cent theoretical studies of the effects of chaos, both classical and quantum, in asymmetric resonant cavities (*10*).

There are also examples of microlaser structures that were first demonstrated with inorganic semiconductors and were subsequently implemented with organics. The GaAs and InP microdisk lasers have the very small volumes and low-threshold power and current densities (*11*). The lasing modes of these thumbtack-shaped lasers are also whispering-gallery modes. Microdisk lasers with molecular doped polymer (*12*) and sublimed organic active layers (*13*) have been recently reported. These microlasers are of interest because of their simple fabrication and potential to lower threshold pump powers as a result of strong emission coupling to low-loss microcavity resonances.

In addition to being useful in investigating the properties of novel resonators, organic solid-state gain media have been considered for possible replacement of liquid dye lasers. This goal was one of the motivations for the earliest work on light amplification in organic films (*1*) and is still an area of active research, and some solid-state organic laser dye materials have been marketed. However, their durability has not been adequate to lead to large-scale replacement of liquid dye lasers.

Developments in organic laser dyes also influenced the design of efficient thin-film organic light-emitting diodes (LEDs) (*14*), in which the emissive layer consists of tris (8-hydroxyquinolinato) aluminum (Alq) doped with small amounts (~1%) of a laser dye such as DCM. The excited states created electrically in the host Alq excite the dye dopant through a process known as Förster energy transfer. The resultant emission spectrum is primarily that of the dye, and the quantum yield is enhanced with respect to that of a film of only the host Alq. Such dye-doped emissive layers are used in the most efficient and reliable organic LEDs reported to date (*15*). Advances in efficiency and lifetime have also been made in LEDs with conjugated-polymer emissive materials (*16*). These improvements in LED performance characteristics have stimulated fresh interest in organic laser research. For example, advances in conjugated-polymer design and synthesis have led to several recent reports of stimulated emission from films that can have high photoluminescence quantum yields and high optical gain (*16, 17*).

Electrically driven lasing in organics is being eagerly sought. Organic materials generally have emission spectra that are red-shifted with respect to their absorption spectra, which makes it easier to create population inversion in comparison with inorganic semiconductors, which must first be made transparent because the large absorption in the unpumped state is several orders of magnitude higher than that in organics. The main difficulty in realizing organic diode lasers is that the low carrier mobilities in these materials make it difficult to create carrier densities high enough to generate the gain necessary to overcome losses in the laser cavity. There are other challenges as well: The device technology is still in its infancy. Important design issues have to be addressed and advances in fabrication technologies are needed. There are encouraging developments. Progress has been made in achieving optically pumped laser action in materials or material combinations that are capable of charge transport (*13, 16, 17*). Photopumped lasing has been demonstrated in resonator structures such as microdisks and planar microcavities, which are quite easily adapted for electrical injection. However, much remains to be done, and considerable effort is being expended to bridge the gap that exists between present technology and a diode laser. This laser research should help LED technology as well.

Organic laser materials, particularly the recently improved materials, will continue to be useful in researching the properties of resonators. There is currently much interest in lasers based on the photonic band-gap concept, where the separation between theory and experiment is remarkably wide (*18*). Organic gain media have a number of properties that are particularly suitable for these geometries. For example, they can be quite easily made to fill the subwavelength-sized holes and crevices that are characteristic features of photonic band-gap structures. The accelerated pace of research will make the future of organic solid-state lasers at least as interesting as the past.

References

1. B. H. Soffer and B. B. McFarland, *Appl. Phys. Lett.* **10**, 266 (1967).
2. P. P. Sorokin and J. R. Lankard, *IBM J. Res. Dev.* **10**, 162 (1966).
3. H. Kogelnik, and C. V. Shank, *ibid.* **18**, 152 (1971).
4. I. P. Kaminov, H. P. Weber, E. A. Chandross, *ibid.*, p. 497.
5. M. Nakamura *et al.*, *ibid.* **22**, 515 (1973).
6. D. R. Scifres *et al.*, *ibid.* **25**, 203 (1974).
7. F. De Martini *et al.*, *Phys. Rev. Lett.* **59**, 2955 (1987).
8. H. Yokoyama, *Science* **256**, 66 (1992).
9. S.-X. Qian *et al.*, *ibid.* **231**, 486 (1986).
10. J. U. Nockel and A. D. Stone, *Nature* **385**, 45 (1997).
11. S. L. McCall *et al.*, *Appl. Phys. Lett.* **60**, 289 (1992); R. E. Slusher *et al.*, *ibid.* **63**, 1310 (1993).
12. M. Gonokami *et al.*, *Opt. Lett.* **20**, 2090 (1985).
13. M. Berggren *et al.*, presented at the Materials Research Society Meeting, San Francisco, CA, 31 March 1997.
14. C. W. Tang *et al.*, *J. Appl. Phys.* **65**, 3610 (1989).
15. C. W. Tang, *SID96 Digest* (Society for Information Display, Santa Ana, CA, 1986), p. 181.
16. R. H. Friend *et al.*, *Solid State Commun.* **102**, 249 (1997).
17. F. Hide *et al.*, *Science* **273**, 1833 (1996); N. Tessler *et al.*, *Nature* **382**, 695 (1996); S. V. Frolov *et al.*, *Phys. Rev. Lett.* **78**, 729 (1997).
18. J. D. Joannopoulos *et al.*, *Solid State Commun.* **102**, 165 (1997).

BIOCHEMISTRY

Creating Isoprenoid Diversity

James C. Sacchettini and C. Dale Poulter

Nature relies on an intricate network of biosynthetic pathways to produce the cornucopia of small organic molecules needed to support life. Among these, the isoprenoids are extraordinarily diverse in chemistry and structure. Over 23,000 individual isoprenoid compounds have been characterized, and hundreds of new structures are reported each year. They serve as visual pigments, reproductive hormones, defensive agents, constituents of membranes, components of signal transduction networks, mating pheromones, and photoprotective agents, to name only a few of their many roles. Abnormalities associated with the pathway can cause coronary heart disease and cancer, while at the same time some isoprenoid compounds, such as taxol, offer promise as potent new drugs.

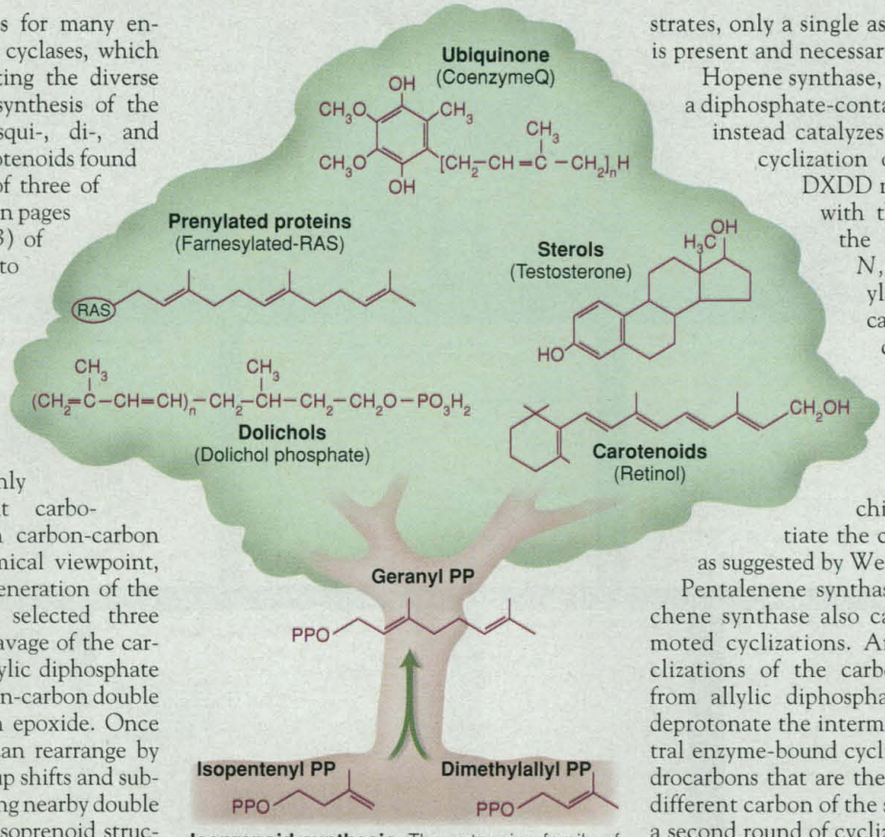
Most of the molecular diversity in the isoprenoid pathway is created from the diphosphate esters of simple linear polyunsaturated allylic alcohols such as dimethylallyl alcohol (a 5-carbon molecule), geraniol (a 10-carbon molecule), farnesol (a 15-carbon molecule), and geranylgeraniol (a 20-carbon molecule). The hydrocarbon chains are constructed one isoprene unit at a time by addition of the allylic moiety to the double bond in isopentenyl diphosphate, the fundamental five-carbon building block in the pathway, to form the next higher member of the series (see the figure). Geranyl, farnesyl, and geranylgeranyl diphosphates lie at multiple branch points in the isoprenoid

J. C. Sacchettini is in the Department of Biochemistry and Biophysics, Texas A & M University, College Station, TX 77843-2128, USA. E-mail: sacchett@seabass.tamu.edu. C. D. Poulter is in the Department of Chemistry, University of Utah, Salt Lake City, UT 84112-0858, USA. E-mail: poulter@chemistry.utah.edu

pathway and are substrates for many enzymes. These are primarily cyclases, which are responsible for generating the diverse carbon skeletons for the synthesis of the thousands of mono-, sesqui-, di-, and triterpenes; sterols; and carotenoids found in nature. The structures of three of these cyclases are reported on pages 1811, 1815, and 1820 (*1*–*3*) of this issue, yielding clues to how this is accomplished.

The chain elongation and cyclization reactions of isoprenoid metabolism are electrophilic alkylations in which a new carbon-carbon single bond is formed by attaching a highly reactive electron-deficient carbocation to an electron-rich carbon-carbon double bond. From a chemical viewpoint, the most difficult step is generation of the carbocations. Nature has selected three strategies for catalysis—cleavage of the carbon-oxygen bond in an allylic diphosphate ester, protonation of a carbon-carbon double bond, or protonation of an epoxide. Once formed, the carbocations can rearrange by hydrogen atom or alkyl group shifts and subsequently cyclize by alkylating nearby double bonds. Diverse families of isoprenoid structures, often formed from the same substrate in an enzyme-specific manner, are thought to arise from differences in (i) the way substrate is folded in the active site, (ii) how carbocationic intermediates are stabilized to encourage or discourage rearrangements, and (iii) how positive charge is quenched when the product is formed.

Only a handful of the hundreds of enzymes involved in isoprenoid chain elongation and cyclization have been studied, and genetic information is available for only a subset of these. DNA and amino acid sequence comparisons offer only a tantalizing hint of possible evolutionary relationships among isoprenoid enzymes that catalyze some of the chain elongation and cyclization reactions. Although there is little overall similarity between amino acid sequences for the chain elongation and cyclization enzymes, proteins from both classes that use allylic diphosphates as substrates contain highly conserved aspartate-rich DDXXD motifs (D is aspartate, X any amino acid) thought to be Mg^{2+} binding sites. Anecdotal studies established that farnesyl diphosphate synthase, a chain elongation enzyme, will cyclize its normal product to a sesquiterpene hydrocarbon under appropriate conditions (*4*). The potential relations among farnesyl diphosphate synthase, pentalenene synthase, epi-aristolochene synthase, and hopene synthase are brought into focus by the three papers in this issue



Isoprenoid synthesis. The extensive family of isoprenoid compounds is synthesized from two precursors—isopentenyl diphosphate and dimethylallyl diphosphate.

that describe crystal structures for three isoprenoid cyclases.

The cyclase domains of three isoprenoid cyclases as well as farnesyl diphosphate synthase [the only other isoprenoid biosynthetic enzyme whose three-dimensional structure is known (*5*)] have a similar structural motif, consisting of 10 to 12 mostly antiparallel α helices that form a large active site cavity. Lesburg *et al.* (*1*) have labeled this motif the "isoprenoid synthase fold." Indeed, the structural similarities may go well beyond the general composition and arrangement of the protein backbone, supporting an evolutionary relationship for at least some chain elongation and cyclase enzymes. Aspartate-rich clusters are present in all four proteins. The three enzymes that use diphosphate-containing substrates (pentalenene synthase, epi-aristolochene synthase, and farnesyl diphosphate synthase) all contain DDXXD on the walls of the active site cavity. The aspartates are involved in binding multiple Mg^{2+} ions, which stabilize binding of diphosphate groups in the substrates. Farnesyl diphosphate synthase has two of these motifs, one for the allylic substrate and the other for isopentenyl diphosphate. For the cyclases that use diphosphate substrates, only a single aspartate-rich region is present and necessary.

Hopene synthase, which does not use a diphosphate-containing substrate but instead catalyzes a proton-initiated cyclization of squalene, has a DXDD motif that interacts with the amino group of the bound inhibitor *N*,*N*-dimethyldodecylamine-*N*-oxide. Because squalene is uncharged, this region may be a remnant of a diphosphate-binding motif, or it may be part of the protonation machinery needed to initiate the cyclization reaction, as suggested by Wendt *et al.* (*2*).

Pentalenene synthase and epi-aristolochene synthase also catalyze proton-promoted cyclizations. After the initial cyclizations of the carbocations generated from allylic diphosphates, both enzymes deprotonate the intermediates to give neutral enzyme-bound cyclic sesquiterpene hydrocarbons that are then reprotonated at a different carbon of the substrate to initiate a second round of cyclizations. Starks *et al.* (*3*) have proposed that Asp^{444}-Tyr^{520}-Asp^{525} of epi-aristolochene synthase serves as a proton shuttle triad for both protonation and deprotonation reactions, whereas, in pentalenene synthase, His^{309} is proposed to serve the function of the triad. Thus, there are still a host of unsolved structural and mechanistic questions about these enzymes.

The similar folds for the chain elongation and cyclase enzymes are, as pointed out by Lesburg *et al.*, consistent with the hypothesis that enzymes that catalyze successive steps in a metabolic pathway evolve one from another. This is a particularly attractive scenario for the isoprene pathway where similar chemical reactions give rise to a variety of different metabolites. A careful alignment of structures for the chain elongation and cyclase enzymes may provide new insights not only about structure and mechanism but about evolutionary relationships.

References

1. C. A. Lesburg, G. Zhai, D. E. Cane, D. W. Christianson, *Science* **277**, 1820 (1997).
2. K. U. Wendt, K. Poralla, G. E. Schulz, *ibid.*, p. 1811.
3. C. M. Starks, K. Back, J. Chappell, J. P. Noel, *ibid.*, p. 1815.
4. A. Saito and H. C. Rilling, *Arch. Biochem. Biophys.* **208**, 508 (1981).
5. L. C. Tarshis, M. Yan, C. D. Poulter, J. C. Sacchettini, *Biochemistry* **33**, 10871 (1994).