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FIRST REACTIONS

Bacteria Do It Differently: An Alternative Path to Squalene

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In eukaryotes, squalene serves as the precursor to the tetracyclic sterols like cholesterol, whereas in bacteria squalene is more commonly converted to pentacyclic hopanoids such as hopane (Figure 1A). Sterols and hopanoids are both key components to order lipid membranes and reduce permeability.[1] The head-to-head condensation of two molecules of farnesyl diphosphate (FPP) to squalene (SQ) is catalyzed by squalene synthase (SQase) in eukaryotes and some bacteria. The enzyme first generates presqualene diphosphate (PSPP), which is then converted by the same enzyme to squalene via an NADPH-dependent reductive rearrangement reaction (Figure 1B, black arrows). Poulter and co-workers now report in this issue a new pathway to squalene that appears widespread in bacteria.[2] The new pathway requires three enzymes and has both similarities and clear differences with the previously characterized route. This discovery provides new opportunities for selective inhibition of hopanoid biosynthesis, offers interesting insights into the evolution of these pathways, and reveals an intriguing new enzyme that catalyzes an unusual biosynthetic reaction. Many bacteria have gene clusters dedicated to hopanoid biosynthesis. These clusters contain genes for FPP synthesis as well as the cyclization steps that convert squalene to the cyclic hopanoid structures. They also contain two copies of squalene synthase-like genes (*hpnCD*), as well as a gene of unknown function (*hpnE*). The authors expressed in *Escherichia coli* the proteins encoded by these genes from two Gram negative bacteria, *Rhodopseudomonas palustris* and the ethanol producing *Zymomonas mobilis*. *In vitro* investigation of their activities with a variety of potential substrates revealed that one of the squalene synthase homologues (HpnD) converts two molecules of FPP into PSPP (Figure 1B). HpnC then takes PSPP and converts it to

Wilfred van der Donk discusses the revelation from Poulter and co-workers that bacteria have a different way to make squalene involving three novel genes.

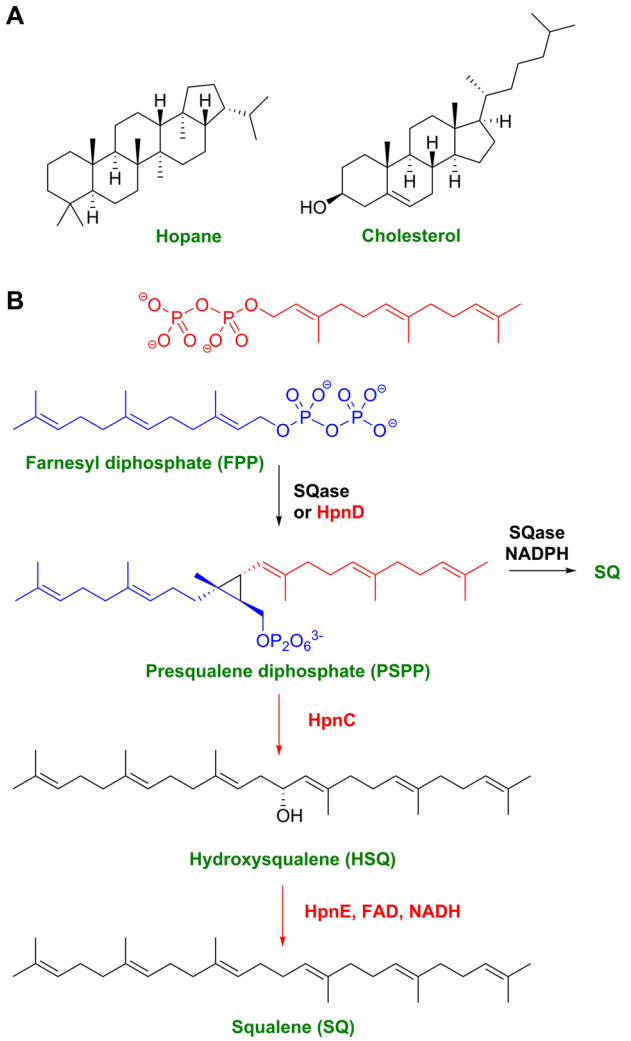

Figure 1. A. Structures of the triterpenes cholesterol and hopane as representative examples of the sterol and hopanoid families that are formed from squalene. B. In eukaryotes, squalene is generated by the condensation of two molecules of farnesyl diphosphate to first generate presqualene diphosphate that undergoes a reductive NADPH-dependent rearrangement to form squalene (black arrows). In bacteria, HpnD converts FPP to PSPP analogous to the squalene synthase reaction. However, HpnC promotes the hydrolytic rearrangement of PSPP to hydroxysqualene, and HpnE then catalyzes the unusual FAD dependent reduction to squalene (red arrows).

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hydroxysqualene (HSQ). Since a typical hopanoid cyclase did not take HSQ as substrate, the authors surmised that the poorly characterized gene product HpnE might convert HSQ into SQ, a previously unknown reaction. They demonstrate that HpnE is indeed an FAD-dependent enzyme that carries out the postulated reaction (Figure 1B). The conclusions reached from these *in vitro* experiments regarding the functions of the enzymes were then corroborated by coexpression of these proteins in *E. coli*, a heterologous host that does not make squalene. HpnCDE were shown to be required and sufficient for squalene biosynthesis. Thus, a new and unanticipated three-enzyme pathway to squalene was uncovered; further genome analysis showed that it is present in both Gram positive and Gram negative bacteria. Based on precedent in other pathways, the authors suggest that the cyclopropanation chemistry carried out by both squalene synthase and HpnD may have evolved from isoprenoid chain elongation enzymes. Although both enzymes probably descended from a common ancestor, the sequences of the canonical squalene synthases and HpnD and its orthologues are quite divergent and they form distinct clades in a phylogenetic tree. The divergence of the two pathways occurs in the transformations of PSPP, with squalene synthase carrying out a reductive rearrangement reaction to squalene and HpnC a hydrolytic rearrangement to hydroxysqualene.

The new pathway requires three enzymes and has both similarities and clear differences with the previously characterized route.

This part of the new pathway raises several questions for future investigations. The acquisition of NADPH binding by squalene synthase is the critical event that allowed squalene synthesis by a single enzyme. Unfortunately, the manner by which squalene synthase binds NADPH is not well understood as the enzyme lacks the typical pyridine dinucleotide binding motif and cocrystal structures have not yet been solved.[3,4] Similarly, the mechanism of selective hydrolysis by HpnC is not yet understood. Hydroxysqualene is also formed by squalene synthase when NADPH is withheld (among a range of other products).[5] The manner by which the HpnC controls the presumably stereospecific hydrolysis reaction will therefore be of interest. Finally, perhaps the most interesting reaction is the reductive displacement of the hydroxyl group of hydroxysqualene by HpnE. The authors propose that perhaps the reduced flavin may activate the alcohol leaving group by protonation resulting in a resonance-stabilized cation that can be reduced by the dihydroflavin anion, a sequence of events that has similarity with other transformations in isoprenoid biosynthesis. Although hopanoids are not essential for growth in several strains of bacteria under normal growth conditions, growth defects were observed under stressed conditions.[6−8] Hence, the different pathway to squalene in bacteria may perhaps be a potential target to selectively disrupt the integrity of cell membranes or facilitate the action of membrane disrupting agents. This may be particularly promising as a new approach to combat Gram negative bacteria that contain the three-enzyme pathway.

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