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Regulation of antibiotic biosynthesis in actinomycetes: Perspectives and challenges

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ABSTRACT

Actinomycetes are the main sources of antibiotics. The onset and level of production of each antibiotic is subject to complex control by multi-level regulators. These regulators exert their functions at hierarchical levels. At the lower level, cluster-situated regulators (CSRs) directly control the transcription of neighboring genes within the gene cluster. Higher-level pleiotropic and global regulators exert their functions mainly through modulating the transcription of CSRs. Advances in understanding of the regulation of antibiotic biosynthesis in actinomycetes have inspired us to engineer these regulators for strain improvement and antibiotic discovery.

1. Introduction

Actinomycetes are the most abundant sources of antibiotics and many other specialized metabolites with industrial, agricultural and medical applications. Typically, genes responsible for the biosynthesis of a specific antibiotic are arranged in clusters [1]. Most genes within the cluster encode enzymes responsible for catalyzing the formation of antibiotics from simple building blocks [2–4]. Moreover, the gene cluster contains genes with regulatory functions. These cluster-situated regulators (CSRs) have major effects on the levels of production of the cognate antibiotic. There are also many other regulators that exert their functions at higher levels [5,6]. Pleiotropic regulators are situated outside the biosynthetic gene clusters (BGCs) and control the production of multiple antibiotics and/or morphological development. Global regulators are scattered throughout the chromosome and control both central metabolic genes and pleiotropic regulatory genes or CSR genes [5]. Here we highlight recent findings on the complex cascade regulation of antibiotic biosynthesis in actinomycetes and summarize progress in genetic manipulation of regulators for strain improvement and antibiotic discovery.

2. Cascade regulation of antibiotic biosynthesis in actinomycetes

2.1. Clusters-situated regulators in antibiotic biosynthesis

Antibiotic biosynthesis is subject to complex regulation involving CSRs and pleiotropic or global regulators (Table 1). Typically, each BGC contains one or more CSRs. *Streptomyces* antibiotic regulatory proteins (SARPs) are the most frequently encountered CSRs among streptomycetes. Representative SARPs include the most well-known ActII-ORF4, RedD and CdaR, the respective activators for the biosynthesis of actinorhodin (ACT), undecylprodigiosin (RED) and calcium-dependent antibiotic (CDA) in the model actinomycete *Streptomyces coelicolor* [7–9]. SARPs exert their functions by directly controlling the transcriptional level of biosynthetic genes or another CSR within the cluster. For example, NosP activates nosiheptide biosynthesis in *Streptomyces actuosus* by directly binding to the bidirectional *nosL-M* promoter region [10,11]. SanG activates nikkomycin biosynthesis in *Streptomyce ansochromogenes* by directly binding to the promoter regions of two diverging transcriptional units (*sanO-V* and *sanN-I*) [12,13]. OtcR activates oxytetracycline biosynthesis in *Streptomyces rimosus* by binding to promoter regions of biosynthetic genes *oxyA*, *oxyI*, *oxyJ*, *oxyR* and *oxyS* [14]. Other examples include two SARPs (PolR and PolY) in polyoxin biosynthesis of *Streptomyces cacaoi* subsp. *asoensis* and three SARPs (PapR1, PapR2 and PapR4) in pristinamycin (pristinamycin I and pristinamycin II) biosynthesis of *Streptomyces*

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Table 1
Representative regulators of antibiotic biosynthesis.

Regulator	Description	Reference
Cluster-situated Regulators		
ActII-ORF4	SARP family, activator of actinorhodin biosynthesis	[7]
RedD	SARP family, activator of undecylprodigiosin biosynthesis	[8]
CdaR	SARP family, activator of calcium-dependent antibiotic biosynthesis	[9]
SanG	SARP family, activator of nikkomycin biosynthesis	[12]
PolR	SARP family, activator of polyoxin biosynthesis	[15]
PolY	SARP family, activator of polyoxin biosynthesis	[16]
ScbR	TetR family, repressor of coelimycin P1 biosynthesis	[18]
JadR2	TetR family, repressors of jadomycin biosynthesis	[20]
JadR*	TetR family, repressors of jadomycin biosynthesis	[22]
ChlF1	TetR family, activator of chlorothricin biosynthesis	[26]
GouR	TetR family, activator of gougerotin biosynthesis	[28]
GdmRIII	TetR family, activator of geldanamycin and repressor of elaiophylin biosynthesis	[27]
PimR	LAL family, activator of pimaricin biosynthesis	[30]
PikD	LAL family, activator of pikromycin biosynthesis	[31]
RapH	LAL family, activator of rapamycin biosynthesis	[32]
NysRI	LAL family, activator of nystatin biosynthesis	[34]
NysRIII	LAL family, activator of nystatin biosynthesis	[34]
SlnR	LAL family, activator of salinomycin biosynthesis	[36]
PimM	PAS-LuxR family, activator of pimaricin biosynthesis	[30]
NysRIV	PAS-LuxR family, activator of nystatin biosynthesis	[34]
PteF	PAS-LuxR family, activator of filipin biosynthesis	[38]
PenR/PntR	MarR family, activator of pentalenolactone biosynthesis	[39]
LmbU	A novel family, activator of lincomycin biosynthesis	[48]
Pleiotropic and global regulators		
ArpA	Repressor of AdpA	[52]
AdpA	Activator of streptomycin biosynthesis	[53]
	Activator of grixazone biosynthesis	[54]
	Activator of nikkomycin biosynthesis	[55]
	Activator of natamycin biosynthesis	[56]
	Repressor of oviedomycin biosynthesis	[57]
WblA	Activator of natamycin biosynthesis	[62]
	Repressor of doxorubicin biosynthesis	[59]
	Repressor of tautomycetin biosynthesis	[60]
	Repressor of daptomycin biosynthesis	[61]
AtrA	Activator of actinorhodin biosynthesis	[63]
	Activator of lidamycin biosynthesis	[66]
	Activator of pristinamycin biosynthesis	[67]
	Activator of daptomycin biosynthesis	[68]
	Repressor of avermectin biosynthesis	[64]

pristinaespiralis. PolR activates polyoxin production by directly binding to the promoter regions of *polC* and *polB*, the respective first genes of two transcriptional units (*polC-polQ2* and *polA-polB*) [15]. Moreover, the transcription of *polR* itself is activated by PolY, another SARP encoded by the neighboring *polY* within the gene cluster [16]. PapR1, PapR2, and PapR4 act as activators of pristinamycin biosynthesis. PapR1 activates pristinamycin production by directly binding to the promoter regions of the pristinamycin I biosynthetic genes *snbA-pipA* and *snbC*, and the pristinamycin II biosynthetic genes *snaB* and *snaE3*. PapR2 activates pristinamycin production by directly binding to the promoter region of *papR1* as well as the target promoters of PapR1 [17]. However, the function of PapR4 and its interplay with the other two SARPs remains unknown.

The TetR family transcriptional regulators represent another important group of CSRs. Unlike SARPs which serve as activators, most TetR family regulators function as repressors of antibiotic biosynthesis. Some TetR family regulators repress antibiotic biosynthesis by directly inhibiting the transcription of cluster-situated activator genes. Representative members include ScbR of coelimycin P1 biosynthesis in *S. coelicolor*, JadR2 and JadR* of jadomycin biosynthesis in *Streptomyces venezuelae*, and PapR3 and PapR5 of pristinamycin biosynthesis in *S. pristinaespiralis*. ScbR represses coelimycin P1 biosynthesis by inhibiting the transcription of *kasO*, which encodes a SARP homologue [18,19]. JadR2 represses jadomycin production by inhibiting the transcription of neighboring *jadR1*, which encodes an OmpR-type activator [20,21]. JadR* represses jadomycin production by inhibiting the transcription of *jadR1*, as well as biosynthetic genes *jadI*, *jadE* and *jadY* [22]. Intriguingly, JadR2 and jadR* act synergistically to repress jadomycin production by inhibiting the transcription of *jadR1* [23]. PapR5 represses pristinamycin biosynthesis by inhibiting the transcription of *papR1* and *papR4*, which encode two of the three SARPs activators mentioned above, while PapR3 represses pristinamycin biosynthesis by inhibiting the transcription of *papR4* and *papR5* [17]. The TetR family regulators also repress antibiotic production by directly inhibiting the transcription of gene encoding a transmembrane efflux protein [24]. There are a few TetR family regulators that act as activators, such as DnrO, ChlF1, GdmRIII and GouR. DnrO activates daunorubicin production via promoting the transcription of *dnrN*, which encodes an atypical response activator [25]. ChlF1 activates chlorothricin production through coordinated modulation of four biosynthetic genes *chlF1*, *chlG*, *chlK* and *chlJ* [26]. GdmRIII activates geldanamycin production via controlling the transcription of two CSR genes and seven biosynthetic genes [27]. GouR activates gougerotin production by coordinating the transcription of the *gouL-B* operon consisting of 11 biosynthetic genes and a major facilitator superfamily (MFS) transporter gene [28].

Regulators of the LALs (Large ATP-binding regulators of the LuxR family) have also been identified as CSRs in many actinomycetes [29]. LALs are characterized by an N-terminal ATP/GTP-binding domain and a C-terminal DNA-binding domain with a LuxR-like helix-turn-helix motif. Many LALs have been identified as activators of antibiotic biosynthesis. Representative examples include PimR of pimaricin biosynthesis in *Streptomyces natalensis* [30], PikD of pikromycin biosynthesis in *S. venezuelae* [31], RapH of rapamycin biosynthesis in *Streptomyces hygroscopicus* [32], NysRI and NysRIII of nystatin biosynthesis in *Streptomyces noursei* [33,34], AmphRI and AmphRIII of amphotericin biosynthesis in *Streptomyces nodosus* [35] and SlnR of salinomycin biosynthesis in *Streptomyces albus* [36]. PAS-LuxR is another interesting family of regulators, which contain a N-terminal PAS sensory domain with a C-terminal helix–turn–helix (HTH) motif of the LuxR type. Examples include PimM of pimaricin biosynthesis in *Streptomyces chattanoogensis* [37], NysRIV of nystatin biosynthesis in *S. noursei* [34], and PteF of filipin biosynthesis in *S. avermitilis* [38]. There are also some regulators, such as MarR family activators of pentalenolactone biosynthesis in *Streptomyces exfoliatus* UC5319 and *Streptomyces arenae* TÜ469 [39], LysR family repressor of ascomycin (FK520) in *S. hygroscopicus* var. *ascomyceticus* FS35 [40], among many others [41].

Of special note is that none CSR has been identified within a few BGCs, such as BGCs for chloramphenicol biosynthesis in *S. venezuelae* [42], erythromycin biosynthesis in *Saccharopolyspora erythraea* [43], lactimidomycin biosynthesis in *Streptomyces amphibiosporus* [44] and albomycin biosynthesis in *Streptomyces griseus* [45]. However, further analysis of uncharacterized genes adjacent to the chloramphenicol BGC identified a StrR-like transcriptional activator in *S. venezuelae* [46]. It is also noteworthy that some CSRs can directly control the expression of genes in other clusters. For example, JadR1 of jadomycin biosynthesis in *S. venezuelae* represses the transcription of biosynthetic genes within the chloramphenicol BGC [20]. Similarly, GdmRIII of geldanamycin biosynthesis in *Streptomyces autolyticus* CGMCC0516 represses the transcription of biosynthetic genes within the elaiophylin BGC [27]. Importantly, a recent study identified a novel transcriptional regulator LmbU of lincomycin biosynthesis in *Streptomyces lincolnensis*. LmbU and its homologues have no significant structural similarities to other known CSRs, suggesting that LmbU represents a new family of regulators [47,48].

2.2. Pleiotropic and global regulators in antibiotic biosynthesis

AdpA is a member of the AraC/XylS family regulators ubiquitously

distributed in streptomycetes [49]. The pleiotropic effects of AdpA are manifested through regulation of hundreds of genes required for antibiotic biosynthesis and morphological differentiation [50–52]. In *S. griseus*, transcription of *adpA* is controlled by ArpA, a TetR family receptor for the γ-butyrolactone (GBL) A-factor. In the early culture stage, ArpA represses the transcription of *adpA* by directly binding to its promoter region. As A-factor accumulates to a threshold concentration, it binds to ArpA and releases the repression of *adpA* [52]. AdpA has been reported to activate CSR genes including *strR* for streptomycin biosynthesis [53], *griR* for grixazone biosynthesis [54] and *sanG* for nikkomycin biosynthesis [55]. Generally considered as an activator for antibiotic biosynthesis, AdpA directly binds the upstream of *sanG* to activate nikkomycin biosynthesis in *S. ansochromogenes*. The role of AdpA in *sanG* transcription is complicated by the presence of five AdpA-binding sites (I–V) in the upstream region of *sanG*; two (I and V) are used for activation of *sanG* transcription, while the other three (II, III, and IV) lead to repression [55]. Similar findings have been reported in AdpA regulation of natamycin biosynthesis in *S. chattanoogensis* [56]. However, a recent study suggests that AdpA acts as a repressor of oviedomycin biosynthesis by directly inhibiting the transcription of cluster-associated activator *ovmZ/ovmW* [57].

WblA, a whiB-like protein identified in *S. coelicolor*, is also widely distributed among actinomycetes [58]. WblA is a major regulator of morphological development and antibiotic biosynthesis. Reports show that WblA functions generally as a global repressor of antibiotic biosynthesis, such as doxorubicin biosynthesis in *Streptomyces peucetius* [59], tautomycetin biosynthesis in *Streptomyces* sp. CK4412 [60], and daptomycin biosynthesis in *S. roseosporus* [61]. However, WblA acts as an activator for natamycin biosynthesis in *S. chattanoogensis* [62].

The TetR-family regulator AtrA is another important group of pleiotropic regulators. AtrA activates ACT production through promoting the transcription of *actII-ORF4* in *S. coelicolor* [63]. In *S. avermitilis*, AveI (an AtrA orthologue) was first identified as a negative regulator for avermectin production. Gel shift assays revealed that AveI binds specifically to the promoter region of *actII-ORF4* but not that of the cluster-associated activator gene *aveR* [64]. However, transcriptomics analysis shows that transcription of *aveR*, as well as several genes in the BGCs for oligomycin and filipin biosynthesis, were elevated in the *aveI* mutant [65]. The complex regulation of AveI on biosynthetic pathways of avermectin, oligomycin and filipin awaits further investigation. In *Streptomyces globisporus*, AtrA binds to the promoter region of the *sgcR1R2* operon and activates the expression of SgcR1 and SgcR2, two of the three CSRs within the lidamycin BGC, thereby stimulating lidamycin production [66]. AtrA was also found to promote pristinamycin biosynthesis via directly activating the transcription of *spbR* and *papR5*, two CSR genes within the pristinamycin BGC [67]. Surprisingly, AtrA binds directly to the promoter region of structural gene *dptE* other than that of *dptR1* and *dptR2*, two regulatory genes situated close to the daptomycin BGC in *S. roseosporus* [68].

3. Genetic manipulation of regulators for strain improvement

Titer improvement is in constant pursuit of fermentation-based bioprocess for antibiotic production. Traditionally, overproducing strains have been obtained by iterative random mutagenesis coupled with screening techniques. Recently, there has been considerable interest in rational engineering of antibiotic producers. Improvement of antibiotic titers can be achieved by overexpression of key structural genes [69], resistance genes [70], ATP-binding cassette transporter genes [71] or amplification of the entire BGCs [72–76].

A better understanding of cascade regulation of antibiotic biosynthesis in actinomycetes have provided the basis for genetic manipulation of transcriptional regulators (Fig. 1). Considering the importance of these regulators, this method is an efficient way for titer improvement. This can be achieved simply by high-level expression of genes encoding activators or deletion of genes encoding repressors. Overexpression of genes encoding SARPs have been used to increase production of nikkomycin in *S. ansochromogenes* TH322 and oxytetracycline in *S. rimosus*. Overproduction of nikkomycin has been achieved by engineering of the CSR activator gene *sanG* with different constitutive promoters [77]. Overproduction of oxytetracycline has been achieved by overexpression of the CSR activator gene *otcR* as tandem copies under the control of constitutive SF14 promoter [14]. As aforementioned, no CSR gene has been identified within the lactimidomycin BGC in *S. amphibiosporus* ATCC 53964. However, two genes encoding SARPs, *mgsA* and *chxA*, have been identified respectively within the iso-migrastatin BGC in *Streptomyces platensis* NRRL 18993 and the cycloheximide BGC in *Streptomyces* sp. YIM56141. Overproduction of lactimidomycin has been achieved by overexpression of *mgsA* or *chxA* in *S. amphibiosporus* ATCC 53964 [44]. Similar strategy has also been used to overproduce tacrolimus (FK506) in *Streptomyces tsukubaensis* NRRL18488 and ansamitocins in *Actinosynnema pretiosum* by overexpression of *bulZ* and *asm18* [78,79]. Overexpression of genes encoding LAL family regulators lead to overproduction of FK506 in *Streptomyces* sp. strain KCTC 11604BP, neomycin in *Streptomyces fradiae* CGMCC 4.7387, milbemycin in *Streptomyces bingchenggensis* and salinomycin in *Streptomyces albus* [80–82]. Other examples include LysR family regulator of ascomycin production in *S. hygroscopicus* var. *ascomyceticus* [40], PAS-LuxR family regulators of wuyiencin production in *Streptomyces wuyiensis* CK-15 and reedsmycin production in *Streptomyces youssoufiensis* OUC6819 [83,84], and Crp/Fnr family regulator of leinamycin production in *Streptomyces atroolivaceus* [85].

Deletion of TetR family repressors has been used to increase avermectin production in *S. avermitilis* [86], calcimycin production in *Streptomyces chartreusis* NRRL 3882 [24], and pristinamycin production in *S. pristinaespiralis* [87]. Deletion of *wblA* leads to overproduction of pikromycin in *S. venezuelae* [88], daptomycin in *S. roseosporus* [61] and antifungal polyene in *Pseudonocardia autotrophica* [89]. Other examples include deletion of genes encoding GntR family regulators for nucleoside antibiotic A201A overproduction in *Marinactinospora thermotolerans* 00652, and platensimycin and platencin overproduction in *Streptomyces platensis* [90,91], GBL receptors for validamycin overproduction in *S. hygroscopicus* 5008 [92], SgcR for lidamycin overproduction (C-1027) in *S. globisporus* [93], and PhaR for daptomycin overproduction in *S. roseosporus* [94]. It is not uncommon that deletion of multiple repressors are required to further increase antibiotic titers [95]. To achieve maximal level of antibiotic production, it requires a systematic manipulation of deletion of repressors in combination with overexpression of activators [93,96,97].

4. Genetic manipulation of regulators for antibiotic discovery

New antibiotics are urgently needed to combat the growing emergence of antimicrobial-resistant pathogens. Uncharacterized biosynthetic pathways embedded in the genome of actinomycetes are appealing sources for antibiotic discovery [98]. Therefore, various strategies have been devised to trigger the expression of these cryptic BGCs for the discovery of new antibiotics [5,98,99]. Among them, genetic manipulation of regulators (activators or repressors) is a simple and effective way to awake these BGCs. For example, a giant type I modular PKS gene cluster of *Streptomyces ambofaciens* ATCC 23877 was activated simply by overexpression of a LAL family regulatory gene, leading to the discovery of a group of novel macrolide antibiotics [100]. Similarly, a type III glycopeptide gene cluster of *Amycolatopsis japonicum* was activated by constitutive expression of a StrR-like regulatory gene, leading to the production of ristomycin A [101]. In another study, a cryptic angucycline biosynthetic gene cluster of *S. chattanoogensis* L10 was activated by overexpressing of an OmpR family regulatory gene, leading to the discovery of two novel angucycline antibiotics [102]. A cryptic mureidomycin cluster of *S. roseosporus* NRRL 15998 was activated by constitutive expression of a foreign activator gene from BGC for the biosynthesis of sansanmycins, a group of close-related

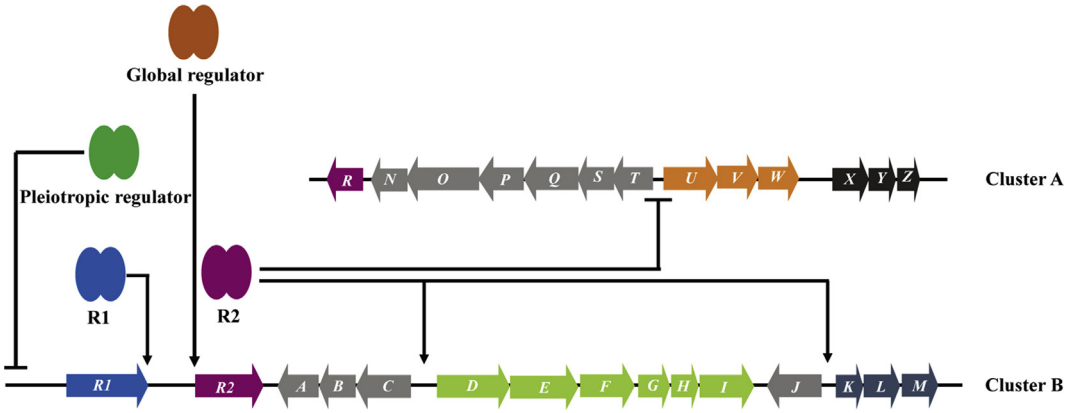

Fig. 1. Cascade regulation of antibiotic biosynthesis. Schematic diagram showing the regulation of antibiotic biosynthesis mediated by cluster-situated regulators and pleiotropic or global regulators. The cluster-situated regulator R2 exert its regulatory function through activating biosynthetic genes within Cluster B. It can also repress the transcription of biosynthetic genes within Cluster A. The transcription of *R2* can be activated by another cluster-situated regulator, which is encoded by the neighboring *R1*, and a global regulator situated outside of Cluster B. The transcription of *R1* is controlled by a pleiotropic regulator situated outside of Cluster B.

metabolites of mureidomycins [103]. There are also many studies involving deletion of genes encoding repressors. For example, deletion of GBL receptors activated an uncharacterized type I PKS gene cluster in *S. coelicolor* A3 (2), the jadomycin gene cluster in *S. venezuelae* ISP5230, and the phthoxazolin A in *S. avermitilis* [20,104,105]. Deletion of *gbnR* encoding a transcriptional repressor in *S. venezuelae* ATCC 10712 resulted in the identification of gaburedins, a novel class of urea natural products [106]. Interestingly, a cryptic chromomycin BGC can be activated by either overexpression of SARP activator gene or deletion of PadR repressor gene in *Streptomyces reseiscleroticus* [107].

Many studies report on genetic manipulation of genes encoding pleiotropic or global regulators. For example, deletion of *wblA* leads to the discovery of two novel tylosin analogues in *S. ansochromogenes* 7100 [108] and a novel dioic acid in *Streptomyces somaliensis* SCSIO ZH66 [109]. Though AdpA and its homologues are generally considered as activators, recent studies suggest that they can also serve as repressors, making them good targets for antibiotic discovery. Deletion of *adpA* in *S. ansochromogenes* activated a cryptic PKS gene cluster, leading to the production of oviedomycin [57]. Similarly, deletion of *adpA* in *Streptomyces argillaceus* activated a Type III PKS gene cluster, leading to the production of germicidins [110]. Another example is deletion of *bldM* in *S. venezuelae* activated a Type I PKS gene cluster, leading to the discovery of a group of unusual biaryl polyketides [111]. Of special note is a study with introduction of a functional *bldA* into *Streptomyces calvus*, which is deficient in the formation of aerial mycelium and spores due to a point mutation in the *bldA* gene. The complementation not only restored sporulation, but also activated a cryptic Type I PKS gene cluster encoding the polyene annimycin [112].

5. Conclusions and perspectives

The importance of understanding the regulation of antibiotic biosynthesis in actinomycetes has encouraged intensive research on transcriptional regulators. The emerging picture shows a complex network of regulators at different hierarchical levels. It is noteworthy that there is a strong association of a specific group of CSRs with pathways for antibiotic biosynthesis of the same family. Examples include SARPs with pathways for polyketide biosynthesis [5], PAS-LuxR with pathways for polyene macrolide biosynthesis [113], and SsaA homologues with pathways for the uridyl peptide antibiotics biosynthesis [103]. However, little is known about regulation at pretranscriptional level (epigenetic regulation) and the posttranscriptional level, via small noncoding RNAs (sRNAs) and the protein degradation machinery (Clp complex and proteasome) [5]. The extracytoplasmic function (ECF) sigma factor SigT serves as a negative regulator of ACT and RED, and SigT is subject to specific proteolysis by the proteasome in *S. coelicolor* [114–116]. It would be interesting to examine the roles of these post-transcriptional regulators in the regulation of antibiotic biosynthesis. Further systematic studies are required to decipher the complex multi-level control of antibiotic biosynthesis.

It is not uncommon that antibiotics and/or intermediates serve as autoregulators of their biosynthesis and as cross-regulators of the biosynthesis of other antibiotics [6]. These autoregulators act on either CSRs or pleiotropic and global regulators. For example, nosiheptide and its intermediates act as signaling molecules to modulate the binding of NosP to its target genes, thereby regulating nosiheptide biosynthesis [10]. Similarly, chlorothricin and its intermediates act as signaling molecules to modulate the binding of ChlF1 to its target genes, thereby regulating chlorothricin biosynthesis in *S. antibioticus* [26]. In another study, jadomycin B binds to ScbR2, the pseudo GBL receptor in *S. coelicolor*, thereby relieving ScbR2-mediated repression of *adpA* and *redD*, which in turn induce *S. coelicolor* to undergo premature differentiation (formation of sporulating aerial mycelium) and early RED production [117]. As aforementioned, AtrA activates the transcription of the *sgcR1R2* operon and stimulate lidamycin production. The binding of AtrA to its target is released by a direct interaction with heptaene, an intermediate of lidamycin from *S. globisporus*, and ACT from *S. coelicolor* [66]. It would be of great interests to examine the effect of antibiotics and/or intermediates on the fine-tuning regulation of antibiotic biosynthesis under physiological conditions.

Based on knowledge from these regulatory studies, considerable efforts have been directed toward rational engineering of regulators for strain improvement and antibiotic discovery. An essential part of activator engineering is the choice of promoters. Currently, there are many native and synthetic promoter available [118]. Among them, the commonly used promoters include *ermE** [44,100,102], *hrdB* [77] and *kasOp** [119–121]. Other expression systems have also been developed for controllable transcription and translation of target gene. Examples include a T7 RNA polymerase-dependent expression system [122] and synthetic riboswitches [123]. This will be helpful in engineering these regulators for strain improvement and antibiotic discovery.

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