including incorporation of unnatural amino acids [19], protein grafting has proven successful in imparting DNA binding selectivity in naturally nonselective proteins, as exemplified by Turner et al. and others [1, 2, 9, 18]. Certainly, a critical requirement for future application of miniature DNA binding peptides is high selectivity for target DNA sites.

The recent advancements in the creation of miniature DNA binding protein hold promise for probing protein folding or for use as artificial transcription factors. To provide even more control, the future generations of miniature DNA binding proteins might incorporate small molecule control of DNA binding or couple with additional activities, like DNA cleavage. For example, fusion of the DNA binding element of the *engrailed* homeodomain peptide with EF hand Ca loop binding domain resulted in the creation of DNA nuclease [20]. In this example, the helix stabilization, DNA binding, and DNA cleavage were dependent on the addition of lanthanide metal, providing an additional level of control for potential applications. In addition, the lanthanide metal mediated DNA cleavage, providing useful activity in a miniature DNA binding protein. As a necessary step toward applications in vivo, like artificial transcription factors, miniature DNA binding proteins must be tested for activity in mammalian cells. Although further development is required, miniature DNA binding proteins represent an exciting tool for exploring various biological and medicinal problems.

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Don't Classify Polyketide Synthases

Polyketide synthases are intensively studied as metabolite factories generating diverse biologically active natural products. Contrary to their current classification as different "types," there is now a growing body of evidence illustrating that nature realized limitless transitional stages during evolution.

Polyketides are a remarkable class of natural products. In addition to an enormous range of functional and structural diversity, they exhibit a wide range of biological activities, applied in the agrochemistry and pharmaceutical industries, which triggered research aimed at a molecular understanding of their biosynthesis. Since the cloning of the first sets of polyketide synthases (PKS) from streptomycetes, numerous different "types" of PKS genes have been identified from a variety of biological sources, mostly bacteria, fungi, and plants, but recently also from protists [1–4]. Although investigations revealed that PKSs show some striking similarities to fatty acid synthase, a more or less systematic approach has been taken to classify the different types of systems based on their source or products as well as biochemical or genetic data.

Recently, it has become evident that transition states between differently classified biosynthetic systems exist. Nature's ingenuity for producing natural products is obviously not restricted to "classes" of natural product biosynthetic systems. For example, the "parallel and convergent universes of PKS and nonribosomal peptide synthetases" (NRPS) [5] were only recognized a few years ago after the first true hybrid PKS/NRPS systems with translationally fused PKS and NRPS modules were

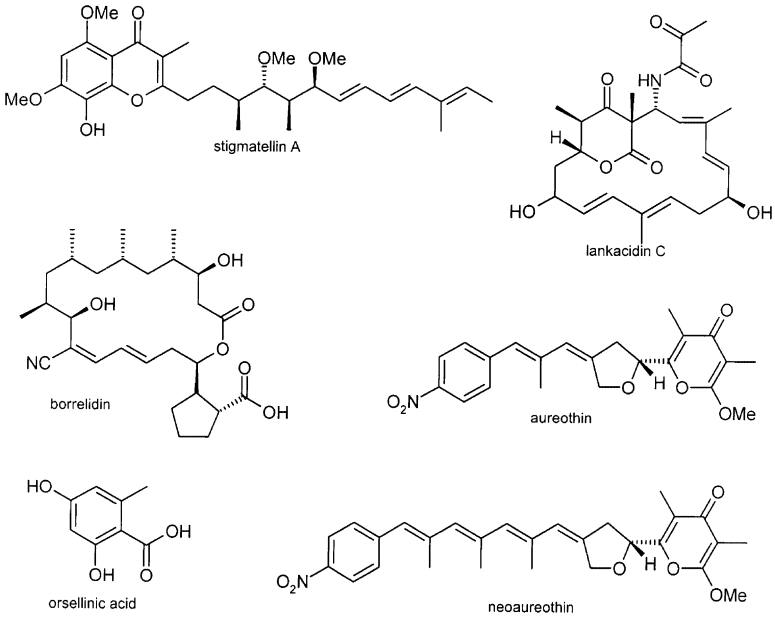

Figure 1. Bacterial Polyketides Synthesized by Type I PKS Acting Iteratively (Not Yet Shown for Neoaureothin)

reported [6–8]. Looking back, one could have argued that some natural PKS systems are also hybrids, because they harbor domains with similarity to peptide synthetases [9, 10].

Another dogma says that type I (or modular) bacterial enzymes act noniteratively, whereas fungal modular enzymes perform iterative rounds of chain extension. In general, in a bacterial modular PKS a correlation exists between the number of modules and the number of extender units used. This correlation is termed colinearity. Bacterial type II (also termed "aromatic" PKS) systems are presumed to produce aromatic compounds (another dogma), whereas type I machineries make lactones, lactames, amides, or free acids. The first evidence for a bacterial iterative type I system was reported by the Bechthold group [11]. AviM from *S. viridochromogenes* strongly resembles fungal PKSs, and it was shown to be an orsellinic acid (Figure 1) synthase by heterologous expression. Next, iterative use of single modules within a modular type I PKS was described and shown to result in the formation of octaketides instead of the usual heptaketides during erythromycin biosynthesis [12]. This process was termed "stuttering," because it was regarded as an aberrant process leading to the production of side products. Stuttering presumably also occurs during epothilone biosynthesis, as 18-membered macrolides occur as minor components in the culture broth of the producing strain [13].

Subsequently, the sequence of the stigmatellin megasynthetase was reported [14]. It represents the first example for a type I system in which iteration occurs as a programmed event by using one module twice. The second peculiarity of this modular system is that it forms a chromone structure, which contradicts the dogma that only type II systems produce aromatic rings. The possibility that the missing module is located elsewhere in the chromosome could not be fully excluded. Nevertheless, a genomic screen for the presence of type I PKS loci and the inactivation of every identified PKS gene cluster in the chromosome of the stigmatellin producer did not affect production [15, 16]. The second example for programmed iterative use of modules within bacterial type I PKS came from the lankacidin biosynthetic gene cluster, which is encoded on a megaplasmid [17]. The latter fact again makes it unlikely that the "missing module" is encoded in the chromosome. Here, eight condensation reactions are performed by five ketosynthase domains, which raises the question of how the number of extension cycles are programmed within the PKS.

Due to the reports from the groups of Salas/Leadlay/Biotica [25] and Hertweck [26] in this issue of *Chemistry & Biology* and last month's issue, respectively, there is now a growing body of evidence that type I PKS modules are indeed used iteratively in bacteria. He and Hertweck show directly, by heterologous expression of the aureothin gene cluster, that the reported set of genes is sufficient to produce the compound. They conclude that AurA is used twice for the extension with methylmalonyl-CoA. Intriguingly, the very similar natural product neoaureothin is known, which might be explained by an almost identical biosynthetic gene cluster that would use the AurA homolog four times instead of catalyzing two extension cycles. The authors find that module 3 may also be used iteratively, because the acyltransferase domain of module 4 is apparently inactive.

There is another theoretical way of solving the "missing module problem," which has only been addressed in the discussion of the reports mentioned above. That would be the presence of nonstoichiometric amounts of PKS proteins, which would imply a different regulation of their expression as well as problems with the processes underlying protein-protein interactions between the PKS modules involved. Studies based on the definition of so-called linker regions [18] or docking domains [19] have recently shed light on this question. The article by Olano et al. in this issue deals with the identification

of the borrelidin PKS, which is characterized by the absence of two modules [25]. Work reported in parallel by the same group addresses both the protein interaction and the stoichiometry question [20]. By chromosomal mutagenesis, they fuse module 5 (which is thought to perform three rounds of condensation) to either module 4, module 6, or both. The authors show that each mutant actively produces borrelidin, which does not support the argument that nonstoichiometric amounts of the PKS proteins solve the missing module problem and makes the presence of separate copies somewhere else in the chromosome improbable.

What do we learn from all this? It has been clear for years that PKS must have evolved from fatty acid synthase. Many researchers have spent a great deal of time going through the enormous body of PKS-related literature and have executed exhaustive classifications to fit the knowledge available at the respective date [e.g., 1, 2, 21]. Nevertheless, the next exception to the rule appeared while the review articles were in preparation or soon after publication.

This could be exemplified by the recent reports on acyltransferase (AT)-less type I PKS [6, 22, 23], which may be regarded as a transition state to PKS systems employing domains instead of modules iteratively. Conceivably, it is believed that a "stand alone" AT loads extender units onto all carrier proteins of the biosynthetic system. Similarly, the lankacidin PKS does not harbor the dehydratase domains that would be needed for product assembly in a typical modular PKS. Instead, one DH domain is found as the stand alone protein in the corresponding gene cluster.

In nature, there appears to be no reason why, for example, a bacterium should not employ a "fungal type of PKS" (which actually implies that no fungal type of PKS exists). We have learned a similar lesson from "plant" (or type III) PKSs, which have been found in bacteria as well [4]. Many additional examples disproving former classifications are available, e.g., related to what was thought to be "plant-specific" metabolism [24]. Why should there be no transition states between all of the PKS types that have been found and whatever one can think of in terms of natural product biosynthesis (e.g., nonribosomal peptide synthetases, isoprenoid biosynthetic proteins, fatty acid synthase, sugar attachment, and biosynthetic proteins)? Because of the ongoing genome-based research, one can conclude that we have to expect more such findings and should be prepared to forget about at least some of the old classifications. In addition, it is clear that more secondary metabolite biosynthetic gene clusters from different sources should be identified and characterized, as they continuously provide novel and relevant information.

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