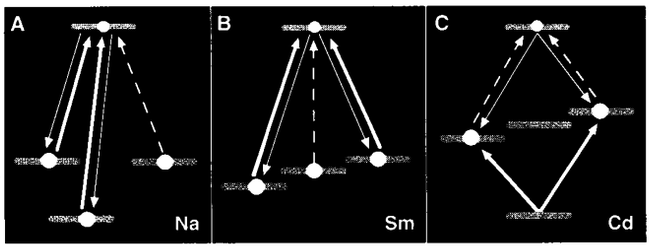

Fig. 2. (**A**) Texas, (**B**) Münster, and (**C**) Amsterdam experiments on LWI. The heavy lines are the coherence-generating fields, the dashed lines indicate pumping into the upper lasing level, and the thin lines are the probe transitions.

started to oscillate with a period determined by the level splitting. Inversionless amplification or absorption of a probe pulse was observed depending on the phase of the oscillating coherence. In the Amsterdam experiment (*8*) on cadmium, a pulse was again used to lift the atoms from the ground state into a coherent superposition of two intermediate magnetic sublevels, and noninversion amplification of a probe pulse was observed.

As is very often the case with new concepts, the study of coherent atomic systems revealed other interesting properties and possibilities. Electromagnetically induced transparency, as predicted by Harris and coworkers (*10*), is a case in point. They proved that when a strong field is present on one transition, such as $b \rightarrow a$ of Fig. 1C, a weak probe will not be absorbed on the $c \rightarrow a$ transition. In this way, they can render an opaque medium transparent. Furthermore, the medium will be highly dispersive, and thus, this effect may be used to slow down the group velocity of short light pulses (*11*) or to make a high-precision magnetometer (*12*) based on the Zeeman level shifts induced by tiny magnetic fields.

It is also possible to use atomic coherence to generate a large index of refraction (*13*), to produce large nonlinear optical coefficients (*14*), and even to quench quantum noise (*15*). In fact, it may be noted that such a phase-coherent atomic ensemble is in a real sense a new state of matter, which has come to be known as "phaseonium."

The physics behind LWI and related effects as explained here is based on atomic coherence and interference. To some extent, it is possible and profitable to recast this picture in terms of so-called "dressed states," such that one state (called the antisymmetric state) does not engage in absorption (or emission), while another (symmetric state) does. If the lower laser-level population is put into the nonabsorbing antisymmetric state, any small amount of population placed in the excited state could be said to lead to lasing with inversion to the empty symmetric state. In this sense, LWI would be lasing with hidden inversion in a dressed basis. However, there is more to the story.

In the original work of Harris (*2*), LWI was based on quantum Fano-interference, for which there is no dressed state description. Likewise, the so-called Raman coherence scheme, as studied experimentally by the Texas group, has no simple dressed state or hidden inversion description. In all cases, however, atomic coherence and interference explains the physics, and we emphasize the utility and generality of this approach. The interplay between atomic coherence effects and quantum optics is a rich field of fundamental and applied research and will be an exciting area of scientific activity for some time.

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Polyketide Biosynthesis: Molecular Recognition or Genetic Programming?

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Polyketides are a large and diverse family of natural products, most of which are produced by Actinomycete bacteria and by fungi. Two classes of polyketides have been extensively studied—the polycyclic aromatic polyketides, typified by the antitumor antibiotic tetracenomycin C (**1**) and the blue-pigmented octaketide actinorhodin (**2**), and the partially reduced, branched-chain fatty acid lactones, known more generally as macrolides and represented by the broad spectrum antibiotic erythromycin A (**3**) (Fig. 1). Complex polyketides are synthesized by a mechanism analogous to the chain-elongation steps of fatty acid biosynthesis, with acetate, propionate, and butyrate forming the fundamental building blocks (*1–3*). The oxidation level and stereochemistry of the growing polyketide chains are adjusted after each condensation step in the chain-elongation process (*1–3*).

The application of molecular genetics to polyketide biosynthesis has yielded dramatic insights into the organization and function of these biosynthetic systems and has allowed the isolation of polyketide synthase proteins. Two landmark developments stand out. Malpartida and Hopwood have reported the cloning of the entire set of actinorhodin biosynthetic genes from *Streptomyces coelicolor*—a 26-kilobase gene cluster (*4*). The polyketide synthase (PKS) genes, identified both by complementation of known mutations as well as by targeted gene disruptions, showed sequence similarities to conserved domains of the genes for fatty acid biosynthesis. More recently, Katz, at Abbott Laboratories (*5*), and Leadlay, at the University of Cambridge (*6*), each cloned and sequenced the genes from *Saccharopolyspora erythraea* responsible for synthesis of the parent macrolide for erythromycin, 6-deoxyerythronolide B (*4*). The *eryA* gene is organized into three large (10-kilobase) open reading frames containing a series of domains, each responsible for one of the individual steps of reduced polyke-

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tide chain assembly. Remarkably, the order of these domains is the same as the order of the biochemical chain assembly and modification steps. Indeed, each of the three open reading frames contains two modules, each of which harbors the appropriate complement of ketosynthase, ketoreductase, dehydrase, enoylreductase, acyl carrier protein, and acyltransferase domains, strongly reminiscent of an animal fatty acid synthase in both overall size and organization. The current "module hypothesis" suggests that each module is responsible for successive steps of chain elongation and functional group modification, an inference borne out by the use of gene deletions to reprogram macrolide biosynthesis, resulting in the rational generation of several erythromycin analogs (*7*). Extensive work in other laboratories has confirmed and extended the original discoveries of the Hopwood, Katz, and Leadlay groups (*8*).

In spite of these substantial advances, the factors responsible for fidelity in the programming of polyketide biosynthesis are still unknown. Unlike simple fatty acid biosynthesis, which involves a simple repetition of a few biochemical transformations, the formation of a complex polyketide requires sophisticated control of a hierarchy of biochemical choices. (i) Substrate. Although aromatic polyketides are derived primarily from acetyl-CoA precursors, activated as the corresponding malonyl-CoA derivatives, the formation of macrolides and related reduced polyketide metabolites requires discrimination among malonyl-CoA, methylmalonyl-CoA, and ethylmalonyl-CoA substrates for each round of elongation. (ii) Reaction sequence. Each condensation step is followed by particular functional group modifications—ketoreductase, dehydrase, and enoylreductase events—or directly by another condensation step. Although for macrolides the timing of these transformations is controlled by distinct genes for each step, formation of aromatic polyketides often requires only a single ketoreduction, which must take place at a specific location in the growing or mature polyketoester chain. In a recent article (*9*), Hopwood and Khosla describe the construction of chimeric PKS genes consisting of different combinations of individual PKS subunits responsible for the formation of distinct aromatic polyketides. The site of ketoreduction is governed by the distance from the carboxyl terminus of the polyketide chain, a conclusion that suggests that reduction occurs subsequent to generation of the full-length polyketide. (iii) Stereochemistry. Reduced polyketides contain numerous stereogenic (chiral) centers, most of which must be introduced during chain elongation and functional group modification. Although there is no apparent stereochemical regularity within any given polyketide product, a pronounced position-specific regularity exists among different macrolide metabolites (*10*). The module hypothesis suggests that the organization of the structural genes directly determines the sequence of the corresponding biochemical transformations. (iv) Chain length. The number of steps in the chain elongation cycle must also be precisely controlled. The chain length of aromatic polyketides is dictated, at least in part, by a single gene product that resembles a ketosynthase but lacks the required domains for acyltransferase and ketosynthase catalytic activity (*9*). Nonetheless, the biochemical mechanism for control of chain length remains obscure. For reduced polyketides, the organization of the *eryA* gene cluster suggests that the length of the eventually formed polyketide chain is the strict consequence of the number (six) of ketosynthase domains. As reasonable as this conclusion may appear, it does not in itself account for the demonstrated ability of macrolide PKS proteins to recognize and properly process exogenously added polyketide chain elongation intermediates (*2, 3*). The latter results suggest that the modular model may ignore significant elements of molecular recognition required for the correct processing of growing polyketide chains.

Until very recently, all attempts to isolate enzymes capable of mediating the formation of complex aromatic or reduced polyketides from simple precursors have failed. Now two groups have reported important progress in achieving these goals. Shen and Hutchinson (*11*) have described the preparation of a cell-free system, generated by the selective expression of cloned PKS genes from *Streptomyces glaucescens*, that supports the in vitro conversion of acetyl- and malonyl-CoA to tetracenomycin F2 (**4**), a partially cyclized intermediate in the biosynthesis of tetracenomycin C (**1**) (Fig. 2). By using a modular expression system, these researchers established plausible functions for individual gene products and have demonstrated the high substrate specificity for the acetyl-CoA starter unit. In the meantime, Leadlay and his collaborators have made substantial progress in the isolation and characterization of the three large multienzymes corresponding to the three 10-kilobase open reading frames that form 6-deoxyerythronolide B (**5**) (Fig. 3) (*12*). In an article in this week's issue of *Science* (*13*), Leadlay sheds new light on the stereochemical specificity of the macrolide synthase. Earlier experiments, in which deuterated substrates were fed to *S. erythraea* (*14*), had

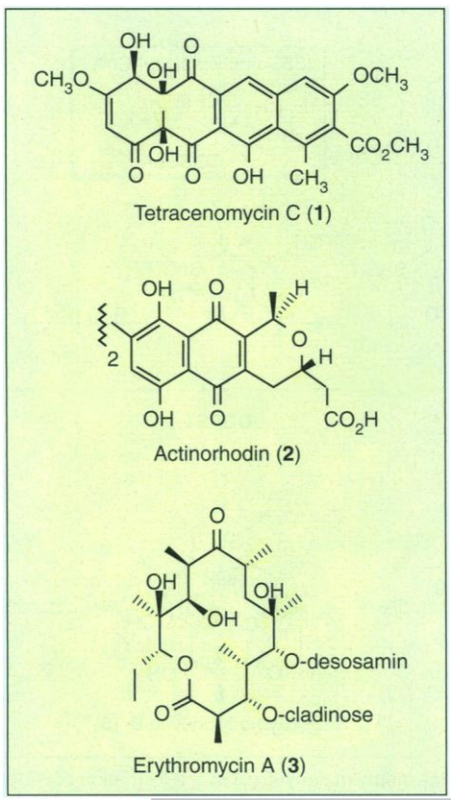

Fig. 1. Typical polyketides. Tetracenomycin C (**1**) and actinorhodin (**2**), and the macrolide erythromycin A (**3**).

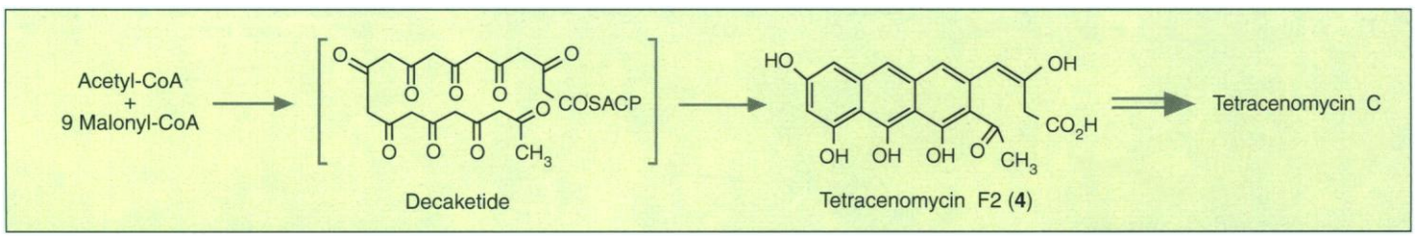

Fig. 2. In vitro polyketide synthesis. Formation of the decaketide tetracenomycin F2 (**4**) from acetyl-CoA and nine equivalents of malonyl-CoA by an in vitro polyketide synthase preparation, which was generated by expression of *Streptomyces glaucescens* tetracenomycin (Tcm) *tcmJKLMN* genes.

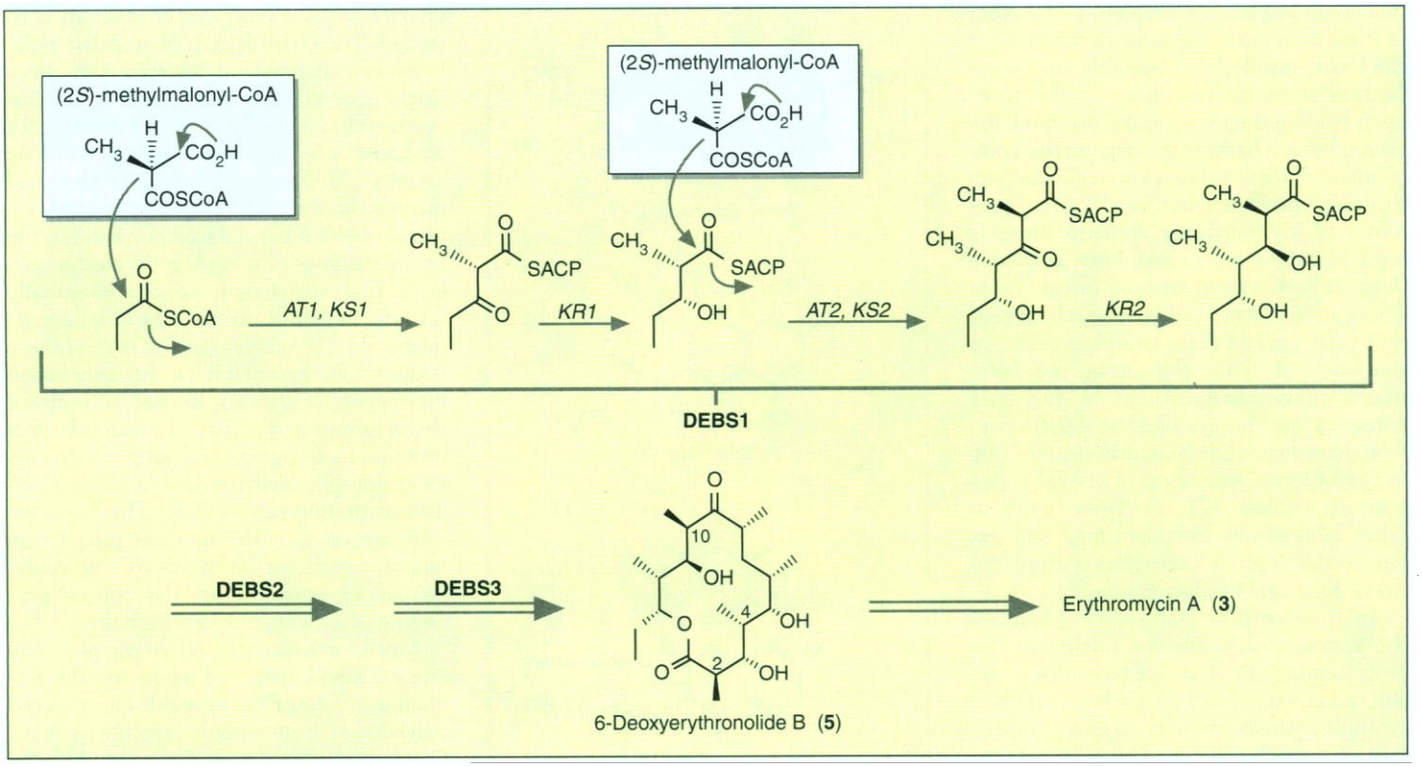

Fig. 3. Synthesis of chiral centers. Utilization of (2*S*)-methylmalonyl-CoA in the formation of 6-deoxyerythronolide B (**5**) by the multienzyme proteins DEBS1, DEBS2, and DEBS3. *AT1* and *AT2* refer to the acyltransferase activities of biosynthetic modules 1 and 2, respectively. Similarly, *KS1* and *KS2* refer to the corresponding ketosynthase activities, while *KR1* and *KR2* refer to the ketoreductase activities. Formation of the first diketide intermediate requires an epimerization of the methyl substituent.

suggested that (2*S*)-methylmalonyl-CoA is the direct precursor of sites in the macrolide aglycone that carry methyl groups in the D configuration (C-2, C-4, and C-10). Although it could not be established directly, it had been assumed that generation of the corresponding L-methyl stereochemistry at C-8 and C-12 might result from complementary condensation of the enantiomeric substrate (2*R*)-methylmalonyl-CoA. Leadlay and his collaborators have now convincingly demonstrated that all six acyltransferase components have an absolute preference for (2*S*)-methylmalonyl-CoA (Fig. 3). This important finding leads inescapably to the conclusion that epimerization of the relevant chiral centers must occur, either prior to or subsequent to ketocondensation, a process reminiscent of the configurational inversions that accompany incorporation of L–amino acids into D–amino acid subunits of nonribosomal polypeptide antibiotics (*15*).

The availability of high-purity cell-free systems supporting complex polyketide biosynthesis will now permit investigation of substrate specificity, the detection of enzyme-bound biosynthetic intermediates, and the rational generation of novel biosynthetic products through the manipulation of gene structure and organization. The results will not only provide solutions for complex and challenging biosynthetic problems but will lead to important insights into fundamental issues of molecular recognition and metabolic control.

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