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Generating Novel Polyene Antifungal Drugs

In this issue of *Chemistry & Biology*, Francisco Malpartida and colleagues [1] report the formation of novel polyene amide derivatives upon transformation of the producer strain with SCP2*-derived vectors carrying the erythromycin resistance gene *ermE*. This unexpected finding provides a new tool for generating antifungal drugs by biotransformation.

Over the last two decades, infections caused by fungi have emerged as a growing threat to human health, especially in persons whose immune systems are compromised in some way. Human immunodeficiency virus infection, leukemia, immunosuppressive therapy, organ transplantation, and cancer chemotherapy contribute to the continuous increase of these numbers. Several classes of agents have been or are currently being evaluated for use in the treatment of invasive mycoses [2, 3], but despite this potentially wide arsenal of antifungal drugs, only a few agents are currently in use; these include the polyenes, the fluorocytosine, and the azole derivatives. One important problem preventing successful antifungal therapy is the dramatic increase in drug resistance, particularly against azole antimycotics and fluorocytosine [2], and this leaves polyene macrolides as the drugs of choice, since resistance to these agents is still a rare event. Polyenes, however, show toxic side effects and poor solubility in water; therefore, there is an urgent need for new, safer, broad-spectrum antifungal antibiotics with improved properties.

Polyene antibiotics are produced mainly by *Streptomyces*, a class of soil-dwelling filamentous bacteria. They are characterized by a hydroxylated macrocyclic lactone ring of amphipathic nature normally containing one sugar (few polyenes have no sugars, and others have two), but their distinct characteristic is the presence of a chromophore formed by a system of three to seven conjugated double bonds in the macrolactone ring [3]. This chromophore is responsible for the characteristic physicochemical properties of these antifungals, including strong UV-visible light absorption, photolability, and poor water solubility. It is generally accepted that the antifungal activity of polyenes is the result of binding to cytoplasmic membrane sterols [4], although the exact mechanism that drives the interaction is not fully understood. It has been proposed that polyenes interact with membrane sterols to form complexes sustained by hydrophobic interaction between the hydrophobic portion of the polyene (chromophore region) and the sterol, as a result yielding transmembrane channels [5, 6], which are responsible for the leakage of inorganic phosphate, small molecules, and monovalent ions, particularly K^+, thus resulting in cell death.

Polyenes belong to the ample and diverse group of macrolides, a class of macrocyclic polyketides [7, 8], and as such, they are synthesized by the sequential assembly of carbon chains from small acyl precursors, in a fashion that mechanistically resembles fatty acid biosynthesis. However, whereas in fatty acid biosynthesis each successive elongation step is followed by a complete sequence of reactions including ketoreduction, dehydration, and enoylreduction, in the synthesis of polyenes and other macrolides, the product of each decarboxylating condensation may undergo all, some, or none of the above-mentioned modifications. As a result, ketones, hydroxyl groups, double bonds, and saturated chains appear at defined positions of the polyketide chain. This process is catalyzed by a complex enzymatic system, the polyketide synthase (PKS). Polyene synthases are of a modular nature, being composed by multifunctional polypeptides that contain sets (or modules) of enzymatic domains for the condensation and reduction steps [9]. Each domain is generally used at a unique step in the biosynthesis, and the extent of processing depends upon the functional domains operating at a given cycle. There is, therefore, a direct correspondence between the catalytic domains present in the modules and the structure of the resulting polyketide product, which provides a powerful model for the rational design and engineered biosynthesis of novel polyketides through genetic manipulation [10, 11]. Biosynthesis can be initiated by using acyl CoA from primary metabolism as starter unit, but in other cases starters such as acetate and propionate can be generated by decarboxylation of malonyl and methylmalonyl groups attached to the so-called loading modules of PKSs. Other more unusual starter units

need to be activated as acyl adenylates before use as primers [12].

Once the polyene macrolide core has been formed, it undergoes a series of post-PKS enzymatic reactions to yield the final bioactive compound. In comparison with other macrolides, polyenes undergo few post-PKS modifications, but they usually require the addition of the special aminosugar mycosamine. It is also common to find a carboxyl group near the glycosylation site that is generated by oxidation of an exocyclic methyl branch, and some hydroxyl or epoxide groups in the polyol region that are not introduced by the PKS. In order to introduce these modifications on the aglycones resulting from the PKS, the polyene biosynthetic clusters express several genes required for mycosamine biosynthesis and glycosylation, and one or more genes (coding for cytochrome P450 monooxygenases and ferredoxins) needed for the specific oxidation of selected positions of the macrolactone ring [9].

The group led by Malpartida has investigated the genetic basis for the biosynthesis of two closely related polyenes, the tetraenes rimocidin and CE-108 in *Streptomyces diastaticus* var. 108 [13]. Both are derived from the same biosynthetic pathway, and only differ in the nature of the starting unit, acetyl-CoA for CE-108 or butyryl-CoA for rimocidin. This means that the loading module is capable of making a choice between two different starter units, a rare peculiarity that has also been reported for the avermectin synthase loading module [14]. Such versatility prompted Malpartida and colleagues to manipulate the gene, *rimA*, that codes for the loading module PKS. The gene was thus cloned into a low copy-number *Streptomyces* vector (pIJ922, an SCP2*-derivative) together with the erythromycin resistance gene *ermE* for the selection of recombinants, and upon transformation of either the parental strain or a *rimA*-disrupted mutant, two novel tetraenes were detected and chemically characterized as the amides of the parental carboxylic acid tetraenes naturally produced by *S. diastaticus*. This finding was totally unexpected, since according to the canonical biosynthetic model, no involvement could be predicted for RimA in the formation of the carboxylic group. Further investigation led to the realization that the generation of the new molecules was actually triggered by the introduction of certain SCP2*-derived vectors, such as pIJ922 or pIJ941, containing the *ermE* gene and that both genetic elements were required [1]. Excitingly, both compounds are bioactive and show increased antifungal activity and reduced hemolytic effect compared to their parental molecules.

The authors propose two plausible mechanisms to explain the formation of the new metabolites. In one, a putative amidotransferase that is poorly expressed under natural conditions would be used as a tailoring enzyme. This is an interesting possibility, although given that the amide derivatives show higher antifungal activity than their parental molecules, one could expect that evolution would have favored a higher expression of such enzyme. Surprisingly, in nature the occurrence of such modifications is rare. The second explanation involves the use of malonamyl-CoA by module 7 of the PKS instead of methylmalonyl-CoA through a nondecarboxylating Claisen condensation. This would imply (1) a broad specificity for module 7 acyl carrier protein transacylase domain (AT), being able to accept both substrates, a property not previously observed for extender ATs [15], and (2) the ability of module 7 ketosynthase domain to catalyze two different types of Claisen condensation (decarboxylating and nondecarboxylating) depending on the substrate, also an unprecedented feature.

Obviously, we are far from understanding the mechanism that drives the conversion of the free carboxylic group into an amide, and this is an exciting challenge for the future. If such a mechanism proves to be present in strains that produce polyenes carrying carboxyl functionalities, the generation of amide derivatives will become a straightforward process.

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