Volume 3, Number 3, 2026
Pages 1421-1436
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

CRISPR-BASED MANIPULATION OF TRANSCRIPTIONAL REGULATORS FOR SILENT ANTIBIOTIC BGC ACTIVATION IN STREPTOMYCES

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https://doi.org/10.5281/zenodo.23971362

Article Information

Article History

Received:	June 19, 2026
Revised:	August 19, 2026
Accepted:	September 07, 2026
Available Online:	October 28, 2026

Keywords:
Streptomyces, silent biosynthetic gene clusters, CRISPRi, CRISPRa, transcriptional regulators, antibiotic discovery

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Abstract

Most biosynthetic gene clusters (BGCs) contained in Streptomyces genomes are not expressed under commonly used laboratory conditions, and thus represent an enormous and largely unexplored source of new antibiotic scaffolds. Promoter replacement, regulator overexpression and heterologous expression have all been shown to be effective conventional activation strategies, but they are limited by operation complexity, limited scalability and the lack of understanding of the native regulatory architecture. New approaches have emerged along with the discovery of CRISPR-based tools that manipulate transcription without physically altering BGC structure – these tools act in trans to rewire the regulatory networks that control the expression of BGCs. In this review we will discuss the shift from conventional gene editing to programmable transcriptional regulation in Streptomyces, focusing on the use of CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), and on the engineering of both pathway-specific and global regulators by CRISPR. We review evidence showing that the capability to activate

silent pathways without chromosomal engineering can be achieved by CRISPRi to repress endogenous repressors or CRISPRa to activate biosynthetic operons, and that targeting pleiotropic regulators like AdpA, DasR, and Bld-family proteins can enable coordinated activation across multiple BGCs. Recent progress with endogenous Type I-E Cascade systems has allowed activation of thirteen cryptic BGCs to be achieved in non-model strains and new scaffold RNA architectures have been developed to allow both activation and repression from the same complex. The translational potential of these approaches is highlighted by case studies such as jadomycin B and auroramycin, and the recently activated linaridin pathways. We conclude that the challenge has shifted from the tool to understanding the regulatory network: the power of CRISPR based activation is now becoming constrained not by the number of programmable effectors available, but by what is known regarding the nodes to target.

INTRODUCTION

Streptomyces are the most diverse and prolific clinically relevant antibiotic-producing bacteria accounting for approximately two thirds of the naturally derived antibacterial compounds currently used. The even greater potential of their genomes is that antiSMASH analysis typically reveals thirty to forty or more BGCs per strain, but only a small number of these are expressed on any particular laboratory culture. The rest, called silent or cryptic, are those coding biosynthetic machinery for polyketides, nonribosomal peptides, ribosomally synthesized and post-translationally modified peptides (RiPPs), and hybrid scaffolds, whose chemical diversity is yet to be explored to a large extent[1].

Traditional approaches to accessing this chemical space have been through the genetic manipulation of BGCs or their close genetic environment. Novel compounds have been obtained by promoter replacement, by over-expression of pathway specific activators or by deletion of pathway specific repressors, such as auroramycin from S. roseosporus or the gaburedins from S. venezuelae. These have been extended beyond species barrier by heterologous expression in surrogate hosts. However, both of these types of approaches have one problem in common: they involve physically modifying the chromosome or inserting large DNA constructs, which is time consuming, strain dependent and not really applicable to multi-plexed or high throughput discovery formats[2].

CRISPR-based transcriptional manipulation alters this problem situation. The catalytically inactive Cas9 (dCas9) can be fused or recruited with the transcriptional effector domains, thus enabling gene repression or activation of the target genes without genome editing. The guide RNA can be readily changed; it's a system that works in trans from a plasmid, and you can design multiple targets to work together. This ability has been transformed in Streptomyces into functional CRISPRi and CRISPRa platforms in the last few years and has been successfully used to activate silent BGCs using perturbation of endogenous regulatory networks[3]. In this review, we explore how this journey has gone from traditional BGC activation to the rewiring of transcription by CRISPR and the regulatory logic that these tools leverage.

2. Transcriptional Regulation of Antibiotic Biosynthesis in Streptomyces

2.1 Architecture and Classification of Antibiotic BGCs

Streptomyces antibiotic BGCs can vary in size from around ten to more than one hundred kilobases and contain not only the genes coding for the enzymatic machinery for scaffold assembly but also genes coding for accessory proteins that modify, transport and, crucially for this discussion, regulate the assembly. BGCs are traditionally classified based on their biosynthetic logic, namely type I and type II polyketide synthases (PKS), nonribosomal peptide synthetases (NRPS), RiPP pathways, and hybrid systems. The classification is practically relevant for activation strategies, as there is a systematic variation in regulatory architecture with the BGC types. In contrast to RiPP clusters, which tend to be subject to pleiotropic or global control, PKS and NRPS clusters often have specific regulators involved in the pathway[4].

Accessibility of BGCs is also influenced by the genomic context. Chromosomal end-proximal clusters or clusters in genomic islands may be under distinct regulation as compared to those in the very centre of the chromosomes. While the genome sequences of hundreds of Streptomyces strains have allowed systematic cataloguing of BGC diversity, the regulatory logic of most of the clusters is yet to be characterised. This is the biggest knowledge gap to overcome for targeted activation strategies[3].

2.2 Pathway-Specific Transcriptional Regulators

Cluster-situated regulators (CSRs) are genes that are encoded within or in the immediate vicinity of BGCs and that directly control the transcription of the biosynthetic genes. The best characterised families are the SARP (Streptomyces antibiotic regulatory protein) family (e.g., ActII-ORF4 and RedD in S. coelicolor) and the LAL family (e.g., ChaI and AstGI). These regulators are usually activators which bind to promoter regions upstream of biosynthetic operons to recruit RNA polymerase and start transcription. A small but important subclass acts as repressors, such as ScbR2, which is part of the cpk cluster in S. coelicolor, which is able to act as a repressor of the pathway-specific activator cpkO, a deletion of which leads to production of the cryptic polyketides abCPK and yCPK[5].

The difference between activator and repressor CSRs has implications for CRISPR-based intervention. It is possible to target repressors for knockdown using CRISPRi and to target activators for upregulation using CRISPRa or to remove native promoters and replace them with constitutive/inducible elements using Cas9-mediated editing. The advantages and disadvantages in terms of predictability and experimental burden are different for each strategy. Some systems have been found to work well, such as the expression of the chaI gene from S. chattanoensis in the presence of constitutive promoters, which resulted in the production of chattamycins A and B, and the expression of the astGI gene from Streptomyces sp. The production of hydroxymycorrionin and thiazinotrienomycin was stimulated by XZQH13. In more recent work, the LuxR family regulator PAI-A and PAI-B were overexpressed in S. globisporus C-1027, which resulted in the activation of a silent type II PKS cluster and production of three ortho-methyl phenyl alkenoic acids[6], [7].

2.3 Global and Pleiotropic Regulatory Networks

Global regulators are organized in layers above the level of CSRs to regulate antibiotic biosynthesis, in addition to developmental progression, nutritional status and signals from the environment. Important nodes include the central activator of the A-factor cascade in S. griseus and its orthologues in other species (AdpA); the GntR-family regulator that responds to N-acetylglucosamine to coordinate both chitin metabolism and antibiotic production (DasR); and the Bld family of developmental regulators, named for the "bald" morphology of mutants lacking the ability to form aerial hyphae. BldD, which was previously described as a repression of development, recently has been demonstrated to induce antibiotic production in S. venezuelae and S. roseosporus, revealing the logic of these networks sometimes seems to be counterintuitive and depends on the context[8].

The DasR-AdpA axis in S. roseosporus is a good example of the complexity of global regulation. DasR is a repressor of daptomycin production, both by binding directly to the dptE operon promoter and by indirectly repressing the transcription of adpA. AdpA, in turn, activates the production of daptomycin by binding to the promoter of dptA and dptR2. The architecture of this system enables the possibility of a double hit in both the direct repression of DasR and the indirect repression of its binding target by targeting DasR; this would be a challenging combination to achieve with conventional genetic methods without permanently destroying the central metabolic regulator[9].

There are two practical implications concerning BGC activation by global regulators. The first is leverage: they can be leveraged over multiple BGCs in a single perturbation, possibly activating multiple pathways that are not evident in a single experiment. Second, they pose risk: their manipulation can generate pleiotropic phenotypes which complicate compound discovery, strain fitness. Systematic investigation of such trade-offs has become possible recently through the development of endogenous Cascade-based activators that allow tunable, as opposed to absolute, perturbation of global regulatory nodes[2], [10].

2.4 Regulatory Mechanisms Underlying BGC Silencing

The mechanisms of silencing are not unique but represent a combination of various mechanisms. Some BGCs are silenced by specific CSRs, which can be deleted to lift silencing. Others do not express the functional activator under the laboratory conditions either due to poor expression of the activator gene or due to a lack of cofactors or signaling molecules. Others are repressed all over the world by nutrition signals: carbon catabolite repression, nitrogen regulation, phosphate limitation. The meaning is that activation should be adapted to the silencing, one based on the absence of the activator and another based on repressor binding at the BGC[11].

Chromatin topology is an additional level of complexity. The linear chromosome in Streptomyces is spatially organized and this can affect the accessibility of BGCs. Recent studies proposed that the implementation of rewiring chromatin topology could break the silencing process and that the use of CRISPR could be able to make use of such chromatin organization. Chromosomal position, nucleoid associated proteins and BGC expression remain an area that is not well studied and has great implication for BGC activation strategies[3], [12].

3. CRISPR Technologies for Transcriptional Manipulation

3.1 CRISPR-Cas9-Mediated Genetic Engineering

The first use of CRISPR in Streptomyces was genome editing, using Cas9 to create double strand breaks and using homologous repair to either delete or insert fragments or replace promoters. This strategy has been successful in activation of silent BGCs, either by the deletion of repressor genes or introduction of constitutive promoters, and it is still a popular tool for strain construction. In the groundbreaking study by Zhang et al., they showed how to precisely introduce the kas O p promoter upstream of silent BGCs in S. roseosporus to activate a polycyclic tetramate macrolactam cluster and a phosphonate cluster, resulting in the production of alteramide A and FR-900098, respectively. This work also showed that CRISPR-Cas9 significantly increased knock-in efficiency in strains which are typically resistant to conventional homologous recombination, allowing genetic manipulation of normally difficult strains[13]. But, editing does need transformation, repair template design, and verification as well as new rounds of strain construction per target. This burden per target is a limitation for discovery applications which require screening of many candidate BGCs. Partially, the more recent development of promoter libraries and modular assembly platforms like mCRISTAR, miCASTAR, and iCASRED has been used to address this, as it allows simultaneous promoter swapping and activation of multiple loci, however, these rely on chromosomal modification[3].

3.2 Catalytically Inactive Cas9 (dCas9) and CRISPR Interference (CRISPRi)

CRISPRi uses dCas9, a nuclease-deactivated Cas9, to sterically inhibit transcription by binding to DNA with the help of a guide-RNA. Targeting the promoter region inhibits initiation; targeting the coding sequence inhibits elongation. A functional CRISPRi system for streptomyces was characterized in S. venezuelae by expressing a codon-optimized dCas9 from a constitutive promoter, and targeting the promoter or coding sequence of a fluorescent reporter with sgRNAs . Repression was strong and design rules, including the window of effective promoter targeting, were quite uniform and similar to what was observed in other bacteria, indicating that the repression mechanism is likely to be portable[2], [14], [15]. The golden key to activate BGC came when CRISPRi was targeted against a repressor of the silent jadomycin BGC. Production of jadomycin B could be detected by LC-MS after repression of the endogenous repressor relieved the silencing of the pathway, while the no-CRISPRi control did not produce any jadomycin B. This established the principle that the silent BGCs can be activated by CRISPRi, relieving the negative regulation, without altering the BGC. This demonstration was important not just for the compound synthesized, but for the experimental paradigm that it created: BGC activation is something to test, not a strain to build[2], [16]

3.3 CRISPR Activation (CRISPRa) Systems

CRISPRa brings transcriptional activation domains to dCas9 to turn it from a repressor into an activator when used upstream of a promoter. To be able to use this functional CRISPRa in S. bacteria, activation

domains had to be identified that could function inside these Gram-positive, high-GC bacteria with a transcriptional machinery different from that of E. coli. Ameruoso et al. were able to do this by genetically engineering dCas9 to include the E. coli SoxS activation domain and were able to activate target genes in S. venezuelae using this system. This CRISPRa system was able to induce the production of jadomycin B when targeted at the promoter of the jadJV biosynthetic operon in a strain that didn't make the antibiotic. It was the first proof of concept for CRISPRa in Streptomyces and the first in any Gram-positive bacterium in general[17].

A problem that arose in the application of the CRISPRa technology in the genus Streptomyces was that most of the bacterial CRISPRa systems were optimized for the E. coli host and did not work well in high-GC, morphologically complex, actinomycotes. The SoxS based approach worked where others have not by virtue of the fact that the SoxS activation domain is able to interact productively with the Streptomyces RNA polymerase, presumably due to conserved surfaces that mediate activator-polymerase interactions in bacteria. Later studies have grown the CRISPRa toolkit. A transcriptionally active Cascade-based transcriptional activator (CTA) was constructed in S. coelicolor, by fusing the Cascade to the activation domain of the SoxS, and demonstrated to activate a PTM BGC, which was previously silent in the wild type[2], [17], [18].

3.4 Endogenous Type I-E Systems and Alternative Regulatory Platforms

An important breakthrough was achieved when endogenous Type I-E CRISPR systems found in the majority of Streptomyces strains were repurposed. In contrast to the class 2 systems, which use one Cas protein, class I systems use a multi-subunit Cascade complex. Zhou et al systematically exploited the Streptomyces Type I-E Cascade and engineered a series of repressors and activators which were successfully used to activate cryptic BGCs in non-model strains. This strategy has several benefits: it does not require the host to express heterologous effectors, it may be possible that the multi-subunit Cascade complex have different targeting rules which may increase the accessible sequence space, and it is naturally occurring in most Streptomyces strains, so it may be easier to implement in different hosts[17], [19].

Other Cas proteins are being adapted for Streptomyces, other than Cascade. Cas12a (Cpf1) is advantageous, because it can be multiplexed, meaning that multiple guides can be processed from a single transcript. The CAPTURE platform is a one-shot cloning system that integrates Cas12a cleavage with Cre-lox recombination to enable high efficiency cloning of large BGCs with near 100% efficiency for clusters of 10 – 113kb. So far, base editors have been used for targeted mutagenesis in Streptomyces, but to a lesser extent than dCas9-based methods for BGC activation. The STAGE toolkit based on TnpB is a step further in the editing toolbox as a small alternative to Cas9[20].

3.5 Scaffold RNA Architectures and Multiplex Regulation

Further modularity of CRISPRa design has been achieved by developing scaffold RNA systems in which the crRNA is modified with MS2 aptamers that bind MCP-fused activation domains. With this architecture, both activation and repression can be achieved with a single Cas complex, depending on the placement of the guide: targeting upstream of the transcription start site enables recruitment of activation domains and stimulation of transcription; targeting the promoter or coding sequence enables steric blocking. This dual capability has not been systematically exploited for BGC activation in Streptomyces, but is a logical next step to pursue for combinatorial regulatory perturbation[21].

An interesting aspect of CRISPR systems is their multiplexing ability, which is relevant to global regulator targeting. Multiple sgRNAs can be delivered by a single plasmid containing CRISPRi or CRISPRa DNA sequences, providing the ability to perform perturbation at multiple points of a regulatory network simultaneously. For instance, it may be engineered to use CRISPRi guides against two different repressors and CRISPRa guide against a global activator, all on a single construct. Technically, the combinatorial approach is possible with the current architecutre of scaffold RNA and the recent work that the Type I-E Cascade system can be evolved to target multiple genes simultaneously indicates that the simultaneous perturbation of multiple regulatory nodes is also feasible[22].

4. CRISPR-Mediated Manipulation of Pathway-Specific Regulators

4.1 Targeting Pathway-Specific Repressors

The most straightforward use of CRISPRi to activate BGCs is to target a CSR that is negatively regulating the CSR cluster. The cpk cluster of S. coelicolor is a well characterized example: the deletion of ScbR2 leads to the activation of cryptic polyketide production which is otherwise repressed by ScbR2. One way to do this is by using CRISPRi, which will not perturb the system but can be made to be either more or less repressive by changing the guide or by varying the amount of dCas9 protein expressed. This strategy was also found to work for the jadomycin BGC in S. venezuelae; disrupting the repressor gene of this BGC resulted in activation of the BGC[23].

The problem with the repressor targeting is practical: identification. There are a number of candidate regulatory genes in many BGCs which cannot be inferred from sequence data alone. The gaburedin BGC in S. venezuelae is a good example: the gbnR gene, which codes for an arpA-like putative transcriptional repressor, was identified by bioinformatic analysis and validated by deletion, which activated the gbn cluster, leading to a new family of γ-aminobutyrate-derived ureas . Although the gene encoding the repressor was known, in the absence of this information, CRISPRi would necessitate screening of a number of candidate guides, which is a helpful but resource-consuming process[24].

4.2 Activation of Pathway-Specific Transcriptional Activators

CRISPRa can be used complementarily where silencing is being caused by low expression of activator and not by actual repression. Instead of directly targeting the BGC structural genes, which necessitates out-competing the native regulatory architecture with the CRISPRa complex, it is possible to target the promoter of a CSR activator, thus increasing the native activation signal. This allows the stoichiometry and timing of biosynthetic gene expression maintained by the native regulatory system to be preserved[25]. The jadJV activation experiment shows the other way: direct CRISPRa targeting of the biosynthetic operon promoter itself, which avoids CSR control altogether. Depending on the objective (mimicking or overriding native regulation), one of these strategies will be chosen. In BGCs that have well-defined CSR activators, it might be better to target the promoter of the activator, to get better co-ordinated expression. In the absence of known regulatory architecture in the BGC, it is more pragmatic to target the biosynthetic operon directly[26].

4.3 Engineering Regulator-Binding Sites and Promoter Architectures

To overcome native control of BGCs, promoter replacement has been achieved via Cas9 to place constitutive or inducible promoters upstream of the BGC. In contrast to previous attempts in a heterologous host that required multiple refactored genes to drive expression in the new host, the Zhang et al. study showed that a single promoter knock-in upstream of the first ORF of a silent PTM cluster in S. roseosporus was enough to drive expression and yield alteramide A. This finding indicates that factors, such as chaperones, availability of precursor molecules, or metabolic states in the native host context, that cannot be fully mimicked by heterologous expression[27].

A important distinction between CRISPRi/CRISPRa and promoter replacement is the difference between cis and trans acting modifications – while promoter replacement is permanent, CRISPR regulation is transient. Promoter replacement might be the better option for production strains where stable, high level expression is desired, while the trans-acting approach is more scalable for applications such as discovery, where many BGCs need to be screened rapidly. There are also possible hybrid approaches - use CRISPRa to screen for initial discovery, and then use promoter replacement to optimize the strain after the BGC is validated[23].

4.4 Case Studies of Pathway-Specific Activation

The discovery of the auroramycins is an example of the “regulator-targeting” paradigm at its best. Promoter insertion using CRISPR-Cas9 was used to activate an uncharacterized type I PKS cluster in S. roseosporus to produce a doubly glycosylated 24-membered polyene macrolactam that shows strong anti-MRSA activity. Although this study was done before the advent of BGC activation in Streptomyces by using CRISPRi/CRISPRa, it provides the chemical reward that these technologies seek to make available.

More recent studies on lavendomycin used tandem-sgRNA CRISPRi to inhibit a second NRPS pathway, uncovering the biosynthetic genes to this antibiotic and showing how CRISPRi can be used as a reverse genetic approach for BGC assignment, in addition to BGC activation. The principle of this logic is not simply repressor-targeting, but rather relieves the competition for precursors or regulatory resources, making a pathway that was previously masked detectable. This method is especially important for strains that synthesize more than one compound; small amounts of products can be masked by the presence of the predominate product[28], [29].

In Streptomyces sp. the activation of the silent ansamycin cluster. By overexpressing astGI, a LAL-family regulator, the diversity of effective strategies is further illustrated by XZQH13 . In this case, the challenge was not from a repressor, but a lack of activator expression. When the activation signal was missing, it was provided by the overexpressing of the genes on a constitutive promoter, giving rise to hydroxymycorrionin and thiazinotrienomycin. These case studies all highlight the same theme: activation is effective if the lesion that maintains silencing can be identified, and CRISPR tools allow for either repression, activation, or editing to address the lesion[30].

5. Engineering Global and Pleiotropic Regulatory Networks

5.1 Manipulation of Global Regulators: AdpA, DasR, and Bld-Family Regulators

Global regulators can provide the promise of coordinated activation across multiple BGCs, but manipulation must be done with a careful consideration of network topology. The A-factor is a cascade and AdpA is located at the top of the cascade to activate both streptomycin biosynthesis and morphological differentiation in S. griseus. Its overexpression can pleiotropically increase the production of antibiotics, but the signaling molecule A-factor must also be present for the cascade to work, and this is not the case in some species that do not have the necessary components in the signaling cascade upstream of AdpA[31].

DasR does not follow this logic. DasR links nutrient sensing to secondary metabolism as it is a repressor of antibiotic production that is relieved upon binding to N-acetylglucosamine. DasR functions to suppress daptomycin production both by directly binding to the dptE promoter and by indirectly repressing adpA in S. roseosporus. This double mechanism may, therefore, make it an attractive target for CRISPRi; the repression of DasR would open up both the direct and indirect blocks at once to give an even greater activation effect than that of targeting either pathway separately. But the pleiotropic effects of influencing a central metabolic regulator are not limited to the ability to produce antibiotics, and the growth defects seen in DasR manipulation may be a downside to the increased production[32].

The Bld regulators are examples of context dependent global regulatory logic. Although previously thought to be a developmental repressor, BldD was demonstrated to activate production of daptomycin in S. roseosporus and of antibiotics in S. venezuelae, perhaps by direct binding to biosynthetic promoters. This dual role repressor of development/activator of secondary metabolism makes BldD an attractive but unpredictable target for CRISPR based intervention. Targeting BldD with CRISPRa might produce antibiotics without causing developmental defects as seen with BldD deletion, however the feedback effects on BldD's developmental regulon are unclear[33].

5.2 Modulation of Stringent Response and Nutritional Signaling

Under nutrient limitation, the stringent response is a global signal through accumulation of ppGpp that diverts the resources of the cell from growth to secondary metabolism. By engineering the levels of ppGpp, using relA or spoT mutations or by modulating their expression by CRISPR can increase antibiotic production but at the expense of growth and viability. The key principle here is the trade-off: what conditions induce secondary metabolism tend to suppress growth and vice versa, and this is an unsolved problem in strain design[34].

CRISPRi represents a possible strategy to semi-complete modulation of the stringent response components without causing a growth defect. It might be possible to achieve intermediate levels of ppGpp that activate secondary metabolism but do not completely halt growth by targeting relA with CRISPRi and tuning the efficiency of the guide or the expression of dCas9. Such an approach using CRISPRi has

not been tested in streptomyces, but it is a logical one to explore the activation space of the stringent response[23].

5.3 Coordinated Regulation of Multiple Silent BGCs

The multiplexing potential of CRISPR systems is especially important when considering targeting global regulators. Multiple sgRNAs can be delivered in a single CRISPRi/-a plasmid, allowing perturbation of multiple regulatory nodes simultaneously. The multiplex GENE activation has been recently demonstrated in the recent Type (I-E) Cascade work and 13 BGCs have been activated in multiple strains using this method. This level of activation: multiple pathways in multiple strains is a major step up from single-BGC demonstrations, and indicates that multi-node perturbation is beginning to be feasible[24].

Multi-node perturbation, which is the combinatorial logic of the perturbation, is powerful but complex. If the pathways do not compete for precursors or regulatory resources, two repressors may be targeted simultaneously, leading to additive or synergistic activation. Combination of targeting a repressor and an activator may result in effects that would not be seen with either perturbation alone. It would be interesting to systematically explore these combinations and perform metabolomic screening with CRISPRi/CRISPRa libraries to create a functional map of regulatory interactions that can't be mapped by sequence analysis alone[35].

5.4 Balancing Growth, Development, and Secondary Metabolism

The main tension of global regulator engineering is that, ecologically speaking, secondary metabolism is a reaction to stress and developmental transition. It may be forced to come on line by regulator intervention, which could produce artificial conditions that are not conducive to continuous production. Thus, the most effective approaches might be those that disrupt a particular pathway locally rather than globally, and that is exactly what CRISPRi/CRISPRa can do that over-expression of global regulators cannot[36].

The idea of "regulatory decoupling" dissociating the initiation of secondary metabolism from the natural cues has been pursued with promoter exchange and heterologous expression; here, CRISPR-based methods provide a more subtle approach. CRISPRi/CRISPRa could be used to fine-tune the sensitivity of regulatory circuits (e.g., lower the threshold for a global activator to respond to a nutritional signal or partially relieve repression without completely removing it). These intermediate perturbations can be able to reach activation without the fitness cost of full deregulation[37].

6. CRISPR-Based Strategies for Silent BGC Activation

6.1 CRISPRi-Mediated Repression of Negative Regulators

The logic of CRISPRi activation of silent BGCs is very easy: identify the repressor, repress it, and observe the pathway. The activation of the jadomycin BGC in S. venezuelae is still the best demonstration of principle. The problem in practice is the identification of the repressor. Many BGCs also harbor genes of unknown function (candidates) which can only be determined by their sequence and some BGCs are repressed by repressors encoded outside the cluster. To identify the relevant nodes, genome-wide approaches might be required, such as transcriptomic correlation or regulatory network inference or systematic CRISPRi screens[38].

With the Type I-E Cascade system, it is possible to take a more systematic look at this problem. Since it is possible to create guide libraries against all predicted TFs in the genome and combine it with metabolomic screening, this will enable the identification of BGC-repressing repressors, even when it is unknown which regulators are responsible for which BGCs. The thirteen BGCs, uncovered in the Zhou et al. study, were identified by a combination of genome mining and targeted perturbation, indicating the potential of scaling the approach up to larger screens[39].

6.2 CRISPRa-Mediated Activation of Silent Biosynthetic Pathways

In the case of biosynthetic operons, direct CRISPRa activation does not require any knowledge of regulation, simply a known promoter is required to target the operon. The jadJV activation experiment was found to be feasible and the CTA system was extended to a PTM BGC in S. coelicolor. However, the

drawback of direct activation is that it cannot necessarily mimic the coordinated expression of multiple operons in a BGC, especially for BGCs that are large and contain multiple regulatory elements[40].
The distance-dependence of CRISPRa activity presents an additional design criterion. In S. venezuelae, the best activation was achieved when the sgRNA was directed to the sequence about 70–90 bp upstream of the transcription start site (TSS), as has previously been found for other bacteria, where the positioning of the activation domain relative to the RNA polymerase (RNAP) binding site is critical. This design rule is general but can be adapted based on varying promoter/effector domain[9].

6.3 Multiplex CRISPR Targeting of Regulatory Networks

Combining multiple different perturbations of regulatory networks is provided by the multiplexing capacity of CRISPR systems. Multiple guides were used in parallel in the Type I-E Cascade system, and the CAPTURE platform showed high efficiency in cloning large BGCs using cas12a. When these capabilities are combined with efficient BGC cloning, a pipeline for systematic exploration of regulatory combinations is created[41].
Combinatorial space is enormous: with a genome of 30 predicted transcription factors that could regulate BGCs, and guides that can be used in pairs, triplets, or more, the number of possible perturbations is larger than can be manually screened. Making multiplex screening tractable requires computational prioritization, based on the transcriptomic and network data. Existing perturbation data could be used to train machine learning methods and generate hypotheses on which regulator combinations are most likely to give synergistic activation[42].

6.4 CRISPR-Assisted Promoter Replacement and Refactoring

However, this promoter replacement approach is still useful in the context of CRISPRi/CRISPRa. Refactoring with constitutive or inducible promoters might be necessary for BGCs whose silencing doesn't easily happen upon regulatory perturbation, e.g. those requiring coordinated silencing of multiple operons. A potentially less disruptive way to modify the promoter is by the use of CRISPR base editors in Streptomyces. Combining promoter engineering and CRISPR-based regulation gives a multi-layered approach. First, BGCs are identified by CRISPRi/CRISPRa screening for their ability to be activated by regulatory perturbation. Secondly, expression of the activated pathways is optimized by promoter replacement or base editing. Third, production can be performed in a clean background using genome-reduced chassis strains, like the S. albus Del14 with 15 native BGCs removed. Every layer brings both precision and yield – and no need to optimize the previous layers first[43].

7. Discovery of Novel Antibiotics Through CRISPR-Based BGC Activation

7.1 Activation of Cryptic PKS and NRPS Clusters

Discovery of the auroramycin is the most completely characterized example investigating a novel antibiotic isolated from a silent PKS cluster of Streptomyces by using the CRISPR chain. The compound was a 24-membered polyene macrolactam with an unusual isobutyrylmalonyl extender unit and two amino sugars, which exhibited strong activity against clinical isolates of MRSA. The activation approach employed promoter insertion by Cas9, as opposed to CRISPRi/CRISPRa, but it provides the chemical benefit that programmable regulatory tools hope to make more commonplace[44].
An example of the latter is the discovery of the alteramide A from S. roseosporus. The PTM cluster was known from S. griseus and the S. roseosporus homolog was predicted by sequence analysis. The cluster was activated by promoter knock-in, and produced alteramide A, which spontaneously cyclizes upon photo-irradiation to a second compound. In particular, the S. griseus cluster was previously refactored in a heterologous host system, but the S. roseosporus native expression resulted in a compound (dihydromaltophilin) that was not detected in the heterologous system, indicating host dependent biosynthetic/processing factors[45].
NRPS clusters pose unique problems. Their biosynthetic logic modular assembly lines with substrate specificity brought about by adenylation domains results in predictable, albeit often structurally complex, products upon silent expression. This lavendomycin work is a showcase on how CRISPRi can be used not only to activate pathways, but to assign BGCs to known compounds—a crucial step in genome mining.

Further extension of the NRPS and RiPP chemistry that can be accessed by the activation of linaridin clusters through the Type I-E Cascade system[46].

7.2 Discovery of Novel RiPPs

Compared to the PKS/NRPS clusters, RiPP BGCs tend to be smaller and more easily genetically manipulated, although they are less well studied for regulation, and may be regulated by global rather than pathway-specific mechanisms. We have shown that CRISPRa can be used to access this class of pathways, as the CTA is able to activate a PTM BGC in S. coelicolor . Compared to PKS/NRPS, RiPP discovery via CRISPR-based activation is still a relatively underdeveloped field, but the structural diversity of RiPPs, and their synthetic biology-friendly nature make them an appealing target for future research.[47]

The pathways of RiPP activation that were identified in the Type I-E Cascade study represent a major increase in RiPP accessibility. The linaridins are a relatively small RiPP family and the fact that multiple linaridin BGCs are encoded in non-model strains implies that the endogenous Cascade system is particularly suited to access to this class of compounds. The explanation could be the regulatory structure of RiPP clusters: CSRs rarely exist and this makes them more vulnerable to global regulatory perturbations[48].

7.3 Metabolomic Profiling and Antimicrobial Screening

The result of a CRISPR activation experiment is a metabolomic change; new peaks in a LC-MS chromatogram, which need to be correlated with biological changes. Prioritization of novel compounds for isolation and characterization can be done using untargeted metabolomics platforms combined with molecular networking. The auroramycin work is a representative example of the pipeline: Activations of the transcriptional clusters are verified, metabolomic analyses are performed to identify the compound resulting from the induced cluster, followed by structural characterization using NMR and chemical derivatization and then a bioassay against clinical pathogens[49].

Automation and computational tools are needed to scale this pipeline, and keep up the pace of the increased number of activation experiments enabled by CRISPR tools. An automated metabolomic analysis was necessary to activate thirteen BGCs in the type I-E Cascade study. Dereplication, the identification of known compounds to avoid the risk of rediscovery, is even more important if multiple pathways are activated during screening and computational tools for spectral matching and molecular networking are now a necessary part of the discovery pipeline[50].

8. Integrating CRISPR with Multi-Omics and Computational Approaches

8.1 Genome Mining and Identification of Silent BGCs

AntiSMASH and similar programs give the initial set of BGCs present in a genome, but will not reveal if the BGCs are silent or which regulatory systems control them. Through transcriptomic data, the RNA-seq data under various conditions, BGCs that are not expressed or have low expression levels can be determined and candidate regulators can be determined by co-expression analysis. From “which clusters are present” to “which clusters are silent and why” is the shift the introduction of genome mining and transcriptomics brings to the problem[51].

The Type I-E Cascade study is a good illustration of such integration. Genome mining of 67 Streptomyces genomes revealed that Type I-E CRISPR systems are prevalent and the construction of Cascade-based regulators allowed systematic activation of BGCs in different strains. The 13 BGCs found in this work were not chosen with the knowledge of the regulatory control of their activity; they were rather identified through a genome mining and screening procedure[52].

8.2 Transcriptomics for Regulator and Target-Gene Prioritization

Predictions for regulation from CRISPRi and CRISPRa experiments can be tested using transcriptomics. When CRISPRi targeting a candidate repressor is capable of activating a BGC, RNA-seq can be used to verify that the biosynthetic genes are indeed activated, and identify other genes in the regulon. The "perturb, measure, refine" process is the heart of functional genomics—and now possible on a large scale

with CRISPR. Off-target effects of CRISPR systems – part of interpreting transcriptomic data. Beyond just the genes of interest, RNA-seq analysis of Cascade-edited strains identified genes that were up or down regulated, such as many transcriptional regulators. While these off target effects could interfere with interpretation of activation experiments, they can also be indirect regulatory relationships that would not be obvious from targeted analysis. To differentiate between direct and indirect effects, multiple guide RNAs against a target and suitable controls are needed to carefully design experiments[53].

8.3 AI-Assisted Prediction of Regulatory Targets

Machine learning methods are now starting to be used to infer regulatory networks in Streptomyces. By building models with data on transcriptomic data, binding sites prediction and known regulatory-target interactions, hypotheses can be produced on what regulators control which BGCs. These predictions can then be tested with CRISPRi/CRISPRa, thus completing a feed forward loop between computational prediction and experimental validation. The problem is lack of available data, as there are few regulator-BGC relationships that have been verified in Streptomyces, the number required for successful supervised learning approaches is still quite small[54].

A potential way forward for the field is the integration of multi-omics data with machine learning. Fluxes of precursors and bottlenecks in the metabolic pathways have been predicted by genome scale metabolic models of S. coelicolor and S. avermitilis and these models could be linked with regulatory network models to predict perturbations most likely to activate silent BGCs. Promoter libraries that have been designed using AI are also becoming available, which could help tailor the expression of activated pathways and help build strains with just the right level of expression for each gene in a BGC[3].

9. Challenges, Limitations, and Future Perspectives

9.1 Genetic Intractability and Strain-Specific Limitations

CRISPR tools created in S. venezuelae or S. coelicolor do not always work effectively in other Streptomyces species. These factors include promoter activity, codon usage, expression levels of cas proteins and host restriction systems, which vary from one strain to another. The development of broad-host-range CRISPR systems is still an ongoing area of research, and the endogenous Type I-E Cascade system can be a source of such systems as it exists naturally in most Streptomyces strains. But even endogenous systems need optimisation for every new host and in reality tool development is often necessary for each new production strain[55].

Many Streptomyces species, including CRISPR, are genetically intractable meaning they are difficult to use all the genetic tools. Strains that are not conjugable, grow slowly or do not have well defined promoters and selection markers are still challenging to manipulate even with the use of editing technology. However, while some of these limitations can be overcome with the use of well characterized genome reduced chassis strains for the expression of heterologous compounds, many interesting compounds are produced by strains that are poorly characterized[56].

9.2 Incomplete Understanding of Regulatory Networks

The main drawback of the CRISPR based regulatory manipulation is that you need to know what regulators to manipulate. This knowledge is available for well characterized BGCs and model strains. Does not, for thousands of BGCs that remain uncharacterized in sequenced Streptomyces genomes. This knowledge could be created through systematic strategies—studying every predicted transcription factor in a genome using a CRISPRi screen, while simultaneously measuring the metabolome changes that result.Although not yet done at scale in Streptomyces, these strategies could be systematic, involving a CRISPRi screen against all predicted transcription factors in a genome, simultaneously measuring metabolome changes[57].

The magnitude of the challenge is overwhelming. The genome of a typical streptomycote contains hundreds of predicted transcription factors and little is known about how these factors interact to regulate each other. Although efficient CRISPR tools are in place, the time and effort to build and screen all the guide libraries targeting all the transcription factors is significant. Such screens need to be prioritized by computational means, based on transcriptomic and network data[58].

9.3 Off-Target Effects and Metabolic Burden

Non-nuclease binding of dCas9 can affect transcription at off-target sites. Partial complementarity of guide RNAs to other genomic locations can sequester dCas9 from the target or result in off-target repression. Off target effects were also demonstrated by RNA-seq analysis of Cascade-edited strains, which revealed that transcriptional regulators are among the genes that are up or down regulated and that off-target effects might be propagated through regulatory networks. These effects can be hard to predict and must be measured on a case by case basis for each guide and construct[59].

There is also a metabolic burden from expressing dCas9 and effector domains that can lead to reduced growth and production. This burden depends on expression levels, number of copies of the construct and host strain. Inducible systems can be used to reduce burden by only expressing the effector during the production phase, although they require additional complexity in experimental design. The production of more efficient effectors and lower burden expression systems would make the use of CRISPR for regulating production more practical[60].

9.4 Delivery, Expression, and Multiplexing Constraints

The main method of entering CRISPR plasmids into Streptomyces is still through conjugation, the success of which depends on the strain. The reliability of CRISPR-based regulation is influenced by plasmid stability, copy number and inducibility. Larger constructs are needed for multiplexing and could be less stable or more challenging to deliver. Some of these constraints would be alleviated by the use of smaller cas-effectors, like the TnpB based STAGE toolkit, and stable integrating vectors[61].

There are pros and cons to the plasmid-based and genome-integrated CRISPR systems. Plasmid based systems are introduced more readily and can be cured after the completion of the experiment, but have the problem of instability and copy number variation. Integrating systems into the genome makes them more stable and consistent but are more complicated to engineer. Generally, plasmid based systems are preferred for screening applications where a large number of guides are tested while integration may be desired for production strains where consistent regulation is needed[62].

9.5 Synthetic Regulatory Circuits and Next-Generation Platforms

Future developments of CRISPR systems in Streptomyces will probably involve synthetically designed regulatory circuits to allow for dynamic and feedback regulation. Since CRISPR activity could be controlled by metabolite levels, circuits that change in response to metabolites could be utilized to control the activity of CRISPR without needing an outside intervention. Independent control of multiple pathways would be achieved by orthogonal transcription factors that don't cross react with native Streptomyces transcription factors[34].

Deeper knowledge of the regulatory dynamics and physiology of Streptomyces than currently available will be required to develop these circuits. The field is moving towards this goal, although the approaches to create and characterize synthetic circuits in Streptomyces are less well developed than those in model organisms such as E. coli. These investment tools – called characterized promoters, ribosome binding sites and reporter systems – are essential to the more advanced capabilities that synthetic biology holds[63].

Conclusion

CRISPR based transcriptional manipulation has developed from proof of principle to actual tool in Streptomyces BGC activation. CRISPRi is able to silence endogenous repressors to remove silencing, CRISPRa can directly activate biosynthetic operons, and both can be multiplexed to disrupt regulatory networks in ways that would be challenging or impossible to accomplish using standard genetic modification. These capabilities have been extended to non-models strains with the development of endogenous Type I-E Cascade systems, which would allow the activation of thirteen BGCs simultaneously, across multiple hosts. These discoveries are important not only for the particular compounds that have been isolated (jadomycin B, alteramide A, the linaridin pathways and potential future discoveries) but because of what they allow in experimental logic. The BGC activation is no longer a strain to be constructed but rather a perturbation to test. Guides can be synthesized and added in

parallel to libraries, and the results can be screened metabolomically. Such scalability is a prerequisite for systematic exploration of the silent BGC space.

But it is no longer the tool that is the limiting factor. It's knowledge of which regulators control which BGCs. Functional characterization of regulatory networks should thus be the next step in this field, either via systematic screens, via enhanced computational prediction, or by combining perturbation data with multi-omics measurements. Regulatory networks in Streptomyces are complex, context dependent and tools to map them are still in development. As this knowledge base develops, the use of CRISPR for activation will stand to become what it was meant to be: not just a tool to achieve one-off successes, but a standard and scalable means of discovering new antibiotics.

By combining CRISPR tools, genome-reduced chassis, AI-designed promoters and multi-omics screening pipelines, the future of novel antibiotic discovery in Streptomyces looks like it could become more of a systematic, high throughput process than a series of lucky discoveries. The number of chemicals that can be written in a silent BGC is enormous and the tools for accessing this are now mostly in place. All that's left is the regulatory expertise to use them effectively.

Author's Contribution: All authors contributed equally
Conflict of Interest: The authors declare no conflict of interest

REFERENCES

[1] Y. Wang et al. , "Unveiling bat-borne viruses: a comprehensive classification and analysis of virome evolution," Microbiome , vol. 12, no. 1, p. 235, Nov. 2024, doi: 10.1186/s40168-024-01955-1 .

[2] A. E. Morales et al. , "Bat genomes illuminate adaptations to viral tolerance and disease resistance," Nature , vol. 638, no. 8050, pp. 449–458, Jan. 2025, doi: 10.1038/s41586-024-08471-0 .

[3] R. Santhoshkumar, H. Mohamed, and M. Jemo, "Integrated multi-omics and genome mining strategies for activating cryptic polyketide biosynthetic gene clusters in Streptomyces: an overview," Frontiers in Microbiology , vol. 17, Sep. 2026, doi: 10.3389/fmicb.2026.1944089 .

[4] P. A. C. la Rosa, J. H. Ramírez-Prado, E. Góngora-Castillo, and A. Prieto-Davó, "Genome mining of coastal cenote sediment-associated Streptomyces sp. NCA360 strain uncovers novel biosynthetic gene clusters and their regulatory architecture," Sep. 10, 2026, Cold Spring Harbor Laboratory . doi: 10.64898/2026.09.08.750260 .

[5] S. Shekoohiyan, F. S. Dariyan, M. Mahdavianpour, M. Pourakbar, and E. Aghayani, "Comparison of response surface method and artificial neural networks in predicting formaldehyde and methanol removal using moving bed sequential batch reactor (MBSBR) and Fixed bed sequential batch reactor (FBSBR): Process optimization and kinetic study," Separation and Purification Technology , vol. 363, p. 132097, Feb. 2025, doi: 10.1016/j.seppur.2025.132097 .

[6] N. S. Alhajeri and A. Tawfik, "Synergistic 3,5-xylenol degradation and resource recovery from phenolic wastewater using a mixotrophic algal-bacterial consortium: Performance and biochar valorization," Journal of environmental chemical engineering , vol. 14, no. 3, p. 122422, Mar. 2026, doi: 10.1016/j.jece.2026.122422 .

[7] S. Hooshmand and S. M. Zamir, "Improved toluene vapor removal in a moving bed biofilm reactor (MBBR): Performance, microbial dynamics, and kinetic study," Biochemical Engineering Journal , vol. 222, p. 109844, Jul. 2025, doi: 10.1016/j.bej.2025.109844 .

[8] L. F. P. Cervantes, C. Caicedo-Montoya, and R. Ríos-Estepa, "Beyond Clavulanic Acid biosynthesis: Exploring the broad regulatory impact of BldD in Streptomyces clavuligerus ATCC 27064," PLoS ONE , vol. 21, no. 4, Apr. 2026, doi: 10.1371/journal.pone.0347564 .

[9] S. Y. Moon, M. R. Kim, N. An, M. H. Noh, and J. Y. Lee, "Dual-mode CRISPRa/i for genome-scale metabolic rewiring in Escherichia coli," Nucleic Acids Research , vol. 53, no. 15, Aug. 2025, doi: 10.1093/nar/gkaf818 .

[10] M. Zhu and X. Dai, "Shaping of microbial phenotypes by trade-offs," Nature Communications , vol. 15, no. 1, p. 4238, May 2024, doi: 10.1038/s41467-024-48591-9 .

[11] Z. Human, R. L. Hoover, C. S. Chan, K. Küsel, and C. Wegner, "High diversity but limited expression of biosynthetic gene clusters in a peatland microbial community," Nature Communications , vol. 17, no. 1, Oct. 2026, doi: 10.1038/s41467-026-78121-8 .

[12] X. Xia, J. Zhang, J. Zheng, G. Liao, Y. Ding, and Y. Li, "Important Role of Bacterial Nucleoid-Associated Proteins in Discovery of Novel Secondary Metabolites," International Journal of Molecular Sciences , vol. 26, no. 6, p. 2393, Mar. 2025, doi: 10.3390/ijms26062393 .

[13] L. Xie et al. , "The visualizable and marker-free gene editing platform mediated by CRISPR/Cas9 in Coleophoma empetri," Applied Microbiology and Biotechnology , vol. 110, no. 1, Feb. 2026, doi: 10.1007/s00253-026-13762-9 .

[14] K. Meier, J. Olejnik, A. J. Hume, and E. Mühlberger, "A Comparative Assessment of the Pathogenic Potential of Newly Discovered Henipaviruses," Pathogens , vol. 13, no. 7, p. 587, Jul. 2024, doi: 10.3390/pathogens13070587 .

[15] G. Prezza, C. Liao, S. Reichardt, C. L. Beisel, and A. J. Westermann, "CRISPR-based screening of small RNA modulators of bile susceptibility in Bacteroides thetaiotaomicron," Proceedings of the National Academy of Sciences , vol. 121, no. 6, Jan. 2024, doi: 10.1073/pnas.2311323121 .

[16] H. Feng, B. Cheng, J. Lim, B. Li, C. Li, and X. Zhang, "Advancements in enhancing resistant starch type 3 (RS3) content in starchy food and its impact on gut microbiota: A review," Comprehensive Reviews in Food Science and Food Safety , 2024, doi: 10.1111/1541-4337.13355 .

[17] C. M. Whitford, P. Gockel, D. Faurdal, T. Gren, R. Sigrist, and T. Weber, "CASCADE-Cas3 enables highly efficient genome engineering in Streptomyces species," Nucleic Acids Research , vol. 53, no. 6, Mar. 2025, doi: 10.1093/nar/gkaf214 .

[18] M. Fekete, A. Lehoczki, A. Kryczyk-poprawa, and V. Zábó, "Functional Foods in Modern Nutrition Science : Mechanisms , Evidence , and Public Health Implications," Nutrients , vol. 17, no. 13, 2025.

[19] C. M. Whitford, P. Gockel, D. Faurdal, T. Gren, R. Sigrist, and T. Weber, "CASCADE-Cas3 Enables Highly Efficient Genome Engineering in Streptomyces Species," May 09, 2023, Cold Spring Harbor Laboratory . doi: 10.1101/2023.05.09.539971 .

[20] J. Luo et al. , "STAGE: A compact and versatile TnpB-based genome editing toolkit for Streptomyces," Proceedings of the National Academy of Sciences , vol. 122, no. 35, Aug. 2025, doi: 10.1073/pnas.2509146122 .

[21] R. Shil, V. Pattapulavar, S. Ramanujam, S. Subburaj, and J. G. Christopher, "Synthetic Biology Strategies for Activating Cryptic BGCs in Streptomyces : Engineering Native and Synthetic Promoters for Antibiotic Discovery," ACS Omega , vol. 11, no. 1, pp. 239–259, Jan. 2026, doi: 10.1021/acsomega.5c08473 .

[22] M. L. Luo, R. T. Leenay, and C. L. Beisel, "Current and future prospects for CRISPR-based tools in bacteria," Biotechnology and Bioengineering , vol. 113, no. 5, pp. 930–943, Oct. 2015, doi: 10.1002/bit.25851 .

[23] A. Ameruoso, M. C. V. Kcam, K. P. Cohen, and J. Chappell, "Activating natural product synthesis using CRISPR interference and activation systems in Streptomyces," Nucleic Acids Research , vol. 50, no. 13, pp. 7751–7760, Jul. 2022, doi: 10.1093/nar/gkac556 .

[24] G. Wang et al. , "CRAGE enables rapid activation of biosynthetic gene clusters in undomesticated bacteria," Nature Microbiology , vol. 4, no. 12, pp. 2498–2510, Oct. 2019, doi: 10.1038/s41564-019-0573-8 .

[25] L. Li, X. Liu, W. Jiang, and Y. Lü, "Recent Advances in Synthetic Biology Approaches to Optimize Production of Bioactive Natural Products in Actinobacteria," Frontiers in Microbiology , vol. 10, p. 2467, Nov. 2019, doi: 10.3389/fmicb.2019.02467 .

[26] K. Scherlach and C. Hertweck, "Mining and unearthing hidden biosynthetic potential," Nature Communications , vol. 12, no. 1, p. 3864, Jun. 2021, doi: 10.1038/s41467-021-24133-5 .

[27] S. Cho, J. Shin, and B. Cho, "Applications of CRISPR/Cas System to Bacterial Metabolic Engineering," International Journal of Molecular Sciences , vol. 19, no. 4, p. 1089, Apr. 2018, doi: 10.3390/ijms19041089 .

[28] T. Hautbergue, E. L. Jamin, L. Debrauwer, O. Puel, and I. P. Oswald, "From genomics to metabolomics, moving toward an integrated strategy for the discovery of fungal secondary metabolites," SPIRE - Sciences Po Institutional REpository , vol. 35, no. 2, pp. 147–173, Jan. 2018, doi: 10.1039/c7np00032d .

[29] D. Mao, B. K. Okada, Y. Wu, F. Xu, and M. R. Seyedsayamdost, "Recent advances in activating silent biosynthetic gene clusters in bacteria," Current Opinion in Microbiology , vol. 45, pp. 156–163, Jun. 2018, doi: 10.1016/j.mib.2018.05.001 .

[30] A. Schüller, L. Studt, and J. Strauss, "How to Completely Squeeze a Fungus—Advanced Genome Mining Tools for Novel Bioactive Substances," Pharmaceutics , vol. 14, no. 9, p. 1837, Aug. 2022, doi: 10.3390/pharmaceutics14091837 .

[31] A. J. Molina-Henares, T. Krell, M. Guazzaroni, A. Segura, and J. L. Ramos, "Members of the IclR family of bacterial transcriptional regulators function as activators and/or repressors," FEMS Microbiology Reviews , vol. 30, no. 2, pp. 157–186, Feb. 2006, doi: 10.1111/j.1574-6976.2005.00008.x .

[32] S. Rigali et al. , "Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces," EMBO Reports , vol. 9, no. 7, pp. 670–675, May 2008, doi: 10.1038/embor.2008.83 .

[33] H. Yan et al. , "BldD, a master developmental repressor, activates antibiotic production in two Streptomyces species," Molecular Microbiology , vol. 113, no. 1, pp. 123–142, Oct. 2019, doi: 10.1111/mmi.14405 .

[34] B. Yang et al. , "Scalable secondary metabolite production in Streptomyces using a plug-and-play system," Nature Biotechnology , vol. 44, no. 6, pp. 1037–1049, Aug. 2025, doi: 10.1038/s41587-025-02762-1 .

[35] A. J. Rubin et al. , "Coupled Single-Cell CRISPR Screening and Epigenomic Profiling Reveals Causal Gene Regulatory Networks," Cell , vol. 176, p. 361, Dec. 2018, doi: 10.1016/j.cell.2018.11.022 .

[36] J. G. Zalatan et al. , "Engineering Complex Synthetic Transcriptional Programs with CRISPR RNA Scaffolds," Cell , vol. 160, pp. 339–350, Dec. 2014, doi: 10.1016/j.cell.2014.11.052 .

[37] P. B. Otoupal and A. Chatterjee, "CRISPR Gene Perturbations Provide Insights for Improving Bacterial Biofuel Tolerance," Frontiers in Bioengineering and Biotechnology , vol. 6, p. 122, Sep. 2018, doi: 10.3389/fbioe.2018.00122 .

[38] M. Letko, S. N. Seifert, K. J. Olival, R. K. Plowright, and V. J. Munster, "Bat-borne virus diversity, spillover and emergence," Nature Reviews Microbiology , vol. 18, no. 8, pp. 461–471, Jun. 2020, doi: 10.1038/s41579-020-0394-z .

[39] H. Zhou et al. , "Identification of novel bat coronaviruses sheds light on the evolutionary origins of SARS-CoV-2 and related viruses," Cell , vol. 184, no. 17, p. 4380, Jun. 2021, doi: 10.1016/j.cell.2021.06.008 .

[40] M. J. Smanski, H. Zhou, J. Claesen, B. Shen, M. A. Fischbach, and C. A. Voigt, "Synthetic biology to access and expand nature's chemical diversity," Nature Reviews Microbiology , vol. 14, no. 3, pp. 135–149, Feb. 2016, doi: 10.1038/nrmicro.2015.24 .

[41] B. Enghiad et al. , "Cas12a-assisted precise targeted cloning using in vivo Cre-lox recombination," Nature Communications , vol. 12, no. 1, p. 1171, Feb. 2021, doi: 10.1038/s41467-021-21275-4 .

[42] Y. Roohani, K. Huang, and J. Leskovec, "Predicting transcriptional outcomes of novel multigene perturbations with GEARS," Nature Biotechnology , vol. 42, no. 6, pp. 927–935, Aug. 2023, doi: 10.1038/s41587-023-01905-6 .

[43] T. Chai, Y. Tao, C. Zhao, and X. Chen, "Hierarchical metabolic engineering for rewiring cellular metabolism," FEMS Microbiology Reviews , vol. 49, Jan. 2025, doi: 10.1093/femsre/fuaf047 .

[44] J. J. Hug, D. Krug, and R. Müller, "Bacteria as genetically programmable producers of bioactive natural products," Nature Reviews Chemistry , vol. 4, no. 4, pp. 172–193, Mar. 2020, doi: 10.1038/s41570-020-0176-1 .

[45] Y. Luo et al. , "Activation and characterization of a cryptic polycyclic tetramate macrolactam biosynthetic gene cluster," Nature Communications , vol. 4, no. 1, p. 2894, Dec. 2013, doi: 10.1038/ncomms3894 .

[46] M. M. Zhang et al. , "CRISPR–Cas9 strategy for activation of silent Streptomyces biosynthetic gene clusters," Nature Chemical Biology , vol. 13, no. 6, pp. 607–609, Apr. 2017, doi: 10.1038/nchembio.2341 .
[47] S. Han and H. Won, "Advancements in the Application of Ribosomally Synthesized and Post-Translationally Modified Peptides (RiPPs)," Biomolecules , vol. 14, no. 4, p. 479, Apr. 2024, doi: 10.3390/biom14040479 .
[48] K. A. Pentón, Y. Mast, and C. Beatriz, "Bacterial biosynthetic superclusters: emerging drivers of synergistic natural products against antimicrobial resistance," Essays in Biochemistry , May 2026, doi: 10.1042/ebc20250019 .
[49] B. Wang, F. Guo, S. Dong, and H. Zhao, "Activation of silent biosynthetic gene clusters using transcription factor decoys," Nature Chemical Biology , vol. 15, no. 2, pp. 111–114, Dec. 2018, doi: 10.1038/s41589-018-0187-0 .
[50] P. Allard et al. , "Integration of Molecular Networking and In-Silico MS/MS Fragmentation for Natural Products Dereplication," Analytical Chemistry , vol. 88, no. 6, pp. 3317–3323, Feb. 2016, doi: 10.1021/acs.analchem.5b04804 .
[51] N. Ziemert, M. Alanjary, and T. Weber, "The evolution of genome mining in microbes – a review," Natural Product Reports , vol. 33, no. 8, pp. 988–1005, Jan. 2016, doi: 10.1039/c6np00025h .
[52] V. Libis et al. , "Multiplexed mobilization and expression of biosynthetic gene clusters," Nature Communications , vol. 13, no. 1, p. 5256, Sep. 2022, doi: 10.1038/s41467-022-32858-0 .
[53] A. W. Cheng et al. , "Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system," Cell Research , vol. 23, no. 10, pp. 1163–1171, Aug. 2013, doi: 10.1038/cr.2013.122 .
[54] H. E. Augustijn, A. M. Roseboom, M. H. Medema, and G. P. van Wezel, "Harnessing regulatory networks in Actinobacteria for natural product discovery," Journal of Industrial Microbiology & Biotechnology , vol. 51, Jan. 2024, doi: 10.1093/jimb/kuae011 .
[55] A. V. Wright, J. K. Nuñez, and J. A. Doudna, "Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering," Cell , vol. 164, pp. 29–44, Jan. 2016, doi: 10.1016/j.cell.2015.12.035 .
[56] M. H. Medema, T. de Rond, and B. S. Moore, "Mining genomes to illuminate the specialized chemistry of life," Nature Reviews Genetics , vol. 22, no. 9, pp. 553–571, Jun. 2021, doi: 10.1038/s41576-021-00363-7 .
[57] H. Xia, X. Li, Z. Li, X. Zhan, X. Mao, and Y. Li, "The Application of Regulatory Cascades in Streptomyces: Yield Enhancement and Metabolite Mining," Frontiers in Microbiology , vol. 11, p. 406, Mar. 2020, doi: 10.3389/fmicb.2020.00406 .
[58] O. Parnas et al. , "A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks," Cell , vol. 162, no. 3, pp. 675–686, Jul. 2015, doi: 10.1016/j.cell.2015.06.059 .
[59] J. Smith et al. , "Quantitative CRISPR interference screens in yeast identify chemical-genetic interactions and new rules for guide RNA design," Genome biology , vol. 17, no. 1, p. 45, Mar. 2016, doi: 10.1186/s13059-016-0900-9 .
[60] F. Ceroni et al. , "Burden-driven feedback control of gene expression," Nature Methods , vol. 15, no. 5, pp. 387–393, Mar. 2018, doi: 10.1038/nmeth.4635 .
[61] B. J. Caliando and C. A. Voigt, "Targeted DNA degradation using a CRISPR device stably carried in the host genome," Nature Communications , vol. 6, no. 1, p. 6989, May 2015, doi: 10.1038/ncomms7989 .
[62] R. G. Dias et al. , "10 years of CRISPR/CAS genomic engineering in Yarrowia lipolytica," Bioprocess and Biosystems Engineering , vol. 49, no. 7, pp. 1767–1783, May 2026, doi: 10.1007/s00449-026-03347-1 .
[63] N. Lee, S. Hwang, Y. Lee, S. Cho, B. Ø. Palsson, and B. Cho, "Synthetic Biology Tools for Novel Secondary Metabolite Discovery in Streptomyces," Journal of Microbiology and Biotechnology , vol. 29, no. 5, pp. 667–686, May 2019, doi: 10.4014/jmb.1904.04015 .