Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

22. Expression plasmids for the human nuclear receptor–GAL4 chimeras were generated by amplification of the cDNA encoding the putative LBDs and insertion into a modified pSG5 expression vector (Stratagene) containing the GAL4 DNA binding domain (amino acids 1 to 147) and the simian virus 40 (SV40) Tag nuclear localization signal (*5*).
23. G. Riddihough and H. R. B. Pelham, *EMBO J.* **6**, 3729 (1987).
24. DNA encoding the human FXR LBD (amino acids 222 to 472; GenBank U68233) was inserted into the pRSET-A expression vector (Invitrogen) and expressed in bacteria. The transformed cells were grown for 12 hours at 25°C, cooled to 9°C, maintained at this temperature until they reached a cell density, as measured by optical density (OD) at 600 nm, of OD_{600} = 14 , induced with 0.25 mM isopropyl β-D-thiogalactoside to a final cell density of OD_{600} = 16, and harvested by centrifugation. Filtered bacterial lysate was applied to an affinity column of Ni^{2+}-charged chelation Sepharose (Amersham Pharmacia Biotechnology, 25 mM tris-HCl, pH 7.2, 150 mM NaCl, and 50 mM imidazole). Protein was eluted with 365 mM imidazole after washing with buffer containing 95 mM imidazole, and further purified by size exclusion chromatography with Superdex S-75 resin (Amersham Pharmacia Biotechnology). The FXR LBD was biotinylated with NHS-LC-biotin reagent (Pierce).
25. The FRET ligand-sensing assay was performed by incubating 10 nM of the biotinylated FXR LBD that was labeled with streptavidin-conjugated allophycocyanin (Molecular Probes) and 10 nM of the SRC1 peptide [amino acids 676 to 700, 5′-biotin-CPSSHSSLTERHKIL-HRLLQEGSPS-$CONH_2$] (*21*) (SynPEP) that was labeled with streptavidin-conjugated europium chelate (Wallac), in 50 mM tris pH 8, 50 mM KCl, 0.1 mg/ml bovine serum albumin, 1 mM EDTA, and 10 mM dithiothreitol, in the presence of test compound for 2 hours at 22°C. Data were collected with a Wallac Victor fluorescence reader in a time-resolved mode. The relative fluorescence was measured at 665 nM, and the indicated values were calculated by subtracting the fluorescence obtained in the absence of test compound from the value obtained in the presence of test compound. Values are expressed as the means ± SD derived from three independent experiments.
26. We thank P. A. Dawson for providing the IBAT expression plasmid pCMV-HISBT, B. W. O'Malley, S. Y. Tsai, M.-Y. Tsai, and M. C. Lewis for critically reading the manuscript. Supported in part by NIH grant RO1 DK53366 to D.D.M. and National Institute of Diabetes and Digestive and Kidney Diseases grant F32 DK09793 to A.M.Z.

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Modulation of Polyketide Synthase Activity by Accessory Proteins During Lovastatin Biosynthesis

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Polyketides, the ubiquitous products of secondary metabolism in microorganisms, are made by a process resembling fatty acid biosynthesis that allows the suppression of reduction or dehydration reactions at specific biosynthetic steps, giving rise to a wide range of often medically useful products. The lovastatin biosynthesis cluster contains two type I polyketide synthase genes. Synthesis of the main nonaketide-derived skeleton was found to require the previously known iterative lovastatin nonaketide synthase (LNKS), plus at least one additional protein (LovC) that interacts with LNKS and is necessary for the correct processing of the growing polyketide chain and production of dihydromonacolin L. The noniterative lovastatin diketide synthase (LDKS) enzyme specifies formation of 2-methylbutyrate and interacts closely with an additional transesterase (LovD) responsible for assembling lovastatin from this polyketide and monacolin J.

Lovastatin is an inhibitor of the enzyme (3*S*)-hydroxymethylglutaryl–coenzyme A (HMG-CoA) reductase that catalyzes the reduction of HMG-CoA to mevalonate, a key step in cholesterol biosynthesis. This activity confers on lovastatin its medicinally important antihypercholesterolemic activity and other potentially important uses (*1*). It is a secondary metabolite from the filamentous fungus *Aspergillus terreus* [American Type Culture Collection (ATCC) 20542] and has been shown to be derived from acetate via a polyketide pathway (*2*). Polyketide biosynthesis in bacteria and fungi is related to fatty acid metabolism but differs in that some of the reduction or dehydration reactions catalyzed by a polyketide synthase (PKS) can be suppressed at specific biosynthetic steps. Substrates besides acetyl-CoA and malonyl-CoA can be used by PKSs to assemble the carbon chain. These attributes result in a much wider range of possible products than fatty acid metabolism, which together with post-PKS modifications provide a very large family of often biologically active secondary metabolites (*3*).

Microbial polyketides are known to be assembled in three different ways. Bacterial PKSs consist of either the modular (type I) or iterative (type II) systems. In a type I PKS, such as that involved in the biosynthesis of erythromycin A (*4*), one distinct group of active sites, called a module (*5*), on a single polypeptide is used to initiate or extend the carbon chain. The active sites present in each module determine the choice of extender unit plus the level of reduction or dehydration for that particular extension cycle, and the number of modules in the polypeptides constituting the PKS determines the length of the polyketide carbon chain. In contrast, for type II PKS systems each activity is encoded by a separate enzyme that is used several times in the biosynthesis of the typically aromatic product [for example, actinorhodin, doxorubicin, and tetracenomycin C (*6*)].

Fungal PKS systems fall into a third group, iterative type I PKSs. Many fungal PKSs make polyketides similar to those produced by the bacterial type II class: polycyclic aromatic compounds, such as 1,3,6,8-tetrahydroxynaphthalene and 6-methylsalicylic acid, whose biosynthesis is primarily an iterative process involving no reduction and dehydration steps (or only one such step). Mammalian and some microbial fatty acid synthases (FASs) are closely related to this class of PKS, having the same order of domains and an iterative nature (*7*). Fungal PKSs also make nonaromatic reduced compounds such as lovastatin (also called mevinolin or monacolin K), brefeldin A (*8*), and T-toxin (*9*) (Fig. 1A). All of these metabolites are derived from polyketide chains that vary in their state of reduction and dehydration, as well as length. How this is accomplished has been a mystery because it is not obvious how a single set of the activities typically found in an iterative type I PKS can make the choice of oxidation level at each chain-extending condensation.

We report here that the interaction of the two fungal PKSs involved in lovastatin biosynthesis with other enzymes of the pathway seems to modulate their overall activity, apparently endowing one iterative PKS with the ability to discriminate between carbon chain intermediates at different stages of assembly and possibly causing the other PKS to behave noniteratively, like a bacterial modular PKS.

In the lovastatin pathway (Fig. 1B), the

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nonaketide carbon chain is derived from one acetate and eight malonate molecules (*2*) and may undergo an electrocyclic cyclization (*10*). Dihydromonacolin L, a predicted PKS product and an established intermediate of lovastatin biosynthesis (*11*), has two hydroxyls, one double bond, and a methyl group at C-6 derived from the methyl of methionine. Most of these functionalities are believed to be created by the PKS while dihydromonacolin L is being synthesized (Fig. 1B, boxed region), consistent with the processive nature of polyketide biosynthesis (*4*–*6*) as documented by recent work on dehydrocurvularin biosynthesis (*12*). When this hypothesis was first put forth (*13*), it was imagined that the hexaketide triene **5** or its nonaketide equivalent could undergo a Diels-Alder–like cyclization to form a precursor octahydronaphthalene ring system. This event has been modeled chemically, but a diastereomeric compound results because of the preference of the methyl group at C-6 to assume an equatorial geometry in the nonenzymatic reaction (*10*). It has been assumed that the PKS involved is able to use different sets of activities at different steps in the assembly of dihydromonacolin L, as specified in Fig. 1B for the formation of **1** through **6**. Because none of the microbial PKSs studied so far exhibit such discriminatory activity for the biosynthesis of a single molecule (they simply make a largely unreduced polyketide of fixed length, or, when different combinations of reduction and dehydration events are carried out, use each active site only once), the biosynthesis of dihydromonacolin L presents an unusual challenge.

Pioneering genetic research on lovastatin biosynthesis by Reeves, McAda, and workers at MDS Panlabs Inc. identified a type I PKS gene essential for lovastatin biosynthesis by *A. terreus* (*14*). Its 335-kD deduced product, originally called the triol PKS but renamed here as the lovastatin nonaketide synthase (LNKS), is predicted to contain the six active sites—KS, AT, DH, ER, KR, and ACP (defined in Fig. 2)—found in type I FASs and many bacterial PKSs and arranged in the same order as in an animal FAS. Key differences are the presence of a methyltransferase domain and replacement of the COOH-terminal thioesterase (TE) domain often found in FASs and PKSs with a ~500–amino acid extension possibly involved in product transfer or cyclization. As noted below, the ER domain of LNKS may not be functional.

Characterization of the LNKS gene set the stage for understanding how the carbon skeletons of dihydromonacolin L and lovastatin are assembled. Moreover, because secondary metabolism genes are invariably clustered in microorganisms, this gene also provided a convenient entry into cloning and characterizing the other genes involved in lovastatin production. Using the previously identified LNKS gene (*lovB*) as a probe, we isolated cosmids containing this gene and the surrounding genomic DNA from an *A. terreus* (ATCC 20542) genomic library (*15*). DNA sequencing and analysis of cosmids pWHM1263 and pWHM1265 (*15*) revealed the presence of 18 potential genes over 64 kb (Fig. 3), the functions of which could largely be predicted by sequence comparisons. Of these genes, two (*lovB* and *lovF*) encode PKSs. As previously noted, the *lovB* gene encodes LNKS (*14*), containing the six domains characteristic of PKSs (Fig. 2), an integrated methyltransferase domain located between the AT and the KR domain, and a domain at the COOH-terminus that is similar to the condensation domain of nonribosomal peptide synthetases (NRPSs) (*16*). Lovastatin has two methyl groups derived from *S*-adenosyl-L-methionine (SAM), one on the nonaketide and the other on the diketide side chain. The presence of methyltransferase domains in LNKS and the LovF (LDKS) protein (see below) indicates that in both cases the methyl groups are likely to be added while the polyketide is being synthesized. These domains are common among NRPSs, where *N*-methylation of amino acids can occur (*16*), but such domains have not been reported for PKSs. The role of the COOH-terminal region of LNKS in the context of lovastatin biosynthesis is unclear, but it could be involved in some of its unusual aspects. The only other PKS system thus far characterized that has such a domain is that for phthiocerol mycoserosate, a *Mycobacterium tuberculosis* cell envelope lipid (*17*). Two condensation domains are present in this system, one located at the end of a type I PKS and the other as a separate gene (*ppsE* and *papA5*, GenBank accession numbers 1781081 and

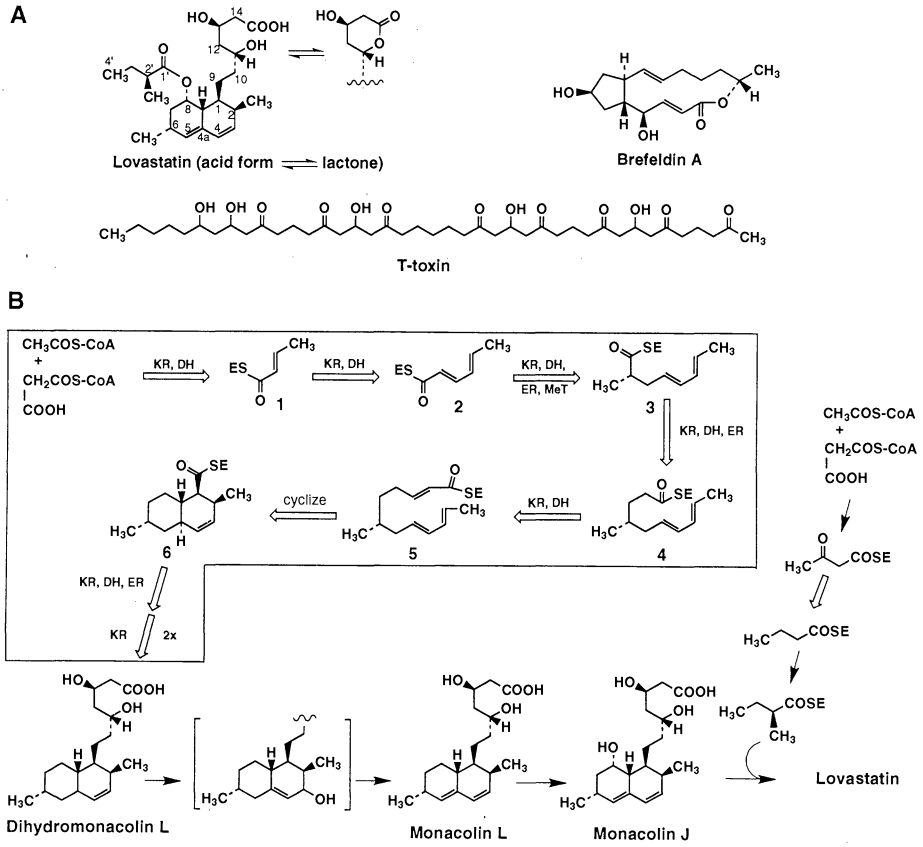

Fig. 1. (**A**) Typical reduced fungal polyketides. (**B**) Lovastatin biosynthetic hypothesis. The boxed region shows the set and order of reactions thought to be necessary for the biosynthesis of dihydromonacolin L, the first recognized intermediate in lovastatin biosynthesis. Abbreviations are defined in Fig. 2.

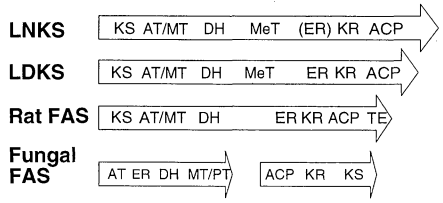

Fig. 2. FASs and fungal PKSs. The arrangement of catalytic domains in fungal PKSs is the same as that for mammalian (rat) FASs. Fungal FASs have two subunits with a different order of catalytic domains. KS, β-ketoacyl synthase; AT, acyltransferase; AT/MT, acetyl/malonyl transferase; DH, dehydratase; MeT, methyltransferase; ER, enoyl reductase [(ER), inactive ER]; KR, ketoreductase; ACP, acyl carrier protein; PT, product transfer; MT/PT, malonyl/palmityl transferase; TE, thioesterase.

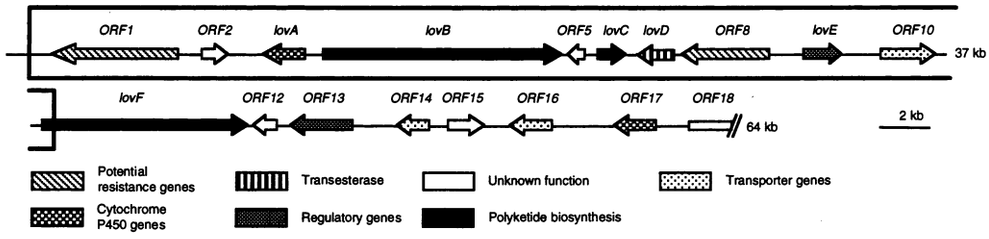

Fig. 3. The lovastatin biosynthesis gene cluster. The region contained in pWHM1263, containing all the genes necessary for monacolin J production, is indicated by the frame. pWHM1265, containing all the other open reading frames (ORFs) shown, overlaps with pWHM1263 at *ORF10*. *lov*, genes proven to be involved in lovastatin production; //, end of region sequenced. Some genes are shaded according to the functions named in the text (*22*). Many of the genes do not have obvious CAAT motifs, TATAAA boxes, or CT motifs (*23*), so in some cases it is possible that the genes have alternative translational starts. The assignment of introns in the genes was determined by a combination of alignments with related genes (or their protein products), codon preference analysis, and looking for intron consensus sequences for intron start, stop, and internal lariat sequence, especially between different ORFs. It is quite possible that some introns may have been missed, especially if no stop codons or frame shifts occurred, or in the genes with no close homologs.

1781086, respectively). One similarity between lovastatin and phthiocerol mycoserosate is that both compounds are composed of more than one polyketide or fatty acid chain joined via ester bonds. Thus, the role in esterification already proposed for the condensation domain in lipopeptide and depsipeptide (*16*) biosynthesis could be extended to lovastatin. Other potential roles are in protein-protein interactions, in the formation of the precursor octahydronaphthalene ring by Diels-Alder cyclization, and as an alternative to the TE domain commonly found in type I bacterial PKSs and NRPSs.

To further investigate the role of *lovB*, we overexpressed it in *A. nidulans* under the control of the *alcA* promoter (*15*). One transformant, WMH1738, which upon SDS–polyacrylamide gel electrophoresis (PAGE) clearly produced a major amount of a high molecular weight protein that was absent from the wild-type strain (Fig. 4A), was selected for further study. This high molecular weight protein cross-reacted with antibodies to LNKS (*14*), confirming the overexpression of LNKS. After growth and induction of the *alcA* promoter, two metabolites were isolated (Fig. 4B): 4-hydroxy-6-[(1*E*,3*E*,5*E*)-1-methylhepta-1,3,5-trien-1-yl]-2-pyrone (**7**) and 4-hydroxy-6-[(1*E*,3*E*,5*E*,7*E*)-3-methylnona-1,3,5,7-tetraen-1-yl]-2-pyrone (**8**). These compounds were fully characterized by the various spectroscopic methods, by incorporation of ^{13}C-labeled acetates, and also by analysis of the chemically reduced products (*18*). The structures of these metabolites are consistent with their origin from acetate via the intermediate shown in Fig. 4B. These results are indicative of a malfunctioning PKS that has retained all activities (apart from the enoyl reductase activity) but is apparently unable to coordinate them properly, resulting in shunt products having a shorter carbon chain and lower degree of reduction than dihydromonacolin L. Both of these metabolites contain a methyl group derived from SAM at the seventh carbon from the methyl terminus, which demonstrates that the integral methyltransferase is functional and positionally specific.

To learn whether the malfunctioning of the PKS was due to the lack of a specific protein component carried by the cluster, we transformed *A. nidulans* strain WMH1738, carrying a copy of the *lovB* gene, to hygromycin resistance using cosmid pWHM1263 (*15*), which contains several other genes from the cluster (Fig. 3). Of 10 transformants tested by fermentation and thin-layer chromatography (TLC) analysis of culture extracts, three (WMH1739, 1740, and 1749) were found to be producing new metabolites (Fig. 5B), one of which had the same mobility as monacolin J. After purification by high-performance liquid chromatography (HPLC), the major new product, present in all three strains, was analyzed by liquid chromatography–electrospray ionization mass spectrometry (LC-ESIMS) and shown to be monacolin J. A second metabolite, believed to be monacolin L, was also observed in one of these transformants (Fig. 5B). Production of the yellow pigments **7** and **8**, produced by strain WMH1738, was stopped or much reduced in these strains. These three producing strains were also tested for their resistance to lovastatin (*15*) and found to be resistant to up to 100 μg/ml. Hence, the genes in cosmid pWHM1263 clearly are sufficient for the production of monacolin J and for resistance to lovastatin and its precursors, although a slight possibility remains that other components of this biosynthesis pathway are being performed by *A. nidulans* enzymes.

Because these results strongly suggest that the aberrant behavior of *lovB* noted above is not due to any effects from heterologous expression in *A. nidulans*, but rather occurs because of the lack of a specific protein from the cluster that interacts with LNKS to pro-

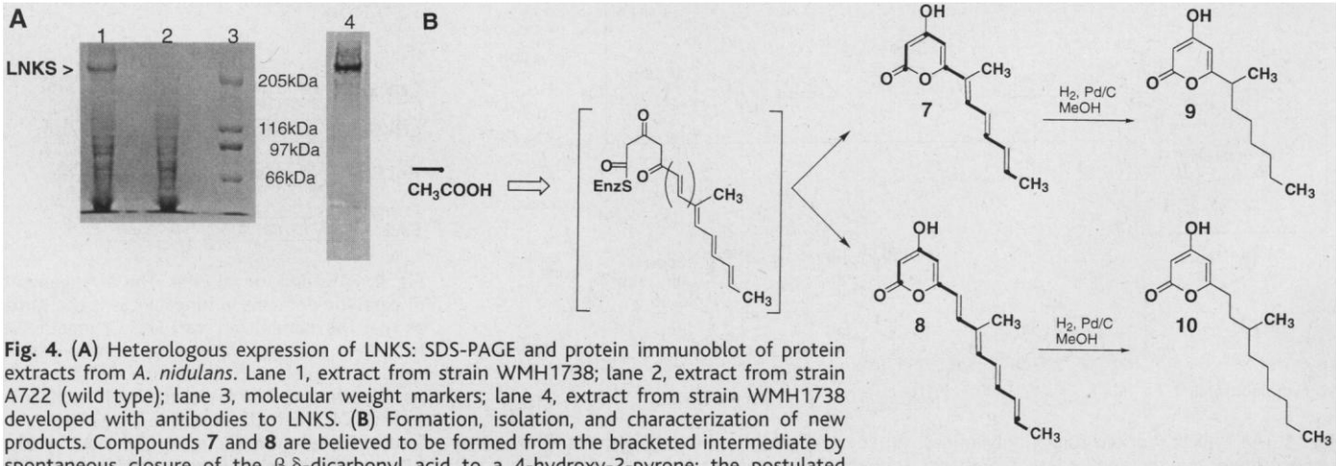

Fig. 4. (**A**) Heterologous expression of LNKS: SDS-PAGE and protein immunoblot of protein extracts from *A. nidulans*. Lane 1, extract from strain WMH1738; lane 2, extract from strain A722 (wild type); lane 3, molecular weight markers; lane 4, extract from strain WMH1738 developed with antibodies to LNKS. (**B**) Formation, isolation, and characterization of new products. Compounds **7** and **8** are believed to be formed from the bracketed intermediate by spontaneous closure of the β,δ-dicarbonyl acid to a 4-hydroxy-2-pyrone; the postulated intermediate itself results from condensation of six or seven acetate-derived C2 units (the parentheses denote the additional C2 unit used to form **8**), as indicated by the ^{13}C labeling pattern.

duce the correct product, this protein was identified as follows. The *lovC* gene adjacent to *lovB* (Fig. 3) encodes a protein of 363 amino acids that has high similarity to the product of the *Cochliobolus carbonum toxD* gene of unknown function from the HC-toxin biosynthesis cluster (*19*), to hormone- and ripening-induced proteins from plants (GenBank accession numbers 2465008 and 1184121), and to ER domains of PKSs. The *lovC* gene was mutated by gene disruption, resulting in strain WMH1734 (*15*). Upon fermentation, this strain was found to be blocked in lovastatin production and to accumulate compound **7** (Fig. 4B). As elaborated above, the formation of this incomplete and incorrectly processed polyketide suggests that the LNKS and (at least) the LovC proteins interact to produce a polyketide of the correct length and with the correct reduction and cyclization pattern.

Formation of the same product or products by overexpression of *lovB* in *A. nidulans* and by the *A. terreus lovC* mutant led us to study whether the products of these two genes interact functionally. The *lovC* gene was placed under the control of the *alcA* promoter and was then used to transform strain WMH1738, containing the LNKS gene under the control of the same promoter, to Zeocin resistance (*15*). Transformant colonies were grown under inducing conditions for the *alcA* promoter, and extracts were analyzed by TLC and HPLC for the production of new metabolites. Several transformants (strain WMH1750 is representative) were clearly producing, by TLC, at least three new compounds and had greatly reduced amounts of compounds **7** and **8**. One of the new metabolites had the same mobility as dihydromonacolin L (Fig. 5B). This material was isolated and characterized in the same manner as for **7** and **8** (*18*), which provided confirmation that this strain was producing authentic dihydromonacolin L. The fact that LNKS and LovC together make up a PKS capable of producing dihydromonacolin L was unexpected and has few precedents (*20*). Dihydromonacolin L has since been observed to be produced by cell-free extracts from WMH1750 (*18*).

The LNKS/LovC PKS represents a curious mixture of type I polyfunctional and type II monofunctional enzymes, and the interaction of these two proteins may explain the remarkable substrate discrimination necessary for the biosynthesis of dihydromonacolin L (Fig. 1B). LNKS may lack ER activity and has a condensation domain possibly involved in product transfer, cyclization, or interaction with another protein. LovC is thought, from sequence similarities and the products found from overexpressing *lovB* alone in *A. nidulans*, to be responsible for the three enoyl reductions predicted to be necessary for dihydromonacolin L production. This enzyme probably acts on the growing polyketide chain still bound to LNKS, because if it acted on a free intermediate we would expect the *lovB*-expressing strain to accumulate a full-length nonaketide. The two enzymes are therefore expected to be closely associated. If, as predicted (Fig. 1B), the ER activity is used only three times in the biosynthesis of dihydromonacolin L, LovC must act at only the tetra-, penta-, and heptaketide stages. LNKS can clearly recognize when to methylate the polyketide intermediate, but it is apparently unable to discriminate correctly once one ER reaction has failed to occur. Furthermore, in the correctly functioning system, LNKS must be able to discriminate the final two steps, when only the KR is used, from the preceding steps when both KR and DH activities are used.

As expected, a second PKS was identified in the lovastatin cluster. The *lovF* gene encodes a 2532–amino acid protein that we designate as the lovastatin diketide synthase (LDKS) (Figs. 2 and 3). This protein contains domains for KS, AT, DH, ER, KR, and ACP typical of PKSs. It also has, like LNKS, a methyltransferase domain. Unlike LNKS, it does not contain the condensation domain, and the ER domain conforms much more closely to the consensus sequence for the nucleotide binding motif (*15*). The function of *lovF* was tested by gene disruption (*15*). A *lovF* mutant strain (WMH1731) of *A. terreus* (ATCC 20542) was fermented and extracts were analyzed for lovastatin production. Strain WMH1731 produced no lovastatin, thus confirming a role for *lovF* in lovastatin biosynthesis; instead, it accumulated a new metabolite that was indistinguishable from monacolin J by TLC (Fig. 5A) and HPLC analyses. A small amount of this metabolite was partially purified by HPLC and its molecular weight was determined by LC-ESIMS analysis. The molecular ion (M+1) of mass/charge ratio (*m/z*) 339 and additional ions of *m/z* 321 and 303 were identical to those seen for authentic monacolin J. The *lovF* gene is therefore highly likely to encode the enzyme responsible for the biosynthesis of the (2*R*)-2-methylbutyryl side chain of lovastatin.

One other gene functionally associated with the LDKS was also identified. The *lovD* gene encodes a protein of 413 amino acids that has 27 to 29% similarity to β-lactamases, carboxypeptidases, lipases, and esterases (GenBank accession numbers 1788770, 1345941, 1552858, and 3649751, respectively). Disruption of this gene led to the isolation of a mutant (WMH1732) that was no longer able to produce lovastatin (*15*). TLC, HPLC, and LC-ESIMS analyses of culture extracts showed that the WMH1732 strain accumulates monacolin J (Fig. 5A), the immediate precursor to lovastatin (Fig. 1B). We propose that *lovD* is responsible for the biosynthesis of the 2-methylbutyryl/monacolin J transesterase that joins together the two polyketide components of lovastatin. An enzyme with this activity has been partially purified from *A. terreus* and found by SDS-PAGE to have a molecular weight of 46 kD, the same as that predicted for the LovD protein (*14*). This enzyme was found to catalyze the transfer of the 2-methylbutyryl side chain from lovastatin to monacolin J, and neither methylbutyrate nor its CoA derivative would substitute for the lovastatin in this reaction (*14*). Hence, we predict that LovD requires as substrates 2-methylbutyrate bound to the LovF protein and monacolin J. This idea accounts for the lack of a TE domain or equivalent product-releasing activity in LovF and also implies that LovD docks with LovF.

In summary, the important HMG-CoA reductase inhibitor lovastatin is synthesized by two separate PKS systems with a larger range of biosynthetic activities than exhibited by the typical fungal iterative type I PKS involved in the biosynthesis of aromatic polyketides. Both of these systems have notable features. The LDKS may be a noniterative type I PKS catalyzing a single Claisen condensation followed by methylation, ketoreduction, dehydration, and enoyl reduction to produce (2*R*)-2-methylbutyrate, which probably remains bound to the enzyme until transferred to monacolin J by a separate transesterase. The dihydromonacolin L synthase consists of at least two enzymes, a type

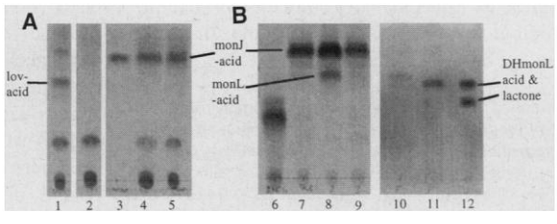

Fig. 5. TLC of extracts from *A. terreus* mutant strains. (**A**) Lane 1, extract from wild type (ATCC 20542); lane 2, extract from *lovC* mutant WMH1734; lane 3, monacolin J (acid form); lane 4, extract from *lovD* mutant WMH1732; lane 5, extract from *lovF* mutant WMH1731. (**B**) TLC of extracts from *A. nidulans* mutant strains carrying lovastatin biosynthesis genes. Lane 6, extract from strain WMH1738; lanes 7 to 9, extracts from strains WMH1739, WMH1740, and WMH1749, respectively. These three strains all produce monacolin J, and strain WMH1740 also produces monacolin L. Lane 10, extract from strain WMH1738; lane 11, extract from strain WMH1750 that produces dihydromonacolin L; lane 12, standard mixture of dihydromonacolin L in acid and lactone forms. Abbreviations: monJ, monacolin J; lov, lovastatin; DHmonL, dihydromonacolin L; monL, monacolin L.

I iterative PKS and a putative enoyl reductase. This system appears to be all that is necessary for the 35 separate reactions [including a possible Diels-Alder cyclization reaction, heretofore precedented only once in nature (*21*)] postulated to be necessary for the biosynthesis of dihydromonacolin L from acetyl-CoA, malonyl-CoA, NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate), and SAM. The LNKS/LovC system exhibits a remarkable discriminatory ability whose catalytic mechanism must be very different from the linear programming found in bacterial modular type I PKS systems (*4, 5*). Lovastatin biosynthesis is completed by the addition of the 2-methylbutyryl side chain to monacolin J by a specific transesterase encoded by *lovD*.

Bacterial type I PKSs have been manipulated genetically to produce novel compounds that are difficult to make by traditional chemical methods (*4–6*). Although much less is known about the molecular recognition powers of the PKSs that make reduced fungal metabolites like lovastatin, the methods of combinatorial biosynthesis may also be applicable to these enzymes. Elimination or inactivation and addition of domains to such PKSs will determine whether this approach can yield useful information on the substrate discriminatory properties of the LNKS/LovC complex in particular and will allow us to determine how it can be manipulated to produce novel compounds. Manipulation of *lovF* to produce compounds with different side chains is much easier to envisage. The deletion of activities in this gene or perhaps addition of further modules could allow the production of various lovastatin analogs in a predictive manner.

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UDP-GlcNAc 2-Epimerase: A Regulator of Cell Surface Sialylation

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Modification of cell surface molecules with sialic acid is crucial for their function in many biological processes, including cell adhesion and signal transduction. Uridine diphosphate-*N*-acetylglucosamine 2-epimerase (UDP-GlcNAc 2-epimerase) is an enzyme that catalyzes an early, rate-limiting step in the sialic acid biosynthetic pathway. UDP-GlcNAc 2-epimerase was found to be a major determinant of cell surface sialylation in human hematopoietic cell lines and a critical regulator of the function of specific cell surface adhesion molecules.

In eukaryotic cells, glycoproteins and glycolipids expressed at the cell surface can be modified to varying degrees by the addition of sialic acids [*N*-acetylneuraminic acid (NeuAc) or other N- and O-substituted neuraminic acids]. Because of their widespread distribution, structural versatility, and peripheral position on oligosaccharide chains of glycoconjugates, sialic acid residues are well suited as molecular determinants of specific biological processes. For example, they are involved in cell-cell interactions, T and B cell activation, and hematopoietic cell differentiation (*1–4*). Differential sialylation, that is, quantitative or linkage-specific differences in sialylation, of cell surface molecules is also implicated in the tumorigenicity and metastatic behavior of malignant cells (*5*).

Sialyltransferases, which reside in the Golgi apparatus, add cytidine monophosphate (CMP)–activated sialic acid residues to specific terminal nonreducing positions on oligosaccharide chains of proteins and lipids (*6*). The differential expression of sialyltransferases explains some (*4*, *7*) but not all examples of differential sialylation (*8*, *9*). In subclones of the human B lymphoma cell line BJA-B, differential sialylation accounts for a reduction of up to 73% in the incorporation of both α-2,6– and α-2,3–linked sialic acid residues into membrane glycoconjugates, even though the activity of the β-galactoside α-2,6-sialyltransferase, ST6Gal I, is not limited (*9*). Also, the different degrees of cell

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