like Munc18-1 and syntaxin, and the sequence of events taking place prior to exocytosis. Clearly, the presence and regulation of lipids cannot be neglected.

Therefore, a main future challenge will be to try to test the biological relevance of lipid actions like the arachidonic acid effect in functional nerve terminals. What are their local concentrations in nerve terminals, and are these levels regulated and how and when? Clearly, the new million dollar question is how to study causal relationships between local lipid metabolism in the nerve terminal and exocytosis. This is a tremendous future challenge because current methodology is definitely incapable of addressing such specific questions. But undoubtedly, the physiological regulation of local lipid levels and their spatial organization are crucial new directions for neuroscience and cell biology, more broadly. And by addressing the new million dollar question, we may also obtain answers for the previous one.

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A New Origin for Chartreusin

Unraveling the sequence of reactions that transforms aromatic polyketide backbones into much more elaborate structures, for example the chartreusin bislactone, is challenging, but in this issue of *Chemistry & Biology*, Xu and coworkers [1] show that sequencing the gene clusters can provide valuable clues.

Aromatic polyketides are a structurally rich group of natural products that exhibit a wide range of valuable biological activities; notable members include the antibiotic tetracycline, the anticancer drug doxorubicin, and the estrogen receptor agonist R1128. Despite their diversity, aromatic polyketides are all assembled from simple small-molecule precursors (most often acetate) by multienzyme complexes called type II polyketide synthases (PKSs). These assemblies minimally include an enzyme for joining the building blocks into long chains (ketosynthase α, KS_α), and a noncatalytic protein to which the growing chain is tethered (acyl carrier protein [ACP]) (Figures 1A and 1B) [2]. This "minimal PKS" also incorporates an enzyme, KS_β or chain length factor, whose precise role is the subject of some dispute, but which has been implicated both in the provision of starter units [3] and the control of chain length [4]. Together, these domains iterate through multiple cycles of chain extension to form a polyketone of a specific length, which is then modified by a suite of accessory PKS enzymes including ketoreductases, cyclases, and aromatases into a polycyclic, aromatic product [5]. Post-PKS elaboration of these structures by, for example, oxidation, methylation, and glycosylation, results in the bioactive compounds.

Considerable progress has been made in redirecting the biosynthesis of aromatic polyketides to the formation of novel compounds by mixing and matching PKS domains through genetic engineering, a concept referred to as "combinatorial biosynthesis" [6]. Early experiments relied on an empirical set of "design rules" which guided the choice of domains to combine to obtain a particular synthetic outcome [7]. More recent studies, however, have begun to illuminate some of the mechanistic and structural aspects of type II PKS domains that influence the choice of starter unit [8], the final chain length, and the mechanisms of chain folding and cyclization [9–11], opening the way to rationally manipulating these features. However, a significant contribution to the structural diversity and biological activity of some aromatic polyketides comes from the variable, and often complex, post-PKS rearrangement of the initially formed skeleton by so-called "tailoring" enzymes [12]. Yet here our knowledge both of the se-

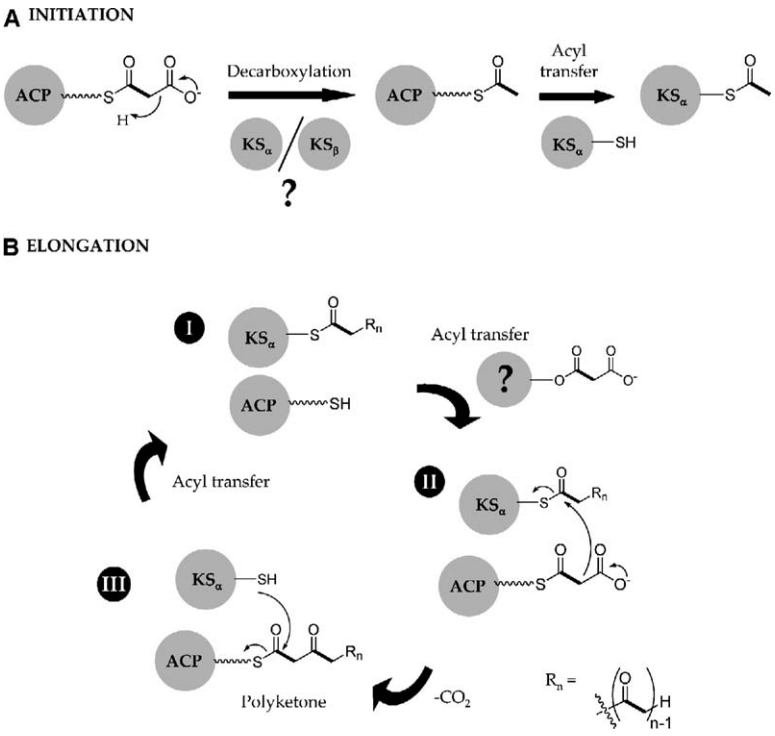


Figure 1. Reactions of the Minimal Polyketide Synthase in the Assembly of Aromatic Polyketides

(A) Biosynthesis is initiated by decarboxylation of acyl carrier protein (ACP) bound malonate to yield acetyl-ACP, in a reaction catalyzed by either ketosynthase α (KS$_\alpha$) or KS$_\beta$. The acetate is then transferred to the active site cysteine of the KS$_\alpha$ domain.

(B) The biosynthesis continues with transfer of the extender unit malonate to the ACP. The KS$_\alpha$ domain then catalyzes decarboxylative condensation to yield a diketide attached to the ACP. The extended chain is passed back to the KS$_\alpha$ domain, and the cycle continues until a chain of the appropriate length is synthesized.

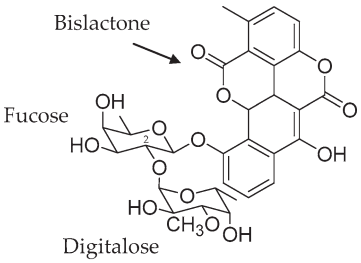
quence of reactions and the mechanistic basis for these transformations is more limited, which has an impact on our ability to manipulate these critical events.

A classic approach to determining the order of post-PKS processing reactions is to add ^{13}C-doubly-labeled acetate to the culture medium of the producing organism, such that each molecule of the target polyketide contains a single molecule of labeled precursor [6]. By NMR analysis, it is then possible to identify sites at which acetate had been incorporated intact into the polyketide backbone, and also positions where some rearrangement process had resulted in cleavage of the acetate carbon-carbon bond. These results are then used to construct a plausible mechanism by which the chain is folded and then subsequently reorganized into the final product. Such theories can gain significant credibility when one of the proposed intermediates in the biosynthesis is then isolated from a "blocked mutant" of the producing strain, in which one of the steps in the pathway has been disabled [13].

The aromatic polyketide chartreusin (Figure 2) has been the object of speculations aimed at elucidating the biosynthetic pathway to its unusual bislactone aglycone, called chartarin. Pharmacological studies have demonstrated that the bislactone system is critical for the high antitumor activity of chartreusin [14], and so it is a very attractive target for modification by both genetic engineering and semisynthesis. Experiments in the 1970s with ^{13}C-acetate yielded a labeling pattern that was difficult to reconcile with models of polyketide folding and cyclization, but nonetheless, it was proposed that chartreusin derives from an initially formed polyketide undecaketide, which is substantially rearranged by a series of oxidative reactions to yield the final bislactone product [15, 16]. However, this mechanistic scheme could not explain why a mutant strain of the chartreusin producer *Streptomyces chartreusis* should produce a metabolite, chrymutasin, whose structure is consistent with originating instead from a decaketide [17].

A more modern strategy for investigating metabolic pathways is to clone and sequence the biosynthetic genes. Gene clusters for type II aromatic polyketides can usually be located within genomic libraries by PCR screening using degenerate probes designed against the well-conserved sequences of the KS$_\alpha$ domains. In this way, Xu and colleagues were able to identify in *S. chartreusis*, a putative ca. 37 kb gene cluster for chartreusin production [1]. To confirm its role in the biosynthesis, they expressed the gene set in the heterologous host *Streptomyces albus* and obtained a fluorescent metabolite that was identical to chartreusin by chromatographic and mass spectrometric analysis.

In the center of the cluster they identified, as anticipated, genes for the minimal PKS components, KS$_\alpha$, KS$_\beta$, and ACP. However, the closest homologs to the KS genes in the sequence databases were from clusters involved in the biosynthesis of decaketides, and not those directing the assembly of longer polyketide chains. Critically, this finding implied that chartreusin must originate from a decaketide and not an undecaketide, and therefore that the proposed pathway to its biosynthesis needed substantial revision. To gain support for their hypothesis, Xu et al. inactivated a gene within the cluster, *chaZ*, a putative FAD-dependent monooxygenase which they suspect initiates the reorganization of the polyketide skeleton by a Baeyer-Vil-



CHARTREUSIN

Figure 2. Structure of the Aromatic Polyketide Chartreusin

Chartreusin incorporates an unusual bislactone skeleton and a disaccharide consisting of fucose and digitalose.

liger-type oxidation. As hoped, the strain accumulated a simpler metabolite, resomycin C—a decaketide. Although for technical reasons the authors could not demonstrate unequivocally that resomycin C is a true intermediate in the pathway (and not simply an aberrant shunt product), taken together, the sequence and structural data show that chartreusin is derived from a decaketide progenitor and not an undecaketide.

Inspiration for constructing a new pathway from resomycin C to the chartarin bislactone that is also consistent with the original ^{13}C-labeling studies comes from the gene cluster. In addition to *chaZ*, the authors found a gene, *chaP*, for a putative dioxygenase; ChaP is a member of the vicinal oxygen chelate (VOC) superfamily, which catalyzes a variety of reactions including oxidative cleavage of C-C bonds [18]. In their new biosynthetic scheme, ChaZ and ChaP perform two as yet unprecedented C-C bond cleavage reactions, which play critical roles in rearranging the polyketide skeleton from the chartarin aglycone. The results of experiments designed to prove this rearrangement cascade by elucidating all of the pathway intermediates are eagerly anticipated, as are mechanistic studies of these fascinating tailoring enzymes.

Chartreusin suffers from poor pharmacokinetics, but its biological activity can be improved by modifying its disaccharide moiety (Figure 2). For example, a natural derivative of chartreusin, elsamicin A, incorporates an amino sugar in place of digitalose and exhibits increased water solubility [19]. With heterologous expression in *S. albus* established, it should be possible to obtain the chartarin aglycone from an appropriately engineered blocked mutant. The aglycone could then be used to construct a library of chartreusin analogs with modified patterns of glycosylation. Sequencing has shown that the cluster contains two putative glycosyltransferases, ChaGT1 for attachment of fucose to the chartarin aglycone, and ChaGT2, which catalyzes the unprecedented 2-glycosylation of fucose with digitalose. If these enzymes exhibit the broad specificity toward the sugar donor observed for other glycosyltransferases from PKS systems [20], they could be invaluable for chartreusin drug discovery efforts.

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