serted sulfur atoms [19], these in vitro studies were not wholly consistent with this premise. The authors were unable to show formation of a lipoyl-ACP intermediate by conducting the reaction in the absence of LipB and/or apo-PDC.

The article by Zhao et al. in last month's issue of *Chemistry & Biology* firmly establishes that the preferred substrate for LipA is not octanoyl-ACP but octanoyl-E2 [20]. By extension of this finding, it can be assumed that LipA has multiple substrates, which are the lipoyl-accepting domains of PDC, KGDC, BCDC, and the H protein of GCS. The strength of their work lies in the inclusion of both in vivo and in vitro experiments in establishing this finding. The authors use strains of *E. coli* with null mutations in *lipA*, *lipB*, and *fadE* to incorporate exogenous deuterated octanoic acid into the lipoyl-accepting domains of a plasmid-encoded PDC. The authors then induce lipoyl synthase activity via transduction of the culture with phage λ particles containing a *lipA* cosmid. Analysis of the isolated E2 domains by mass spectroscopy revealed that the deuterium-containing octanoyl groups had become lipoylated. No labeled lipoyl domains were observed in cultures that were not transduced with the *lipA* cosmid.

The authors arrive at the same conclusion in in vitro studies using purified LipA. They monitored cleavage of SAM into methionine and 5′-deoxyadenosine as well as lipoyl-E2 formation. Significant production of 5′-deoxyadenosine was observed when octanoyl-E2 was the starting substrate, while only trace amounts were observed when octanoyl-E2 was replaced with octanoyl-ACP. Moreover, lipoyl-E2 production was observed only in the presence of octanoyl-E2.

The enzymology of sulfur insertion into unactivated C-H bonds is unchartered territory and portends new and exciting chemistry to be unraveled. The seminal experiments of Miller et al. suggest that the immediate sulfur donor is already associated with the protein, since activity was observed in the absence of exogenous sulfur sources [6]. Lipoyl synthase has two sets of conserved cysteine residues. One set contains the motif that is common to all Radical SAM enzymes, while the second set resides in a CXXXXCXXXXXC motif, which is common only to lipoyl synthases. In analogy to biotin synthase, this motif could house a second iron-sulfur cluster that acts as the sulfur donor in the reaction [18]. The intermediates and sequence of events associated with sulfur insertion are presently only speculative in both of these enzyme systems. In addition, the exact mechanism by which SAM is cleaved to generate a 5′-dA• is currently unknown in all Radical SAM enzymes [16]. The conclusions reached by Zhao et al. now enable these questions to be addressed because they resolve the major issue and limitation associated with lipoyl synthase: the nature of the true substrate.

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Profound Insights into Squalene Cyclization

In this issue of *Chemistry & Biology*, our understanding of the formation of pentacyclic hopene from the linear squalene is enhanced by an X-ray structure of a complex between squalene-hopene cyclase and the substrate analog 2-azasqualene [13].

A classic example of the interface between chemistry and biology is the carbocationic transformation of squalene and (*3S*)-2,3-oxidosqualene to polycyclic triterpenes. In 1955, seminal papers were published that described the chemical mechanism of the cyclization reaction [1, 2]. These studies highlighted important reac-

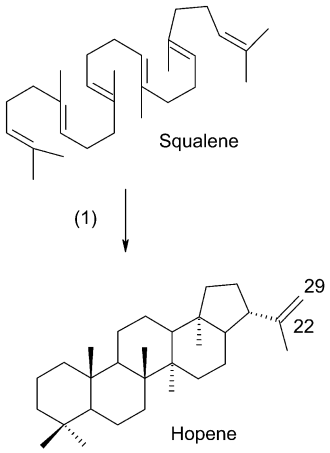

Figure 1. Scheme Depicting the Cyclization of Squalene to Hopene by the Squalene-Hopene Cyclase

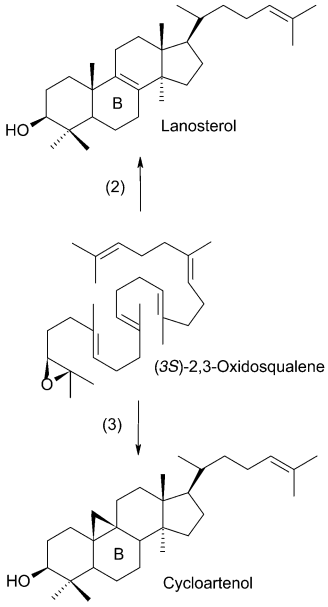

Figure 2. Scheme Depicting the Cyclization Reactions Carried Out by Oxidosqualene-Lanosterol Cyclase (2) and Oxidosqualene-Cycloartenol (3)

Both enzymes use (S)-2,3-oxidosqualene as a substrate.

tions carried out by the prokaryotic squalene-hopene cyclase (SHC) (Figure 1, 1), the oxidosqualene-lanosterol cyclase (Figure 2, 2), and the oxidosqualene-cycloartenol cyclase (Figure 2, 3).

These and other cyclization reactions generate a treasure trove of more than 100 triterpenes, which are important by themselves or after further conversion as plant surface components, phytoalexins, membrane rigidifiers, raft components, hormones, and pheromones. Furthermore, some triterpenes serve as molecular fossils, facilitating dating and diagenesis of early membrane-bound life trapped in sediments [3]. The oxygen component of oxidosqualene (see Figure 2) is introduced by a monooxygenase reaction from molecular oxygen. Since molecular oxygen appeared in significant concentrations only relatively late on our planet, i.e., after the invention of oxygenic photosynthesis by cyanobacteria, all compounds derived from oxidosqualene are considered late innovations in evolution [4]. Consequently, it is logical to propose that most of them were not necessary for early forms of life.

As shown in Figure 1, nature had already invented an oxygen-independent pathway for synthesizing cyclic triterpenes. In a large number of *Bacteria* (not yet in *Archaea*), squalene is directly cyclized to hopene by SHC (Figure 1), an evolutionary progenitor of oxidosqualene cyclases [4]. Strong evidence for this comes from phylogenetic trees for eukaryotic and prokaryotic sterol cyclases, which have their root in SHC [5].

Nature's one-step reactions are fascinating in their complexity: in each of the cyclization reactions discussed here, four to five rings were formed, seven to nine stereocenters were established, and 14 or more covalent bonds were opened and closed. However, the squalene-hopene cyclase also generates side products [6], including diplopterol and, in significantly lower concentrations, a variety of 6,6,6,5-tetracyclic compounds. Such inefficiency suggests that a triterpene may not necessarily be produced by one specific cyclase in an organism.

An interesting feature of the SHC reaction is that its high exothermicity of 40–50 kcal/mol exceeds by far the usual protein stabilization energy [7]. Seven to eight nontandem repeat motifs (QW motifs) seem to help the enzyme maintain its integrity [7, 8]. The energy released may actually help to melt lipophilic side chains in the channel through which the bulky product exits. The very low turnover number of 0.3^{-sec} correlates with the intricate structure of the enzyme. It is conceivable it takes a long time to thread squalene into and through the channel, to fold the compound correctly in the catalytic cavity, and for pentacyclic hopene to leave the cavity after transformation.

To date, the most extensively studied triterpene cyclase is SHC from the thermophilic bacterium *Alicyclobacillus acidocaldarius*, which is easily cultured at 60°C [9, 10]. Georg Schulz and coworkers solved the X-ray structures of this enzyme at 2.9 and 2.0 Å resolution [8, 11, 12]. However, our understanding of the nature of the specific interaction between squalene and the enzyme catalytic cavity has been hampered by the lack of a crystallized enzyme:substrate complex.. In this issue of *Chemistry & Biology*, the Schulz group presents data that overcomes this obstacle by cocrystallizing SHC with 2-azasqualene, a very near structural analog of squalene [13] (Figure 3).

The structure of the folded substrate is now "visible" at 2.13 Å resolution, and the amino acid environment of the catalytic cavity that houses its carbon skeleton is established, permitting a better interpretation and calculation of properties of existing mutant SHCs ([14, 15] and citations therein) and even of mutant oxidosqualene cyclases ([16] and citations therein). Furthermore, the cyclization products of squalene analogs can now better be predicted using this information ([15] and citations therein). Significantly, this cocrystal structure has implications for the pharmaceutical sector, facilitating ratio-

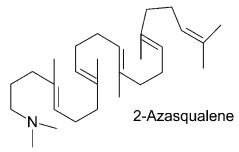

Figure 3. The Structure of 2-Azasqualene

nal design of sterol cyclase inhibitors as hypercholesteremic drugs.

Energetic and kinetic aspects of the SHC reaction are profoundly discussed in a very recent paper by Rajamani and Gao [17]. Reinert et al. [13] and Rajamani and Gao should be read together, because they nicely complement each other. The first article gives us a more static and less dynamic view of the reaction as compared to the second paper, which attempts to simulate the enzyme dynamics and energetics. Both papers agree that the 6,6,5-cyclic carbocation is not an intermediate during cyclization, but they disagree on the role played by the 6,6,6,5-cyclic carbocation. For the Schulz group, it represents an intermediate side product, and for Rajamani and Gao, a minor dead-end side product.

Two questions remain to be solved for oxidosqualene cyclases producing sterols. (1) How do sterol cyclases induce the boat conformation in ring B? (2) How do the sterol cyclases manage to orient the substrate oxidosqualene when it enters the catalytic cavity? Substrate orientation should not be problematic for the SHC, because the substrate is symmetrical and therefore it does not matter which end reaches the protonation site first. But in contrast, if one offers SHC oxidosqualene as an alternative substrate, cylization always begins from the oxido end [18]. Surprisingly, the enzyme retains a mechanism to select for the "correct" site.

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New Probes for Microtubule Dynamics

A phenotype-based screen identifies a purine analog, named diminutol, that perturbs the microtubule cytoskeleton in cells. An affinity-based approach identifies a protein target of this small molecule and leads to the characterization of a new pathway that may regulate cytoskeleton dynamics.

The treatment of cells with the small molecule colchicine, a natural product, results in dramatic phenotypes in dividing cells. In early studies these phenotypes were described as "explosions" of the mitotic apparatus (reviewed in [1]). Ed Taylor and coworkers used an affinity-based approach to identify the protein target of colchicine, and their research led to the discovery of tubulin [2, 3]. This landmark work, carried out in the 1960s, involved the use of a small molecule to unravel a key biological mechanism. In appreciation of the similarity between such a strategy and conventional genetics, in which one modulates protein function by introducing mutations in genes rather than by using cell-permeable small molecules, the term "chemical genetics" has been coined [4, 5]. Recently, several examples of the successful application of chemical genetics in the examination of a range of biological processes have been reported (for example, see [6]). Using phenotype-based screens and a battery of powerful in vitro and cell-based assays,