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COMMENTARY

New Kid on the Block: LmbU Expands the Repertoire of Specialized Metabolic Regulators in *Streptomyces*

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ABSTRACT *Streptomyces* has an extensive natural product repertoire, including most of the naturally derived antibiotics. Understanding the control of natural product biosynthesis is central to antibiotic discovery and production optimization. Here, Hou et al. (J. Bacteriol. 200:00447-17, 2018, https://doi.org/10.1128/JB.00447-17) report the identification and characterization of a novel regulator—LmbU—that functions primarily as an activator of lincomycin production in *Streptomyces lincolnensis*. Importantly, members of this new regulator family are associated with natural product biosynthetic clusters throughout the streptomycetes and their actinomycete relatives.

KEYWORDS *Streptomyces*, antibiotic, cluster-situated regulator, lincomycin, natural product, regulator, transcription factor

Natural products are among the cornerstones of medicine and agriculture today, given their antibacterial, antifungal, antiparasitic, herbicidal, immunosuppressive, and cancer-therapeutic properties. Bacteria are major producers of antibiotics and other natural products, with the streptomycetes being among the most prolific contributors (1). The rise in antibiotic resistance has led to a renewed focus on understanding the fundamental principles governing natural product biosynthesis by these bacteria, with a view to developing strategies that improve and accelerate antibiotic discovery (2–4).

Combined genetic and biochemical investigations into the biosynthesis of natural products has revealed that the production of these compounds is directed by genes that are generally clustered together on the bacterial chromosome (5). Depending on the size and structural complexity of the natural products, their biosynthetic gene clusters can span anywhere from several kilobases to more than 100 kb. Located within these clusters are genes specifying enzymes that direct the synthesis of the core metabolite scaffold and its subsequent modification and functionalization, as well as proteins that function as transporters, resistance determinants, and transcription regulators.

The dawn of the genomic era led to the sequencing of many *Streptomyces* and related actinobacterial chromosomes and revealed a striking dichotomy: the number of natural product gene clusters present in their genomes far exceeds the number of compounds identified from each characterized strain (6–9). Our ability to access and characterize this incredible treasure trove of bioactive compounds has been hampered by low titers of production. Indeed, the majority of these "cryptic" biosynthetic gene clusters are expressed at very low levels, with a significant proportion of them being transcriptionally "silent" under laboratory conditions.

Highly productive strategies aimed at stimulating or enhancing natural product levels have involved circumventing or modifying the native regulation of biosyn-

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TABLE 1 Common classes and examples of cluster-situated regulators in the streptomycetes

Regulator family	Type of regulator	Binding site(s)	Classic example(s)	Reference(s)
SARP	Activator	5- to 7-nt direct repeats	ActII-ORF4 (actinorhodin), RedD (undecylprodigiosin), DnrO (daunorubicin)	13–15
LAL	Activator		AveR (avermectin)	16
TetR	Repressor	Inverted repeats	ActR (actinorhodin)	17
GntR	Repressor		PmtR1 (platensimycin)	18
Two-component regulator	Activator/repressor		AbsA2 (calcium-dependent antibiotic; repressor), RedZ (undecylprodigiosin; activator)	19, 20
StrR (ParB-like)	Activator	Inverted repeats	StrR (streptomycin), CmlR (chloramphenicol)	21, 22
LmbU	Activator/repressor	Short palindrome	LmbU (lincomycin)	23

thetic gene clusters. These strategies have included everything from heterologous expression of gene clusters within natural and engineered hosts to synthetic biology approaches that completely refactor the pathway (e.g., removing noncoding DNA and transcription factors and replacing them with synthetic regulatory components) (10, 11). Within native strains, a range of genetic strategies have been employed to improve metabolite production, including the use of promoter knock-ins, overexpression of cluster-specific activators or global regulators, and deletion of cluster-specific repressors (11, 12). While these strategies often yield some level of metabolite production, it is commonly coupled with suboptimal regulation and an unbalanced metabolic burden, which can lead to the accumulation of undesired intermediates or side products. Consequently, a comprehensive understanding of metabolic regulation is critically needed in order to fully exploit the metabolic potential of the streptomycetes.

There is considerable flexibility in the innate cluster regulatory schemes: some metabolic clusters lack any obvious regulatory genes, while others harbor multiple regulators. While regulators of many types have been found within biosynthetic clusters, a few classes are represented far more frequently than others (Table 1). Known as either "pathway-specific" or "cluster-situated" regulators, these transcription factors primarily act to modulate the expression of genes within their cognate cluster. The SARPs—or *Streptomyces* antibiotic regulatory proteins—are among the most common and the best studied in the streptomycetes. These proteins are classified as members of the OmpR family of regulators, having an N-terminal winged helix-turn-helix DNA binding domain (24). They are potent activators of their associated biosynthetic pathways, and overexpressing these genes has proven to be a powerful means of enhancing the production of their cognate metabolite (25–27). Similarly, the members of the LAL family of regulators (for large ATP-binding regulators of the LuxR family) are also largely confined to the streptomycetes and, like the SARPs, function to activate the expression of their specific biosynthetic pathway (28). Less well understood are the StrR-like regulators, which also have cluster-specific activator functions (21, 22).

Negative cluster regulation is commonly mediated by TetR and GntR family members. Members of the TetR family in particular are frequently found in association with—and controlling—the expression of adjacent resistance/transporter determinants (29). The activity of both TetR and GntR regulators is mediated by ligand binding (29, 30). This typically results in a conformational change in the regulator, release of DNA binding, and a concomitant relief of repression.

Despite significant advances in our understanding of *Streptomyces* gene regulation, a reasonable proportion of natural product biosynthetic gene clusters do not appear to encode any obvious pathway-specific regulators. This suggests that our knowledge of gene expression in these bacteria—and, in particular, the control of these biosynthetic clusters—remains incomplete. This also raises several important questions. How are these natural product pathways regulated? How many novel regulators have yet to be identified, and how do they function?

One natural product biosynthetic gene cluster whose regulation is poorly understood is

that corresponding to lincomycin. Originally isolated in 1962 from *Streptomyces lincolnensis* (31), lincomycin is used as a clinically approved treatment for Gram-positive bacterial and *Mycoplasma* infections. It is the archetype of the lincosamide family of antibiotics (32). Lincomycin has been subjected to considerable derivatization, the most notable manifestation of which is clindamycin, a chlorinated variant with improved antibiotic activity. To date, genetic and biochemical studies have focused on elucidating the genes and enzymes responsible for synthesizing the precursors propylhygric acid (a nonproteogenic amino acid) and the sugar methylthiolincosamide and their subsequent conjugation to form the mature antibiotic (32).

In this issue, Hou et al. revisit the lincomycin biosynthetic pathway and shed light on its regulation (23). Bioinformatic analysis of the lincomycin gene cluster in *S. lincolnensis* suggested that gene *lmbU* encodes a novel cluster-situated regulator. To test this hypothesis, the authors first inactivated *lmbU* and found that this mutation abolished lincomycin production. In contrast, overexpressing this gene resulted in a 5-fold increase in the lincomycin titer compared with the wild-type strain. To determine whether this effect stemmed from altered regulation, transcript levels for select genes throughout the cluster were assessed in the wild-type, *lmbU* mutant, and *lmbU* overexpression strains. Consistent with the lincomycin production experiments, transcript levels were significantly decreased for four of the biosynthetic genes tested (*lmbA*, *lmbC*, *lmbJ*, and *lmbW*) in the *lmbU* disruption strain, while the opposite effect was seen for several genes in the overexpression strain, where the expression of *lmbA* and *lmbJ* was increased. To validate these observations and to gain further insight into the *in vivo* function of *lmbU*, each predicted promoter region in the lincomycin gene cluster was fused to a neomycin resistance (Neo^r) gene cassette and subsequently introduced into both the wild-type *S. lincolnensis* strain and *lmbU* mutant strain. The results largely confirmed the previous experiments; however, it also became apparent that LmbU did not function solely as an activator. Higher kanamycin resistance (reporter activity) was observed for the *lmbU* and *lmbK* promoter fusions in the *lmbU* mutant background, suggesting that LmbU may serve (directly or indirectly) to repress the expression of these genes.

To address the mechanistic basis of LmbU activity, recombinant LmbU was overexpressed and purified. Electrophoretic mobility shift assays (EMSAs) were performed to test whether the observed effects were due to direct control by LmbU. LmbU was found to bind directly to the putative promoter sequences of *lmbA* and *lmbW*. Systematic deletion and mutagenesis of the sequence within the *lmbV-lmbW* intergenic region ultimately identified a palindromic DNA motif (5′-CGCCGGCG-3′) critical for LmbU binding. Indeed, *S. lincolnensis* strains containing the Neo^r gene fused to an *lmbA* promoter variant lacking this sequence were sensitive to kanamycin, confirming the *in vivo* function of the motif for LmbU regulation. It appeared that the LmbU effect on the expression of *lmbC*, *lmbJ*, *lmbK*, and *lmbU* was indirect, as no binding to these promoter regions was observed using EMSAs. Intriguingly, this suggests that LmbU comprises only one piece of a complex network governing lincomycin biosynthesis.

With the discovery and characterization of LmbU activity, that study has established a new branch in the regulatory hierarchy governing natural product biosynthesis in the streptomycetes and their relatives. Future investigations into LmbU biochemistry and its protein structure will ultimately uncover the molecular mechanisms underpinning its function. Still unknown is how the activity of LmbU is integrated with that of other regulators and where it fits in the regulatory interplay coordinating *Streptomyces* differentiation and specialized metabolism. Nevertheless, a comprehensive understanding of LmbU function may expand the repertoire of tools available for use in the genetic engineering of these bacteria. Excitingly, *lmbU* homologs are associated with diverse natural product gene clusters in taxonomically disparate strains. Manipulating these genes may provide access to new natural product leads and ultimately help with replenishing the dwindling supply of effective antibiotics available to combat drug-resistant pathogens.

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