Terpenoid Synthases—From Chemical Ecology and Forest Fires to Biofuels and Bioproducts

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Terpenoids are a diverse group of natural products with a myriad functions in ecological interactions of living organisms. Terpenoid synthases provide a unique reservoir of enzymes for the metabolic engineering of advanced biofuels and high-value bioproducts (McAndrew et al. in this issue of *Structure*).

The field of chemical ecology is concerned with the ecological interactions between living organisms mediated by chemicals produced in nature. This exciting field of science arose largely from a marriage of chemistry and biology to establish fundamental knowledge of the functions of naturally occurring chemicals in the biological world. With boundless opportunities for practical applications, curiosity-driven discovery remains a primary impetus for much of the modern research in chemical ecology. For example, interactions of plants with their living environment involve tens of thousands of specialized metabolites (i.e., secondary metabolites, natural products, or small molecules) produced in diverse metabolic systems. Prominent classes of specialized chemicals of plant origin include the terpenoids, alkaloids, glucosinolates, cyanogenic glycosides, and phenolics. These chemicals can serve a wide array of different functions, for example, in the protection of sessile plants against herbivores and pathogens, the attraction of pollinators or seed dispersing animals, or the orchestration of symbiotic or parasitic relationships of plants with microorganisms, animals, or other plants. While they may not contribute directly to plant growth and development, these specialized metabolites are essential for the survival of plants in their natural environments.

Another modern and interdisciplinary field of science receiving great attention deals with biofuels and bioproducts. It encompasses, among others, again the disciplines of chemistry and biology, as well as chemical and metabolic engineering. The same amazing array of tens of thousands of specialized small molecules that evolved in nature in ecological interactions of plants with other organisms are fair game for the green production of advanced biofuels and bioproducts in engineered and synthetic biological systems. This area of science is largely driven by practical needs to develop and improve production of desired small molecules for a society that may become less dependent on petrochemicals.

Terpenoids, or isoprenoids, are a highly diverse group of specialized chemicals produced in nature with a variety of functions in the chemo-ecological interactions of living organisms (e.g., Gershenzon and Dudareva, 2007). Terpnoids are also well known for an array of traditional and modern uses as pharmaceuticals, natural pesticides, fragrances, flavoring compounds, and a wide range of industrial raw materials (e.g., Bohlmann and Keeling, 2008). As low molecular weight hydrocarbons, natural terpenoids are also notorious for fueling forest fires, as anyone who has seen oleoresin (i.e., turpentine)-soaked conifer trees or oil-laden *Eucalyptus* landscapes go up in flames will know. The same reservoir of terpenoid natural products provides immense opportunities for the metabolic engineering and synthetic biology of advanced biofuels and high-value bioproducts (e.g., Peralta-Yahya et al., 2010). Key to some recent advances in terpenoid metabolic engineering are insights into the structures and functions of a diverse family of terpenoid synthase (TPS) enzymes that evolved in nature in a context of chemical ecology. TPSs, also known as terpene cyclases, produce the manifold core structures of terpenoids found in nature (Christianson, 2006). TPSs of plant origins have been explored for some considerable time as important enzymes for the microbial engineering of terpenoid metabolites with a particular focus on the cost-effective and sustainable production of natural products that are in high demand and short or unstable supply. Examples include the anticancer diterpenoid metabolite taxol (Ajikumar et al., 2010) or the antimalarial sesquiterpenoid artemisinin (Ro et al., 2006).

Recent work has highlighted some of the potentially superior qualities of terpenoid-based biofuels compared to conventional biofuels and identified a suite of opportunities for microbial production of terpenoid-based tailor-made, advanced biofuels using plant TPS enzymes (Peralta-Yahya et al., 2011). An enzyme of interest for the possible production of an alternative to D2 diesel is the sesquiterpene synthase (*E*)-α-bisabolene synthase (Peralta-Yahya et al., 2011). (*E*)-α-bisabolene synthase was first cloned and characterized more than a decade ago from the conifer tree grand fir (*Abies grandis*) in an effort to decipher the complex chemical defenses produced by conifers for their protection against pests and pathogens (Bohlmann et al., 1998). In the chemical ecology of conifers, (*E*)-α-bisabolene is a precursor to todomatuic acid, an insect juvenile hormone analog (Bohlmann et al., 1998); in the synthetic biology of biofuels, (*E*)-α-bisabolene is a direct precursor to the production of bisabolane (Peralta-Yahya et al., 2011).

In this issue, McAndrew et al. (2011) report the three-dimensional structure of grand fir (*E*)-α-bisabolene synthase. This work completes a triad of three-dimensional protein structures resolved for three-domain TPS enzymes, which also includes the two diterpene synthases taxadiene synthase for the formation of

taxol (Köksal et al., 2011a) and *ent*-copalyl diphosphate synthase for plant gibberellin hormone biosynthesis (Köksal et al., 2011b). These TPS enzymes share a common α-helical three-domain structure which is thought to represent the archetype structure from which all plant TPS enzymes may have evolved. Fundamental insights into the three-dimensional structures of a handful of simpler TPS enzymes have previously facilitated the successful in vitro evolution of new TPS functions (e.g., Greenhagen et al., 2006). Likewise, the specific new structures of the three-domain TPSs, such as those of (*E*)-α-bisabolene synthase and taxadiene synthase, may now allow for the improved in vitro evolution and metabolic engineering of enzymes for high-yield production of biofuels and bioproducts.

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