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MINI REVIEW

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Genetic and regulatory mechanisms of antibiotic production in microbes

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ABSTRACT

The increasing global crisis of antimicrobial resistance (AMR), projected to maintain 10 million lives annually by 2050, has the discovery of novel antibiotics as a pressing biomedical imperative. Microorganisms, especially actinobacteria of the genus Streptomyces remain the most creative natural sources of clinically applicable to antibiotics, with their biosynthetic potential encoded within discrete genomic loci termed biosynthetic gene clusters (BGCs). The identification of thousands of BGCs through advanced genome mining platforms such as antiSMASH and BiG-SCAPE, a substantial proportion of this biosynthetic repertoire remains transcriptionally silent under conventional laboratory conditions, representing an underexploited reservoir of pharmacological diversity.

Antibiotic biosynthesis is observed by a multilayered regulatory architecture integrating pathway-specific transcriptional activators (e.g., ActII-ORF4, RedD), global regulators (e.g., AdpA, DasR, AfsR), quorum sensing molecules including γ-butyrolactones, and post-transcriptional mechanisms mediated by small regulatory RNAs and riboswitches. The environmental sign mainly carbon catabolite repression and phosphate limitation through the PhoP/PhoR two-component system; exert critical modulation over secondary metabolite output.

The synthetic biology approaches, including CRISPR-dCas9-mediated transcriptional activation and heterologous BGC expression, are discussed as transformative strategies for unlocking cryptic clusters.

This mini review aims to combine the recent study on the genetic architecture of BGCs encompassing polyketide synthase (PKS), non-ribosomal peptide synthetase (NRPS), and hybrid biosynthetic machineries and to negatively examine the hierarchical regulatory networks managing antibiotic production, including cluster-situated regulators, global pleiotropic regulators, quorum sensing cascades, and epigenetic mechanisms.

KEY WORDS

Antimicrobial resistance (AMR); Biosynthetic gene clusters (BGCs); Antibiotic biosynthesis; Streptomyces; Genome mining; Regulatory mechanisms

ARTICLE HISTORY

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Introduction

Research related to antibiotics is one of the most transformative milestones in the past of modern medicine. Alexander Fleming's discovery of penicillin from a mold in 1928 and its ensuring development into a medicine in the 1940s laid the foundation for microbial natural products as drugs. The decades that ensued witnessed a new era of antibiotic discovery, with structurally different compounds including aminoglycosides, tetracyclines, macrolides, glycopeptides, and ansamycins successively entering clinical use. Beyond their therapeutic value, antibiotics play an important ecological role, mediating competitive interactions among microbial communities in soil, aquatic sediments, and the rhizosphere, thereby structuring microbial biodiversity and modulating community dynamics [1]. The ecological function of these molecules as chemical signals and competitive agents underscores their biological significance well beyond the clinical domain. The actinomycetes, particularly species from the genus Streptomyces, have proved to be a tremendous high-impact source of valuable chemicals, yielding many clinically important antimicrobial compounds including streptomycin, actinomycin, and streptothricin. These organisms collectively account for the majority of clinically deployed antibiotics, representing an exceptional biological source for pharmaceutical science [2].

The global burden of AMR has appeared as a critical threat to public health systems worldwide. This projected to cause 10 million deaths annually by 2050 if left unaddressed, posing a severe threat to global health and advance medicine. The rate of resistance acquisition has dramatically overtaken the pace of novel antibiotic development, creating a widening therapeutic gap that endangers the management of common bacterial infections. Most of the pharmaceutical companies generate antibiotics on a large scale, and the antibiotic market is expected to be worth approximately $47–50 billion with an annual growth rate of around 5–6%. The economic disincentives and the formidable technical challenges of developing structurally novel scaffolds have driven many pharmaceutical companies to reduce investment in antibiotic pipelines [3]. Due to excess use of antibiotics, the types and numbers of drug-resistant bacteria, represented by multidrug resistant (MDR) and extensively drug resistant (XDR) bacteria, have increased in clinical settings, posing a significant threat to human survival. This alarming epidemiological trajectory compels a fundamental reorientation toward understanding and exploiting the genetic and regulatory foundations of microbial antibiotic biosynthesis [4].

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Microorganisms most notably actinobacteria, filamentous fungi, and select Gram-negative bacteria remain the mainly architects of antibiotic chemical diversity. The two-thirds of all known antibiotics are generated by actinomycetes, specifically by Streptomyces, and it is assumed that actinomycetes are the source of some 61% of all microorganism bioactive substances are found. In these organisms, the biosynthetic machinery liable for antibiotic generation is encoded within discrete, contiguous genomic loci termed BGCs [5]. These clusters harbor core biosynthetic enzymes mainly PKS, NRPS and hybrid PKS-NRPS systems alongside tailoring enzymes, transporter proteins, and pathway-specific regulatory elements that collectively orchestrate the assembly of structurally complex secondary metabolites.

The literature has established the wide outlines of BGC architecture and regulation in model Streptomyces species. Genomic analyses suggest that Streptomyces species can synthesize more than 150,000 bioactive metabolites, many of which are coded in BGCs that are cryptic that is, transcriptionally silent or poorly expressed under standard laboratory conditions [6]. Analysis of sequenced Streptomyces genome data showed that a single Streptomyces genome generally encodes 25–50 BGCs, of which approximately 90% are silent or cryptic under standard laboratory growth conditions. The studies have characterized a hierarchy of control mechanisms, including cluster-situated regulators such as the Streptomyces antibiotic regulatory proteins (SARPs — e.g., ActII-ORF4, RedD, CpkO), global pleiotropic regulators (AdpA, AfsR, DasR), two-component signal transduction systems (PhoP/PhoR, AfsQ1/Q2), and quorum-sensing molecules including γ-butyrolactones [7].

The regulatory cascade governing coelimycin production in Streptomyces coelicolor A3(2) responds to stringent culture density and the timing of the transition phase through the quorum-sensing butanolide system and nutrient accessibility signals mediated by global pleiotropic regulators, many of which are two-component signal transduction systems. In parallel, post-transcriptional regulatory layers involving small regulatory RNAs (sRNAs), the alarmone ppGpp, and ribosomal engineering have been reported to modulate secondary metabolite output. Recent advances in native and synthetic promoter engineering have enabled precise transcriptional control over silent gene clusters, with CRISPR-based activation systems and machine-learning-guided promoter design emerging as transformative strategies for BGC activation [8,9].

The present mini review aims to provide a combined account of genetic architecture of antibiotic-producing BGCs across major microbial lineages, with emphasis on PKS, NRPS, and hybrid biosynthetic systems critically examine the multilayered regulatory networks that govern BGC expression, encompassing pathway-specific regulators, global pleiotropic regulators, quorum sensing cascades, and epigenetic mechanisms; discuss the interplay between environmental signals and secondary metabolite induction; and the emerging synthetic biology and genome mining strategies for the rational activation of silent BGCs [10].

BGCs: The Genetic Foundation

BGCs are physically groups of genes that encode the enzymatic pathways necessary to construct specific secondary metabolites. A canonical BGC encompasses core biosynthetic genes, tailoring enzymes responsible for structural modification, transporter proteins, self-resistance determinants, and pathway-specific regulatory elements all co-localized within a discrete chromosomal locus. The most common documented microbial BGC types include PKS, NRPS, ribosomally synthesized and post-translationally modified peptides (RiPPs), terpenoids, and saccharides, along with hybrid chemical entities [11]. PKS systems operate through iterative condensation of acyl-CoA units to yield structurally complex polyketides such as erythromycin and tetracycline, the NRPS systems function as multifunctional enzyme complexes that collects the proteinogenic and non-proteinogenic amino acids into the final peptide structure in an assembly-line fashion, with each module containing minimally a condensation (C), adenylation (A), and peptidyl carrier protein (PCP) domain [12]. PubMed Central Hybrid PKS-NRPS systems further expand chemical diversity, exemplified by bleomycin and epothilone biosynthesis.

The diversity of BGCs is greatly driven by horizontal gene transfer (HGT), module duplication, domain shuffling, and gene gain or loss events. Four primary evolutionary mechanisms shape NRPS and PKS BGC diversity: module duplications, iterative elongation, concerted evolution, and gene insertions or deletions with functional consequences. PubMed Central These processes account for the remarkable structural chemodiversity observed across phylogenetically distant microbial taxa.

The advanced genome mining has basically changed the pace of BGC discovery. Since its initial release in 2011, antiSMASH (Antibiotics and Secondary Metabolite Analysis Shell) has become the mostly used and gold-standard tool used in mining microbial genomes for secondary metabolite BGCs, with version 6 incorporating detection rules for 71 distinct BGC types [13]. Oxford Academic Complementary platforms including PRISM, which employs profile hidden Markov models for structure prediction and BiG-SCAPE, which constructs sequence similarity networks to delineate Gene Cluster Families (GCFs) and generates multi-locus phylogenies of BGCs across large genomic datasets, PubMed Central have collectively enabled comparative genomic analyses at an unprecedented scale, revealing a vast reservoir of uncharacterized biosynthetic potential awaiting functional validation.

Regulatory Mechanisms Controlling Antibiotic Production

Antibiotic production in microorganisms is observed by a complex, multilayered regulatory system that unites genetic, cellular, and environmental signals. The pathway-specific regulators particularly cluster-situated regulators (CSRs) such as SARPs directly control BGCs. These regulators activate or repress transcription of genes liable for specific antibiotics, as seen with actII-ORF4 in actinorhodin production and redD/redZ in undecylprodigiosin synthesis [14]. Negative regulators like AbsA2 can inhibit multiple antibiotic pathways by targeting CSR genes.

Interplay Between Primary and Secondary Metabolism

The interplay in between primary and secondary metabolism is an analytic determinant of antibiotic biosynthesis, as both processes are tightly interconnected through shared

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precursors, energy pools, and regulatory networks. Primary metabolism supplies important building blocks such as amino acids, acetyl-CoA, malonyl-CoA, and other key intermediates that are diverted toward secondary metabolite biosynthesis under specific physiological conditions. The allocation of metabolic flux toward these precursors is therefore a decisive factor influencing antibiotic yield and diversity.

Central carbon metabolism, including glycolysis, the tricarboxylic acid (TCA) cycle, and the pentose phosphate pathway, plays a vital role in this context. These pathways not only generate precursor molecules but also provide reducing equivalents (e.g., NADPH) and ATP required for energetically demanding biosynthetic reactions [15]. Modulation of carbon flux through these pathways often moderate by global regulators and nutrient-sensing systems can significantly increase or repress secondary metabolite production. An increased flux toward acetyl-CoA and malonyl-CoA pools directly supports polyketide and fatty acid-derived antibiotic biosynthesis.

An important feature of this metabolic interplay is the temporal coordination between growth phase and antibiotic production. In many microorganisms, particularly Streptomyces species, secondary metabolism is started during the transition from exponential to stationary phase. This change reflects a reprogramming of cellular priorities from biomass accumulation to survival and competition [16]. Regulatory signals such as nutrient limitation, accumulation of metabolic byproducts, and stress responses trigger the activation of BGCs.

Biotechnological and Synthetic Biology Approaches

The global regulatory networks coordinate broader cellular processes. Regulators such as AdpA influence thousands of genes, linking primary metabolism, development, and secondary metabolite generation. Other systems, including AfsR/AfsS and DasR, corporate nutrient availability with antibiotic biosynthesis, while two-component systems (e.g., PhoP/PhoR) and sigma factors adjust transcription in response to external stimuli [17,18]. Nucleoid-associated proteins can silence gene clusters, while small RNAs and riboswitches modulate mRNA stability and translation based on intracellular conditions. Factors like carbon source, phosphate limitation, and stress responses particularly mediated by (p)ppGpp strongly influence antibiotic biosynthesis. These interconnected systems secure that antibiotic production is tightly regulated and enhanced for survival.

Challenges and Future Perspectives

The significant advances in genome mining and synthetic biology, several challenges continue to force the full misuse of microbial BGCs. An important limitation is the activation of silent or poorly expressed BGCs in native producers. These clusters are often fixed within difficult regulatory frameworks and remain transcriptionally inactive under standard laboratory conditions [19].

The combination of multi-omics approaches the total transcriptomics, proteomics, and metabolomics offers a systems-level understanding of secondary metabolism. The explanation of large-scale datasets and the initiation of causal relationships between gene expression, protein abundance, and metabolite profiles present substantial analytical and computational challenges.

Emerging approaches in synthetic ecology, particularly co-culture systems, provide promising avenues to mimic natural microbial interactions that stimulate secondary metabolite production. Interspecies signaling and competitive or symbiotic interactions have been shown to induce otherwise silent pathways; however, reproducibility and scalability of such systems remain limiting factors [20,21].

The application of AI and ML is transforming BGC prediction and regulatory network modeling. These tools enable high-throughput identification of candidate clusters and predictive modeling of gene regulation; their accuracy is constrained by limited annotated datasets and incomplete biological knowledge.

Future research should prioritize integrative frameworks combining experimental and computational methodologies to systematically activate cryptic pathways, thereby advancing antibiotic discovery and biosynthetic innovation.

Conclusion

The antibiotic biosynthesis in microorganisms is observed by a highly intricate genetic and regulatory landscape encompassing pathway-specific regulators, global transcriptional networks, quorum sensing systems, and several layers of post-transcriptional and environmental control. These interconnected mechanisms secure that secondary metabolite production is coordinated with cellular physiology, nutrient availability, and ecological context. Improvement in the molecular genetics and systems biology have notable expanded our understanding of these regulatory circuits, revealing the complexity underlying BGC expression.

This knowledge base has opened new avenues for the discovery of novel antibiotics. The identification of numerous cryptic and silent BGCs through genome sequencing efforts highlights an immense, largely untapped reservoir of chemical diversity. Coupled with appearing biotechnological and synthetic biology tools that includes genome editing, pathway refactoring, and heterologous expression there is substantial potential to unlock these hidden metabolites and address the need for new antimicrobial agents in the face of rising AMR.

Disclosure Statement

No potential conflict of interest was reported by the author.

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