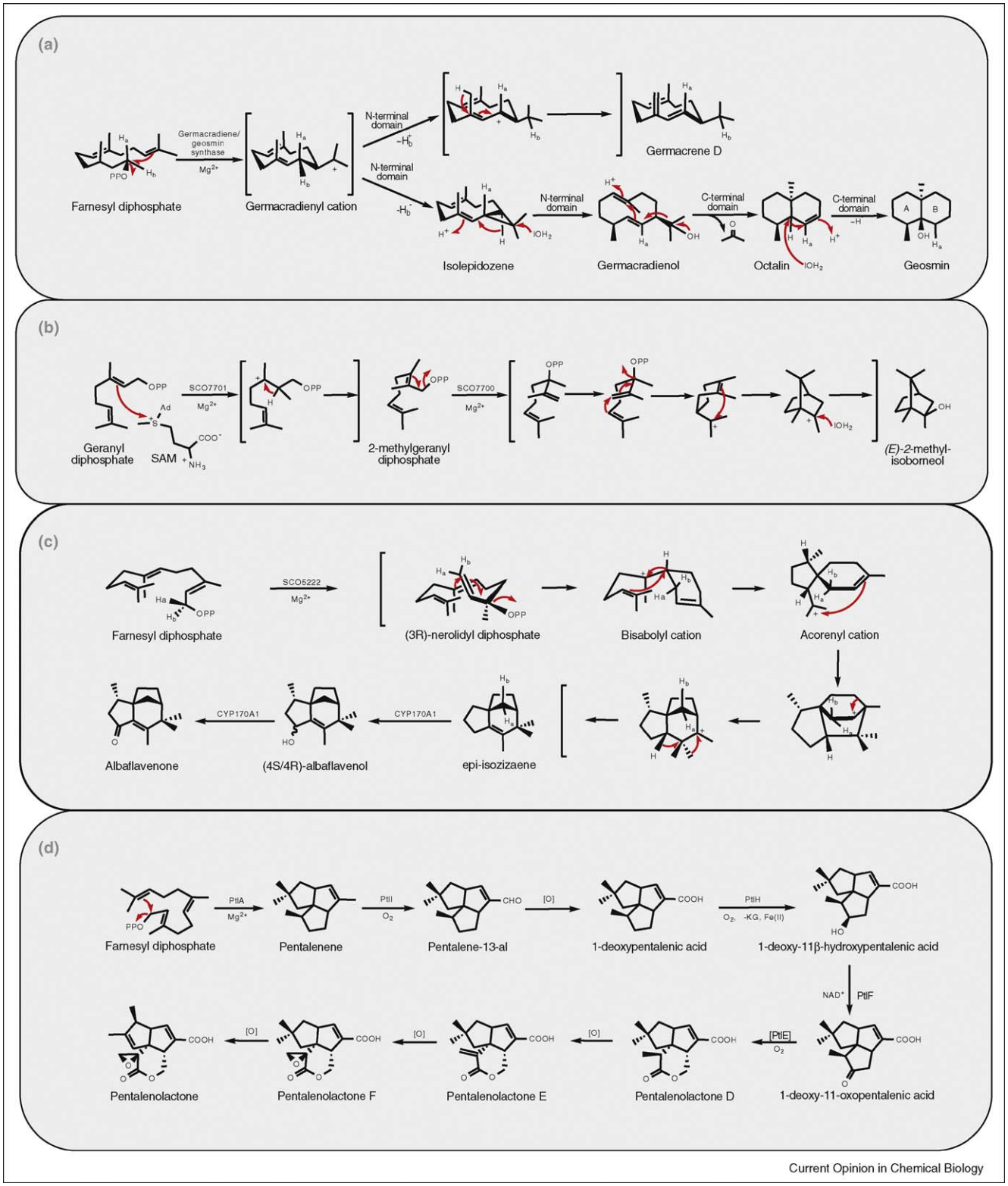

The architecture of several bacterial isoprenoid biosynthetic gene clusters.

detailed studies regarding the conversion of FPP to germacrene D, germacradienol, octalin, and geosmin in *Streptomyces coelicolor* and *Streptomyces avermitilis* [32,33•]. Of particular note was the work of Cane and collaborators who found that geosmin is not the product of a multistep biosynthetic pathway as previously thought, but that the protein encoded by SCO6073 can catalyze the entire conversion of FPP to geosmin. They postulated that germacradienol must be released before rebinding to the protein for subsequent conversion to geosmin [32]. Two independently functioning active sites were identified, one located in each of the N-termini and C-termini in the protein. The N-terminal active site is responsible for the conversion of FPP to germacradienol while the C-terminal active site catalyzes substrate transfer and the final step(s) to generate geosmin [34••]. This is in contrast to the work of Chater and coworkers who described that a mutant carrying an in-frame deletion in the C-terminal domain of SCO6073 still produces geosmin [35]. Further studies revealed that nerolidyl diphosphate is not an intermediate of geosmin biosynthesis [36], but rather of isolepidozene which was isolated from a site-directed mutant of *S. avermitilis* (S233A) [34••]. Jiang *et al.* clarified that the latter reaction comprises a release of acetone via retro-Prins fragmentation and an involvement of a 1,2-hydride shift of the bridgehead hydrogen exclusively into ring B [37•]. It should be mentioned that in cyanobacteria a comparable germacradienol/geosmin synthase (FJ010203) has been discovered and characterized [38].

The investigation of two enzymes that are responsible for a two-step conversion of geranyl diphosphate (GPP) to 2-methylisoborneol in *S. coelicolor* and *Nannocystis exedens* resulted in the unprecedented finding that C-methylation of GPP takes place before its cyclization to generate (*E*)-methylisoborneol [39,40••].

A novel epi-isozizaene synthase (SCO5222) from *S. coelicolor* was recently discovered [41•]; epi-isozizaene is an intermediate of albaflavenone biosynthesis, and the mechanism for the cyclization of FPP to epi-isozizaene was examined by the incubation of the recombinant enzyme with deuterated FPP derivatives. On the basis of the isotope-labeling results a biosynthetic pathway to albaflavenone was proposed involving the oxygenase

**Figure 4**




Recently delineated isoprenoid biosynthetic pathways with key mechanistic steps highlighted. Enzymes and cofactors in brackets are not experimentally proven. Postulated structures given in brackets are consistent with the labeling of products isolated and analyzed after incubation of the respective enzyme with deuterated biosynthetic precursors. **(a)** Cyclization of farnesyl diphosphate to geosmin catalyzed by the bifunctional germacradienyl/geosmin synthase from *Streptomyces coelicolor* and *Streptomyces avermitilis* with a focus on H-1 ($H_a/H_b$) hydride migration. Action of the N-terminal or C-terminal domain of this enzyme is highlighted [32,33•,34••,37•]; **(b)** biosynthesis of (E)-2-methylisoborneol in *Streptomyces coelicolor* comprises an unprecedented methylation of geranyl diphosphate before its cyclization to the product [39]; **(c)** cyclization of farnesyl diphosphate to epi-isozizaene and further reaction steps to yield albaflavenone in *Streptomyces coelicolor* [41•,42]; **(d)** demonstration of pentalenolactone biosynthesis in *Streptomyces avermitilis* including isolated intermediates; [9•,43,45,46]. *S*-Adenosyl methionine (SAM), α-ketoglutarate (α-KG).

gene CYP170A1 which is clustered with the SCO5222 encoding gene [42].

Recent investigations of enzymes responsible for the biosynthesis of pentalenolactone have been reported, and the function of PtlA as a pentalenene synthase converting FPP to pentalenene has been experimentally proven [9•]. Studies on enzymes linking pentalenene to pentalenolactone enabled the isolation of a variety of oxidized metabolites that are plausible intermediates in the conversion. PtlI, a cytochrome P450 monooxygenase, converts the 13-methyl group of pentalenene to the 13-aldehyde [43]. PtlH, whose crystal structure has been solved [44•], catalyzes the hydroxylation of 1-desoxypentalenic acid [45] to yield 1-deoxy-11β-hydroxypentalenic acid. The short chain dehydrogenase PtlF is responsible for the formation of 1-deoxy-11-oxopentalenic acid [46] that putatively serves as substrate for PtlE that is believed to catalyze lactone formation via a Baeyer–Villiger-like oxidation.

## Conclusions

During the last five years the identification and sequencing of many biosynthetic gene clusters for bacterial terpenoids has provided invaluable information about the origin of IPP units, and of the complex cyclization processes and tailoring reactions responsible for the exceptional structural diversity of the isoprene metabolites. In the process new enzymes have been identified and novel reaction mechanisms elucidated. The future of bacterial terpenoid research lies in extending further our understanding of key enzyme mechanisms, including their ability to channel reactive intermediates selectivity toward a single product, and in unraveling the complex interactions of the enzymes that make up the isoprenoid biosynthetic machinery.

## Acknowledgements

This work was supported by a BMBF (GenoMik-plus) grant to A.B., and by a stipend from a Landesgraduiertenförderung Baden Württemberg grant to M.D.

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- •• of outstanding interest


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