Synthetic and Systems Biotechnology 3 (2018) 163–178

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: http://www.keaipublishing.com/synbio

Activation of microbial secondary metabolic pathways: Avenues and challenges

Bikash Baral, Amir Akhgari, Mikko Metsä-Ketelä*

Department of Biochemistry, University of Turku, FIN-20014, Turku, Finland

ARTICLE INFO

Keywords:
Biosynthesis
Chemical diversity
Genome mining
Metabolic engineering
Synthetic biology

ABSTRACT

Microbial natural products are a tremendous source of new bioactive chemical entities for drug discovery. Next generation sequencing has revealed an unprecedented genomic potential for production of secondary metabolites by diverse micro-organisms found in the environment and in the microbiota. Genome mining has further led to the discovery of numerous uncharacterized 'cryptic' metabolic pathways in the classical producers of natural products such as Actinobacteria and fungi. These biosynthetic gene clusters may code for improved biologically active metabolites, but harnessing the full genetic potential has been hindered by the observation that many of the pathways are 'silent' under laboratory conditions. Here we provide an overview of the various biotechnological methodologies, which can be divided to pleiotropic, biosynthetic gene cluster specific, and targeted genome-wide approaches that have been developed for the awakening of microbial secondary metabolic pathways.

1. Microbes: promising and prolific source of new drug leads

Natural products (also referred to as secondary metabolites or specialized metabolites; SMs) represent a group of low-molecular weight structurally diverse and complex bioactive compounds occupying an unusual chemical property space [1]. Microbes in particular are prolific antibiotic factories [2], and have proven to be a bountiful source of SMs that have been successfully developed as crucial drug leads [3,4]. To date, more than 5000 antibiotics have been identified from the genus *Actinobacteria*, while 500 natural products have been isolated from *Myxobacteria* [5–7]. Soil-dwelling *Streptomyces* bacteria are particularly proficient producers with 7600 SMs identified until 2005 [8], while computational predictions have estimated that these bacteria may have the capability to produce 150,000 chemically distinct antimicrobial agents [5–7]. In addition to classical antibiotics, microbial cultures have been a source for immune-suppressive agents [9], lantibiotics, anti-proliferative [10], cytotoxic [11], anti-hypertensive [12], antiviral compounds [13,14] and various enzyme inhibitors [15]. *Circa* 35% of drugs approved by the FDA/EMA are estimated to be either natural products or their derivatives [16], and > 50% of clinical antibiotics are of Actinomycetes origin [8]. Widely used antibiotics include erythromycin A, penicillin G and streptomycin, while examples of microbial metabolites that have been successfully launched for other therapeutic areas comprise the anticancer agent doxorubicin, the immunosuppressants rapamycin and cyclosporine and the anthelmintic drug avermectin B1 (Fig. 1).

The chemical entities described above (Fig. 1) were discovered several decades ago, most during the "Golden Era of Antibiotics" in the 1950s and 1960s. Starting from the 1980s and 1990s, traditional bioactivity-based screening of microbial culture extracts became severely affected by diminishing returns due to high probability of discovering previously known metabolites [17]. Structure elucidation of hit molecules is time consuming and ultimately the high rediscovery rate of known molecules led to decreased interest of pharmaceutical companies in natural products [2], which turned their attention to structure-based drug design and combinatorial chemistry instead [18,19]. However, despite very strong investments into this field in the last 15 years, these efforts have not provided a single novel synthetic antimicrobial agent that has proceeded beyond preliminary clinical trials [20]. One key problem has been that while many synthetic compounds with high efficacies towards their molecular targets have been successfully developed, it has become apparent that these compounds have difficulties in reaching their target site *in vivo* and in penetrating bacterial cell membranes [17].

However, recent technological advancements have created a renaissance of interest in natural products and new chemical entities with

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author.
E-mail address: mianme@utu.fi (M. Metsä-Ketelä).

https://doi.org/10.1016/j.synbio.2018.09.001
Received 13 July 2018; Received in revised form 28 August 2018; Accepted 4 September 2018

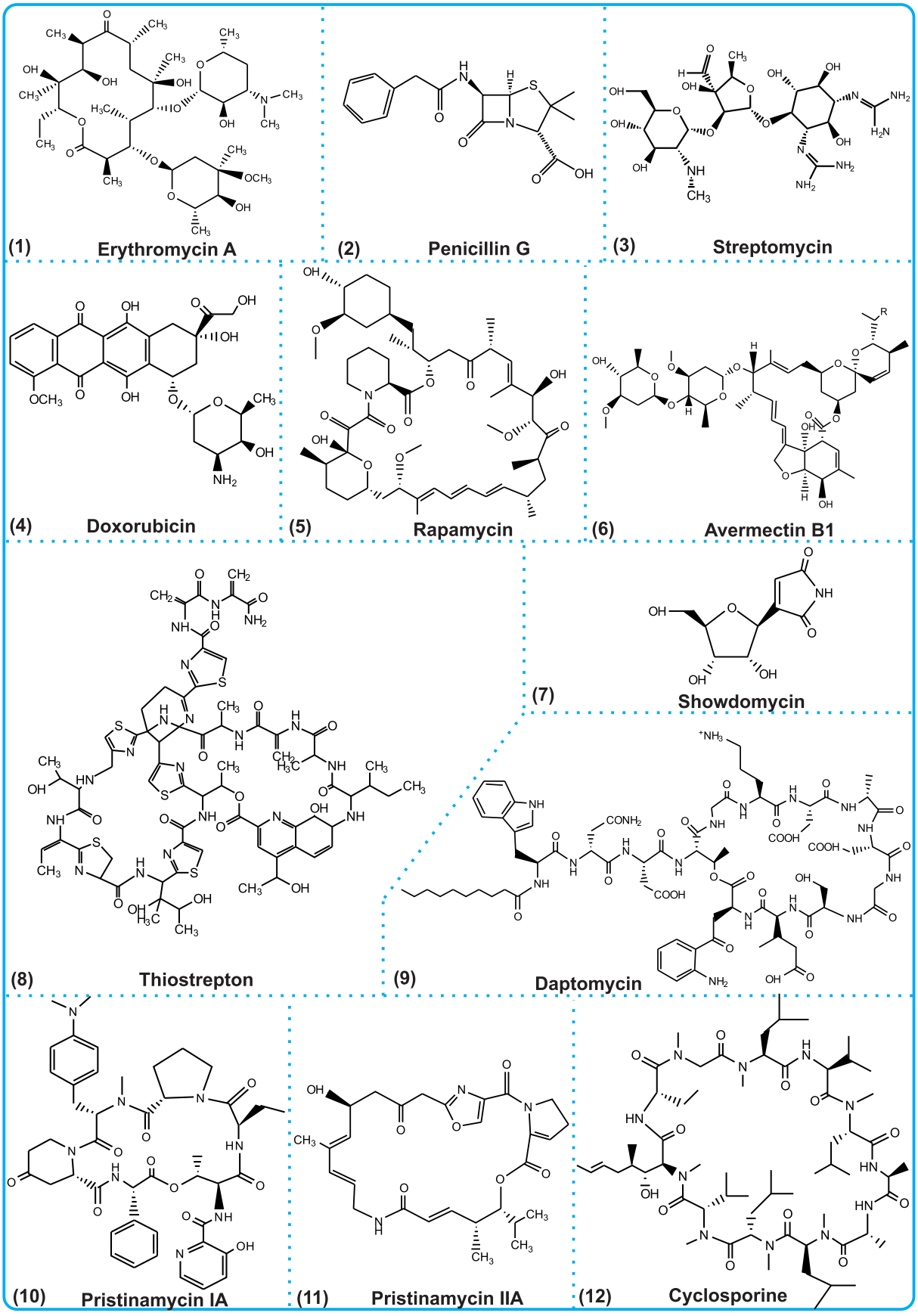

Fig. 1. Chemical structures of selected microbial natural products.

novel mode of action [21,22]. The foundation was laid in the 2000s by the diligent work of the Academic sector in elucidating the biosynthetic logic of the main classes of microbial natural products made by polyketide synthase (PKS) [23,24], non-ribosomal peptide synthetase (NRPS) [25] and ribosomally synthesized and post-translationally modified peptide (RiPP) pathways [26]. The classical pre-genomics view was that an individual microbial strain could produce only a limited number of SMs, but the first genome sequences of *Streptomyces* [27] and *Aspergillus* [28] published in the 2000s and the subsequent explosion of genome sequencing data in the 2010s has demonstrated that these micro-organisms have the genetic capability to produce a significantly larger number of compounds with great strain-to-strain variations. These unknown metabolic pathways are likely to encode numerous bioactive molecules, which could be used to solve the current problems with drug resistant pathogens and to obtain improved chemotherapy agents with reduced side effects. The key question remaining to answer in the field is how to access this hidden potential, since it would appear that the most of these cryptic pathways are silent or poorly expressed under laboratory conditions. In our current discourse, we attempt to provide crucial insights into methodologies that have been developed in an attempt to harness this tremendous chemical diversity.

2. Genomics-driven natural products discovery

The paradigm shift to genomics-based discovery of microbial SMs may be considered to be initiated by the sequencing of the genomes of *S. coelicolor* and *S. avermitilis*, which led to the discovery of 22 [27] and 25 [29] putative biosynthetic gene clusters (BGC), respectively, that could code for secondary metabolic pathways. The genome of the filamentous fungi *Aspergillus nidulans* harbored an even greater potential with 56 putative pathways, as observed by genome mining [30]. However, recent next generation sequencing efforts have vastly expanded this phenomenon in an unparalleled scale to diverse microbial genus that have not typically been associated with natural products such as *Burkholderia*, *Clostridium* and *Pseudomonas* [31,32]. In addition, probing microbial communities in various ecological niches such as the human microbiota [33] and environmental samples [34] have revealed exceptional metabolic diversity. Many lactic acid bacteria, including species of *Enterococcus*, *Lactobacillus* and *Streptococcus* associated with the gastrointestinal tract have been reported to produce bioactive modified peptides [33], in particular those synthesized via RiPP pathways [35,36]. The marine sponge *Theonella swinhoei* has been shown to host many uncultivated bacterial symbionts, which harbor pathways for production of exotic SMs [37]. It should also be noted that the potential of soil microbes for production of SMs remains elusive, since *ca.* 99% of environmental microbes are un-cultured under laboratory settings. In order to circumvent this barrier, environmental DNA isolated directly from soil samples may be used for cloning and expression of BGCs, as in the case of the malacidins pathway [38]. In another approach, the broad spectrum antibiotic teixobactin was discovered from an uncultured soil bacteria '*Eleftheria terrae*' by growing the microbe directly *in situ* in the soil using a bacterial iChip [39].

The development of bioinformatics software for analysis of sequencing data for the presence of BGCs has been instrumental in genomics-driven natural products discovery. Programs such as antiSMASH [40,41], SMURF [42], BAGEL3 [43] and PRISM [44,45] are able to detect and decipher all of the most common biosynthetic types. In some instances, such as type I polyketides and NRPSs and RiPPs, these programs are able to even provide predictions for the chemical scaffolds of the metabolites [45], but for other compound classes such as type II PKSs and glycosylated natural product, the information acquired through computational studies is limited. Linking genomic data to chemistry has also been aided by community standards such as MIBiG (Minimum Information about a Biosynthetic Gene cluster) [46], which define standardized annotation and metadata procedures for characterization of pathways thus facilitating future research efforts.

3. Metabolic gene clusters in microbes

The genome mapping approaches are facilitated by the fact that all genes required for the biosynthesis of the metabolite, its regulation, resistance and transport are typically located in within the BGCs [47–49]. The size of metabolic gene clusters varies greatly depending on the complexity of the end product of the pathway. As an example, the gene cluster responsible for biosynthesis of the glycosylated anthracycline nogalamycin encodes 32 enzymes [50]. In addition, the type of biosynthetic machinery has a significant influence to the size of the BGC with large differences noted between the 30-kb thiostrepton and 128-kb daptomycin gene clusters [51,52], even though both are assembled from amino acid building blocks and are of similar complexity. The difference can be explained with the fact that thiostrepton is RiPP [51], whereas daptomycin is made through a NRPS pathway. Wide variation may also be found within the same classes of biosynthetic machineries, since the chemically simple NRP showdomycin is encoded within a 12-kb fragment of DNA, while the pristinamycin supercluster, which encodes two complex hybrid PK-NRP molecules, spans as large as 210-kb [53].

The onset of SMs production typically occurs during the early stationary phase, and involves complex metabolic changes within the organism [54]. Pleiotropic (or global) regulators are localized distantly from the biosynthetic cluster, and control the expression of secondary metabolism by responding to diverse signals [55]. These pleiotropic regulators have wider coverage, simultaneously controlling the expression of several BGCs. Events of nitrogen or phosphate starvation or distinct signaling compounds like *N*-acetylglucosamine (GlcNAc), stressors like heat, pH and damage to the cell wall provide proper stimuli for triggering the expression of these regulatory genes [56,57]. The production is also guided by regulatory genes present in the biosynthetic clusters, which are pathway-specific and may either be activators or repressors [58].

The genes responsible for providing resistance and for transporting SMs outside of the cells are also typically clustered within BGCs. In particular, if the end product of the pathway has biological activity against the producing host, then the resistance genes for that particular metabolite may be encoded in the genomic *locus*. Similarly, genes encoding efflux pumps (for instance, ATP-binding cassette (ABC) and Major Facilitator Superfamily proteins) have also been traced along the BGCs [59] and are responsible for the reduction of both toxicity [60] and feedback inhibition effects. For instance, in *S. avermitilis*, the ABC transporter AvtAB responsible for the exporting avermectin is found within the biosynthesis gene cluster [61].

4. Strategies for the activation of unknown metabolic pathways

A growing body of evidence has indicated that the activation of gene clusters has the potential to greatly facilitate discovery of new natural products of high-therapeutic leads. Several methods have been developed for the activation of silent or poorly expressed cryptic gene clusters, which can be broadly classified to three major categories (Fig. 2). In one group, there are methodologies that aim to modify the whole metabolome of the target strain and generate pleiotropic effects to activate randomly any pathway residing in the strain. These methods are generally technically simple and are therefore suitable for scaling up to high-throughput systems. Unfortunately, these techniques generally forfeit the advantages provided by next-generation sequencing and genome mining, since they are not able to target the most interesting gene clusters for activation. They also suffer from the same metabolite rediscovery issues as traditional natural products discovery. In contrast, the methodologies lying under the second category are able to focus on desired pathways, but are technically challenging and suffer from low throughput. Finally, the techniques in the third group aim to

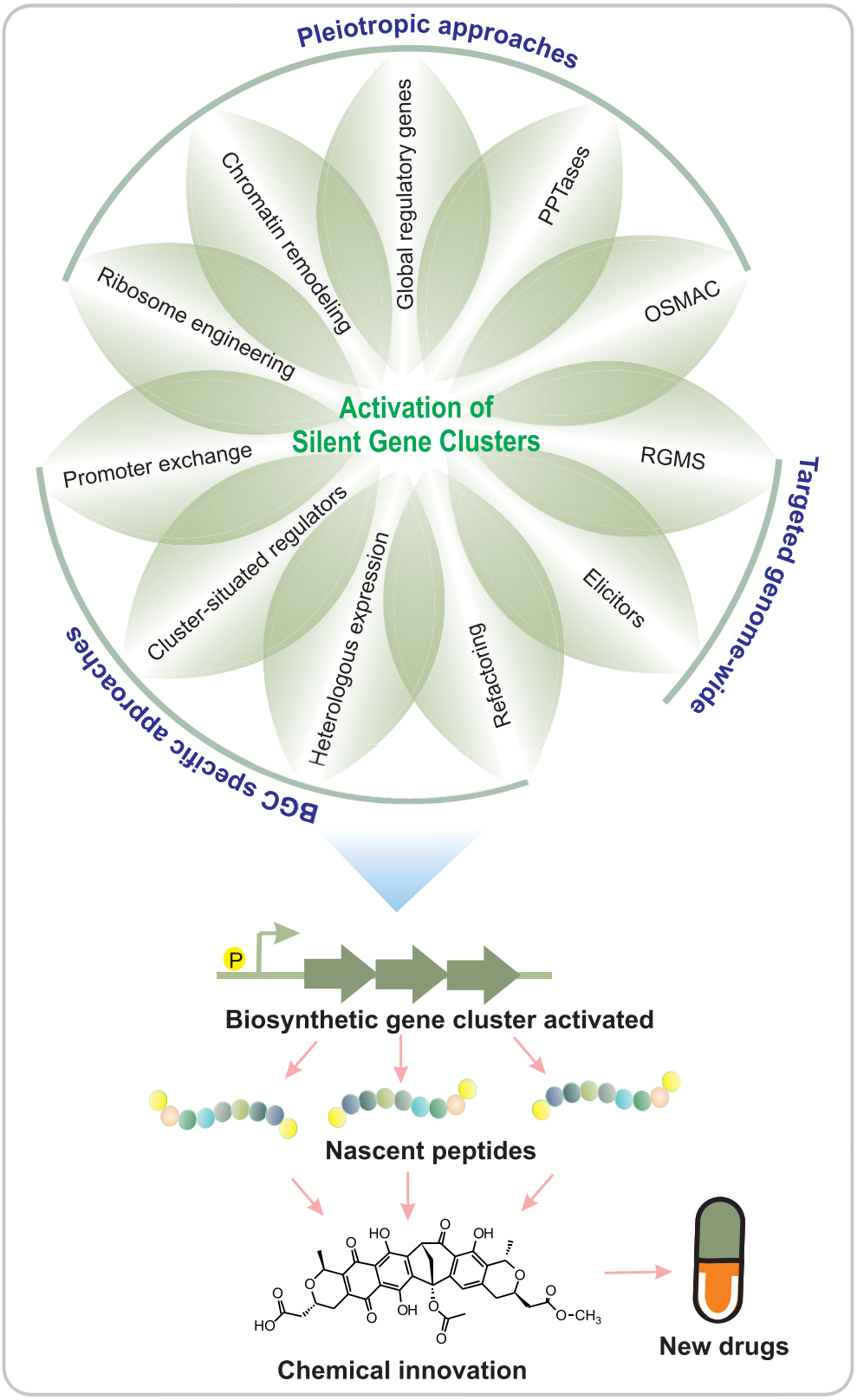

Fig. 2. Methodologies for the activation of silent gene clusters to obtain new natural products. Petals within the circle represents different techniques that have been developed with three major categories, viz., pleiotropic, BGC-specific and targeted-genome wide approaches highlighted. Abbreviations: OSMAC, One strain many compounds; P, Promoters; PPtases, Phosphopantetheinyl transferases; RGMS, Reporter-Guided Mutant Selection.

gain the benefits of both of the approaches mentioned above, usually by combining pleiotropic activation methods to gene cluster specific reporter systems. The diversity of these methodologies offer many tools for the research community, but to date no single superior method for activation of biosynthetic pathways has been presented.

4.1. Genome-wide pleiotropic methods

4.1.1. Ribosome engineering and its applicability

One of the most important adaptations in bacteria to environmental stresses, e.g. depletion of amino acids, is the "stringent response" governed by an intracellular transient accumulation of guanosine tetraphosphate (ppGpp) [62]. The bacterial alarmone ppGpp is synthesized by the ribosomal enzyme ppGpp synthetase (*relA*) and is initiated by the binding of an uncharged tRNA molecule to the aminoacyl binding site (A-site) on the ribosome [63]. Subsequent binding of ppGpp to the RNA polymerase (RNAP) modulates transcription by upregulating and downregulating various promoters [64,65] through mechanisms that can vary between species [66]. Investigations into the RNA polymerase/ppGpp complex in a thermophilic bacterium *Thermus thermophilus* revealed three modes of transcriptional regulation by ppGpp depending on the nucleic acid composition of the promoter, which influenced the conformation of the RNAP [64]. Therefore it is unsurprising that ppGpp has also been linked to secondary metabolism and initiation of antibiotic production [67]. In *Streptomyces*, *relA/relC* mutants with significantly decreased ppGpp contents display lowered production of SMs when transferred to a media with nutritional shift-down [68–70] (Fig. 3A and B). It has been revealed that mutations conferring resistance to rifampicin in the *rpoB* gene, which encodes the RNA polymerase β-subunit, alter the conformation of the RNAP to resemble ppGpp bound status. Therefore, the requirement for ppGpp for the expression of silent or poorly expressed gene clusters is bypassed in these mutants in various actinomycetes [71–73].

In an analogous manner, the acquisition of mutations in ribosomal proteins conferring resistance to antibiotics targeting the ribosome has also been reported to promote the expression of silent genes. However, the molecular mechanism of this phenomena remains obscure. The introduction of streptomycin-resistance mutations in *rel* mutants of *S. coelicolor* and *S. griseus* reinstated actinorhodin production without the requirement of ppGpp [74,75]. On the contrary, elevated concentrations of ppGpp (30-fold) and actinorhodin (180-fold) has been detected in *S. coelicolor*, when the strain was exposed to eight antibiotics in a successive manner, where all of the antibiotics were targeting the translational machinery with the exception of rifampicin [76]. In addition, *relA* disruption resulted in the total loss of ppGpp accumulation as well as a significant decrease in actinorhodin production, indicating the key role of ppGpp in the activation of biosynthesis. Cluster-activated strains resulting from mutations in the ribosome have been reported in Streptomycetes and other bacteria including *Bacillus* spp., *Mycobacterium* spp. and *Pseudomonas* spp. [74,77,78]. Strikingly, two single *rpoB* mutants, an *rpoB*+*rpsL* double mutant, and a single gentamicin-resistant mutant induced the production of piperidamycins in *S. mauvecolor*, whereas the wild-type does not produce these metabolites at any detectable level in various media [79].

4.1.2. Chromatin remodeling

Chromatin remodeling is a vital mechanism for the modulation of transcription of eukaryotic genomes [80]. Chromatin is composed of repeating nucleosome units, which consist of histones (2 copies each of H2A, and H2B, H3 and H4 [80]) wrapped around chromosomal DNA and linked together through a linker histone (H1) (Fig. 3C) [81,82]. Histones cause dynamic variations to packaging and accessibility of DNA in response to physiological and developmental cues [83,84]. These are mediated via post-translational modifications such as deacetylation and methylation of histone tails, which ultimately regulate gene expression [85]. Gene clusters for secondary metabolism are clustered at subtelomeric regions and are co-regulated [86], where histone acetylation and methylation largely impact transcription [87].

For activation of SMs, the state of chromatin packaging has been influenced by artificial expression of histone modification genes and by utilizing small molecule inhibitors against histone deacetylase enzymes. In *A. nidulans*, inactivation of the histone deacetylase (HDAC) *hdaA* down-regulated secondary metabolic pathways [88], while loss-in-function of *cclA* (an ortholog of *bre2* involved in histone H3 lysine 4 methylation) activated the expression of the cryptic secondary metabolic clusters generating monodictyphenone, emodin and its derivatives [89]. The use of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) led to stimulation of production of new cladochromes and calphostin B in *Cladosporium cladosporioides* [90] and histone deacetylase (HDAC) inhibitors have also caused over-expression of SM genes in *A. nidulans* [88,91].

Unlike in fungi and other eukaryotes, prokaryotes are entirely deprived of histones. However, the bacterial genome, for instance, *Streptomyces* sp. comprises their own versions of HDAC [92] and the structure of the nucleoid may have a role in the global regulation of metabolism [93]. Access to chromosomal DNA may be restricted by nucleoid-associated proteins, various RNAs and differential supercoiling in the nucleoid, which may prevent aberrant transcription of many gene clusters [94]. In *Streptomyces*, a bifunctional nucleoid-associated protein (DdbA) has also been characterized that comprises of an N-terminal DNA-binding histone H1-like domain and a C-terminal DksA-like domain, which could modulate the RNA polymerase activity along with ppGpp to induce transcription [95].

4.1.3. OSMAC approach and environmental cues

One strain many compounds "OSMAC" is a relatively simple and versatile approach that allows activation of diverse-metabolic pathways [96]. The biosynthesis of SMs is stimulated through alterations in cultivation parameters [97,98], e.g. media composition, aeration rate, type of culturing vessel or a combination of these factors. In some cases, even subtle changes may result in drastic changes, as demonstrated in fermentations of *Paraphaeosphaeria quadriseptata*, where switching from the use of tap water to distilled water lead to the isolation of six new SMs [99]. These experiments aim to mimic more precisely the natural growth conditions found in the environment to trigger the onset of secondary metabolism, and has been successfully applied to both bacteria and fungi, as reviewed elsewhere [97,98].

The classical OSMAC approach may be extended to more drastic measures by modulating the culture conditions with stress or chemicals. Heat shock has been shown to induce jadomycin production [100] and increase the yields of validamycin [101], whereas limitation of nutrients such as alanine and/or an acidic pH shock led to methylenomycin production in *Streptomyces coelicolor* A3(2) [102]. In particular, numerous SMs have been shown to be regulated negatively by inorganic phosphate concentrations [57], while GlcNAc appears to function as an important sensory molecule for the onset of secondary metabolism [103]. Both biotic (microbial lysates or soil extracts) and abiotic (antibiotics, synthetic compounds) chemical elicitors have been proved to be effective strategies for the induction of silent gene clusters. Two small molecule elicitors have been particularly successful; ARC2, a synthetic compound discovered through large scale screening [104], and goadsporin [105,106], a natural product that promotes secondary metabolism in *Streptomyces*. Sub-inhibitory concentrations of other xenobiotic antibiotics, such as jadomycin B [107] and monensin [108] have been shown to induce endogenous production of SMs. Interestingly, it has been shown that the addition of rare earth elements, e.g. scandium and lanthanum, at low concentrations resulted in the activation of the cryptic secondary metabolite biosynthetic gene clusters [78,109].

Co-cultivation, mimicking the ecological habitats of micro-organisms, has been emerged as an effective strategy to increase the chemical diversity of secondary metabolites (Fig. 4A). It is believed that the

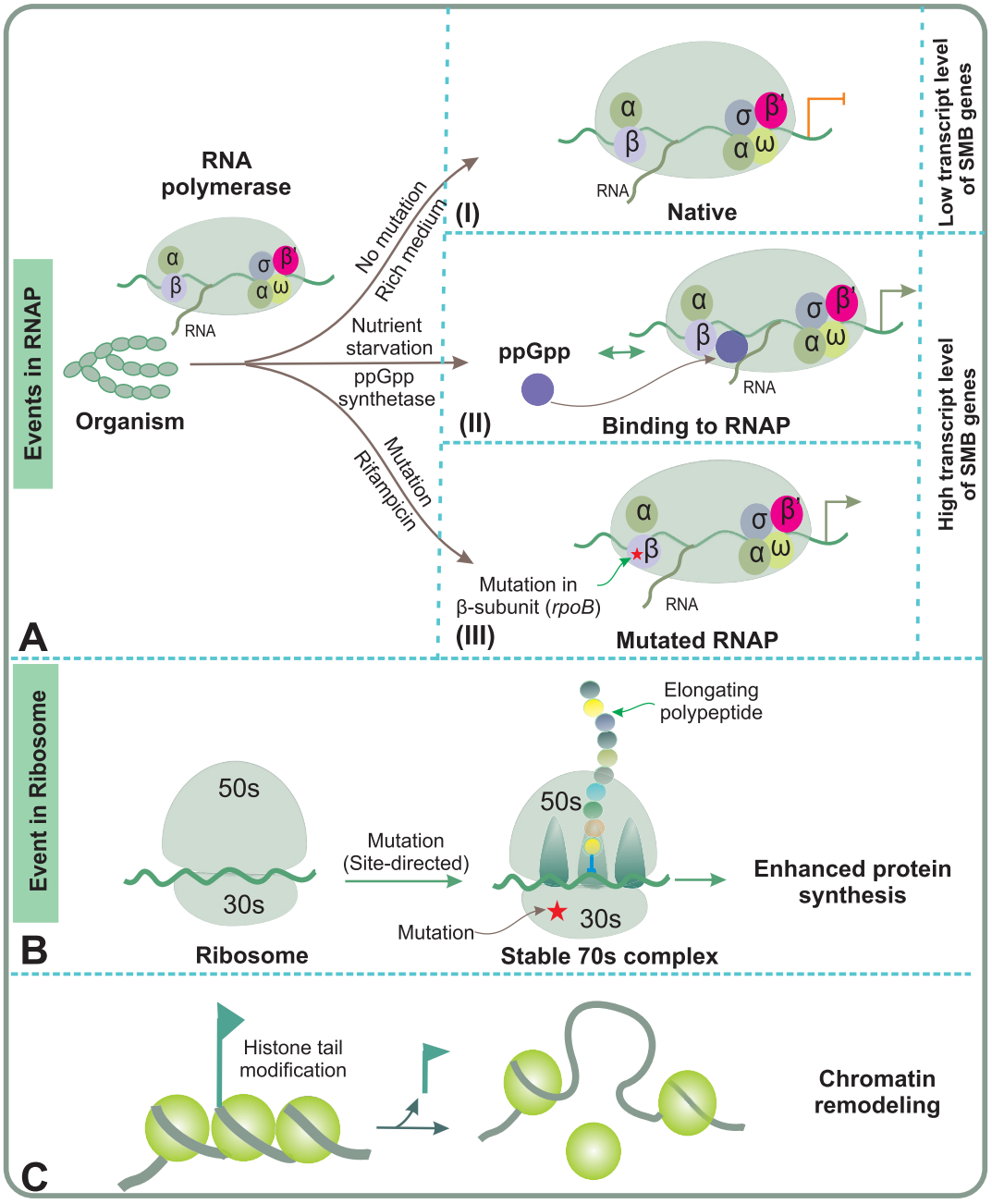

Fig. 3. Pleiotropic approaches for activation of silent gene clusters: A) Events that occur in the RNAP when an organism is grown in alternative conditions: (i) Nutrient rich-medium, where the conditions suppress secondary metabolism and transcription levels from BGCs are low; (ii) Microbe subjected to nutritional down-shift (depletion of amino-acids) conditions, which leads to the production of ppGpp (Guainosine tetraphosphate), that ultimately binds to RNAP, giving rise to high transcription levels from the BGC; (iii) Addition of antibiotics like rifampicin induces a mutation in the β-subunit of the polymerase, which in turn increases the transcription level of the BGC. B) A mutation in the 30S subunit of ribosome stabilizes the 70S ribosomal complex and causes upregulation of protein synthesis in the stationary phase. C) Packaging of DNA around histones prevents gene transcription and is regulated through post-translational modifications of histone tails. In chromatin remodeling, enzymes responsible for histone modifications are manipulated to allow histone release, which enables expression of BGCs from the uncovered stretch of DNA.

social networking in ecological environment is mediated by diffusible low-molecular-weight signaling molecules that are secreted from and received by cells [110,111]. For example, co-cultivation of the marine-derived fungal isolate *A. fumigatus* MR2012 with *S. leeuwenhoekii* strain C34 led to induction of metabolite production in both organisms, while co-cultivation of the fungus with bacterial strain C58 resulted in the detection of pentalenic acid from the bacteria [112]. Another marine-derived fungus *Emericella* sp. was induced to produce emericellamides A and B upon co-culture with *Salinispora arenicola* [113], whereas promomycin produced by one *Streptomyces* strain led to initiation of antibiotic production in another strain by cross-feeding [114]. Interestingly, dialysis experiments and electron microscopy have revealed that not only diffusible signals, but also intimate physical interactions contribute to the networking and induction of silent clusters during co-cultures of *A. nidulans* with *S. hygroscopicus* [115] or *A. fumigatus* with *S. rapamycinicus* [116].

4.1.4. Phosphopantheteine transferases

The biosynthesis of polyketides and nonribosomal peptides only occurs if the essential acyl - and peptidyl carrier proteins, respectively,

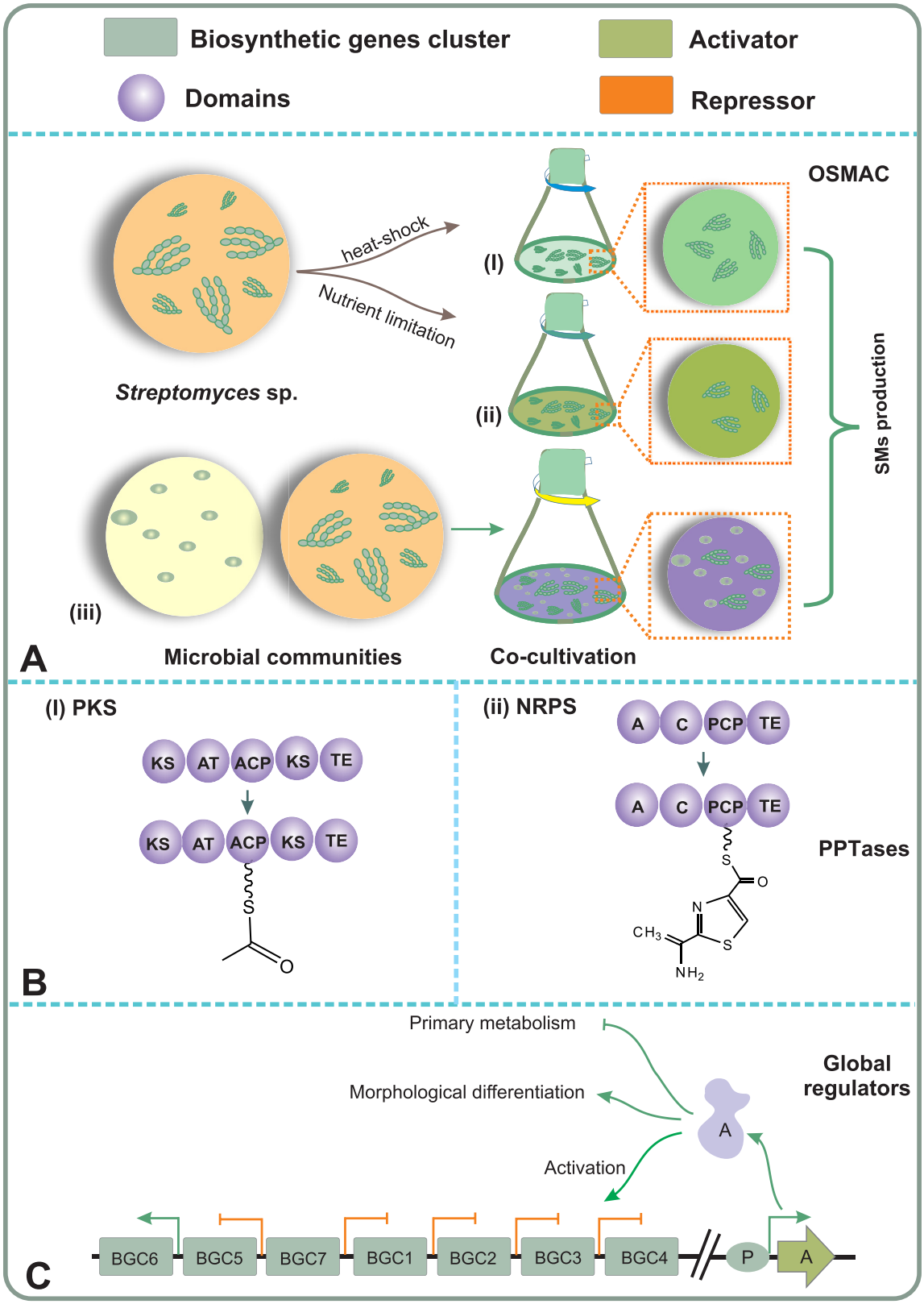

Fig. 4. Pleiotropic approaches for the activation of the silent gene clusters: Global regulators, PPTases and the OSMAC approach. A) In the OSMAC approach, cultivation conditions are manipulated to uncover the environmental signal required for activation of biosynthesis. As an example, co-cultivation of two or more microbes may induce microbial chemical communication, where one or even both participants produce novel compounds. Abbreviations: A, Activator; BGC, Biosynthetic gene cluster; P, Promoter. B) PPTases catalyze the post-translational modification of carrier proteins, which is essential for the biosynthesis of polyketides and nonribosomal peptides. Overexpression of PPTases may influence the production of SMs under conditions where the native PPTase may be down-regulated. Abbreviations: A, Adenylation domain; ACP, Acyl-carrier protein; AT, Acyltransferase; C, Condensation domain; KS, Ketosynthase domain; NRPS, Non-ribosomal peptide synthetase; PCP, Peptidyl-carrier protein; PKS, Polyketide synthases; TE, Thioesterase domain. C) Pleiotropic regulators control many aspects in the life cycle of microbes. Manipulation of these regulatory networks may lead to the activation of BGCs.

are post-translationally modified into active *holo*-form [117]. This reaction is catalyzed by phosphopantetheinyl transferases (PPtases) through covalent attachment of a 4′-phosphopantetheine group to a highly conserved serine residue (Fig. 4B). Microbial genomes may contain up to three PPtases, which harbor specificity to various biosynthetic pathways [118], but these genes are typically not found within BGCs. Since PPtases regulate primary metabolism that leads to physiological changes and metabolic flux alterations, overexpression of PPtases may also influence secondary metabolism [119]. Indeed, expression of two PPtase genes *sfp* and *svp* resulted in activation of silent pathways with a success rate of 70% in a study using 33 Actinomycetes strains [120]. The *sfp* and *svp* gene products, from *Bacillus subtilis* and *Streptomyces verticillus*, respectively, exhibit broad substrate specificity to guarantee sufficient phosphopantetheinylation of diverse biosynthetic enzymes. Recently, PPtase-based activation also led to the identification of three nucleosides including puromycin A and two new congeners in *S. alboniger* NRRL B-1832 [121]. Interestingly, the biosynthetic pathways of nucleosides do not contain carrier proteins, suggesting that the result was achieved indirectly via modulation of cellular regulatory networks by the PPtase.

4.1.5. Manipulation of global regulatory systems

The engineering of global regulatory genes aims to influence the same regulatory networks as the OSMAC approach, but instead of trying to find the environmental cues, it does it at a more refined molecular level through over-expression or inactivation of the genes controlling the cellular response to these phenomena (Fig. 4C). Investigations into pleiotropic regulatory networks in bacteria and fungi have revealed that production of SMs is linked to morphological development and nutrient availability (carbon and nitrogen sources, phosphate starvation, amino acid and iron availability) [55]. Other examples include response to pH stress in fungi mediated by the PacC zinc finger transcription factor [122] and the master regulator DasR in *Streptomyces*, which responds to the presence of *N*-acetylglucosamine that may be considered as a signal for autolytic degradation of the vegetative mycelium [103]. An important distinction to cluster situated regulatory genes is that the global regulatory systems also control the transcription of many other genes not related to secondary metabolism. In fungi, pathway-specific regulatory genes have been discovered only in about 60% BGCs [123], which indicates that the production of many SMs is modulated strictly by global regulatory networks. Similarly, the moenomycin biosynthetic gene cluster does not harbor a pathway-specific regulator and is under the control of pleiotropic regulators in *Streptomyces ghanaensis* [124].

In *Streptomyces*, manipulation of regulatory elements that respond to butyrolactone hormones such as *adpA* [125] have been particularly successful with cryptic oviedomycin [126] and germicidin [127] gene clusters recently activated. Disruption of *whiB*-like regulatory genes, which are associated with control of morphological development [128], have also led to the discovery novel violapyrones [129] and antimycin-type depsipeptides [130].

4.2. Biosynthetic gene cluster specific approaches

4.2.1. Transcription factors and promoter exchange experiments

In eukaryotes, each gene is transcribed from its own promoter, whereas in prokaryotes (e.g., *Streptomyces* sp.) several genes are often clustered together to form operons (Fig. 5A). In all bacterial species, the promoter sequences residing within BGCs are typically associated with specific sigma factors (σ-factors) that are vital for binding of the RNA polymerase (RNAP) in the formation of the translation initiation complex [131]. The various σ-factors confer promoter selectivity and are thereby responsible for switching on specific regulons [132]. For instance, the filamentous prokaryotes, *S. albus* J1074, *S. coelicolor* and *S. avermitilis* comprise 35, 65 and 60 σ-factors, respectively [27,133]. On the other hand, promoter recognition in fungi relies on more diverse transcription factors. Eighty families of transcription factors were revealed through a whole-genome analysis of more than 200 fungal genomes [134]. For example, the filamentous fungi *A. nidulans* and *N. crassa* contain 81 and 58 transcription factors, respectively [135]. These aforementioned mechanisms enable alterations in transcription patterns in response to environmental cues.

The σ-factors influence the expression of larger groups of genes, therefore σ-factor-based engineering methods have been utilized to activate gene expressions associated with secondary metabolite biosynthesis. For example, overexpression of the σ-factor 'Orf21' in *Streptomyces clavuligerus* NRRL3585 resulted in a slight increase of clavulanic acid production through the induction of early biosynthetic genes (*ceas2*, *cas2*) and the activator gene *ccaR* [136]. Moreover, the overexpression of σ-factors led to the accumulation of higher level of FK506, a hybrid polyketide-nonribosomal peptide and carotenoid productions in *Streptomyces* sp. KCCM 11116P [137] and *Corynebacterium glutamicum*, respectively [138]. Successful expression of a polyketide biosynthetic pathway (oxytetracycline) was achieved for the first time in a surrogate host *E. coli* by overexpressing the host σ^{54}-factor [139]. The deletion of σ-factor 'Sig6' caused over-production of avermectin by 2–2.7 fold in *S. avermitilis*, while overexpression of an extra copy lead to significant decrease in yields [140]. In addition, *S. avermitilis hrdB* mutants lacking σ^{hrdB} exhibited higher levels of avermectin production than the parental strain [141].

The promoter sequences residing in BGCs do not typically promote transcription in all growth conditions and are highly influenced by intracellular regulation networks. Manipulation of promoter sequences by exchanging native silent promoters with functionally active ones directly in the producing organism has proven to be a useful strategy to drive expression of the silent genes by circumventing the complexity of the regulatory genes (Fig. 5B). The methodology requires advanced genome editing tools that might not be available for the target organisms and is best suited for small BGCs that are composed of very few operons. The activation of a silent polycyclic tetramate macrolactam (PTM) gene cluster in *Streptomyces albus* has been achieved by the independent introduction of *ermE*p* upstream of *ftdA* and *ftdB* encoding putative desaturase and hybrid polyketide synthase-nonribosomal peptide synthetase, respectively, and led to the identification of 6-*epi*-alteramides A and B. Furthermore, the biosynthesis of a blue pigment 'indigoidine', which is not typically produced in standard laboratory conditions, has been induced in *S. albus* by expressing the NRPS gene *bpsA* under *ermE*p* promoter [142]. The introduction of strong engineered promoter *kasO*p* also resulted in successful activation of silent lycopene biosynthetic cluster in *S. avermitilis* [143]. Recently, replacement of the native promoters of the silent actinorhodin, undecylprodigiosin and PTM clusters with *kasO*p* produced targeted compounds in *S. albus*, *S. lividans* and *S. roseosporus*, respectively [144].

In *Aspergillus nidulans*, replacing the native promoter of the nonribosomalpeptide synthetase, *acvA*, with a promoter from the ethanol dehydrogenase, *alcA*p, from the same host led to a 30-fold increase in penicillin production [145]. In addition, sequential exchange of promoters for six genes in the silent fellutamide B gene cluster (*inp*) with *alcA*p enabled the activation of the cluster and led to the production of the target compound in *A. nidulans* [146].

4.2.2. Manipulation of pathway-specific regulatory genes

Pathway-specific transcriptional regulatory genes are typically found within the BGCs and they may either repress or activate the biosynthesis of the corresponding SM. These genes represent the lowest organizational level in the cellular regulatory networks and are typically tightly regulated by environmental sensors and pleiotropic regulators. However, in reality the regulatory circuits are likely to be even more complex with cross-talk between distinct pathways [147] and even between global and BGC specific regulatory genes reported [148]. The co-localization of pathway-specific regulatory genes offers a possibility for targeted activation of the desired BGC either by inactivation

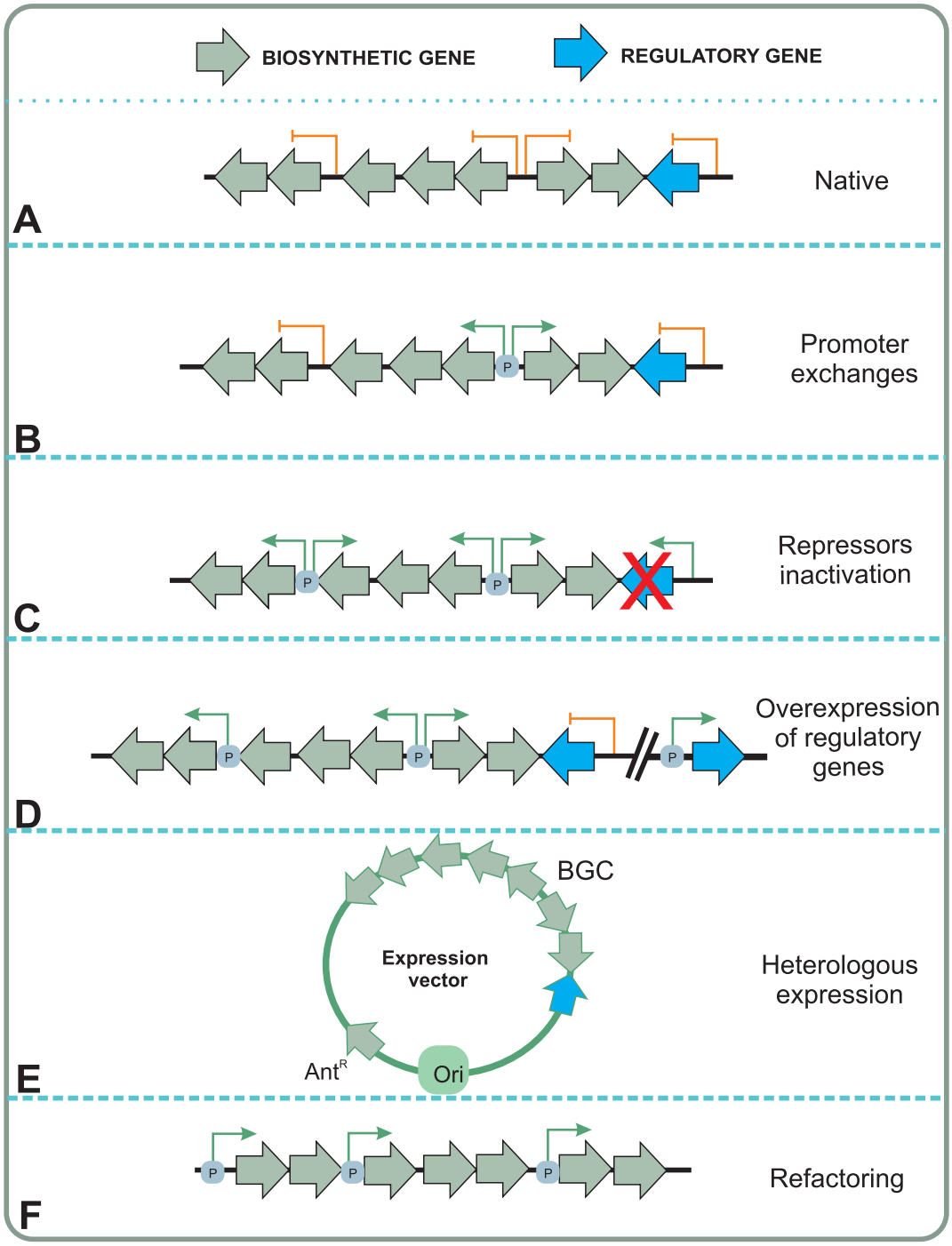

Fig. 5. Gene cluster specific approaches for activating silent biosynthetic pathways. A) The target gene cluster is silent under laboratory conditions and no transcription or product formation can be observed. B) The gene cluster may be activated by exchanging the promoters from the silent gene cluster to constitutively active promoters. C) In instances where the gene cluster is controlled by a repressor, inactivation of the regulatory element may lead to expression of the biosynthetic genes. D) Overexpression of cluster situated activator regulatory genes may also lead to activation of biosynthesis. E) Cloning of the whole biosynthetic gene cluster and heterologous expression in a surrogate host can be used for artificial activation of the BGC. Abbreviations: Ant^R, Antibiotic selection marker; BGC, Biosynthetic gene cluster; Ori, Origin of replication; P, Promoters. F) Synthetic approaches include refactoring of the gene cluster and the use of constitutive or strong promoters to drive expression of the silent gene cluster.

of the repressor or over-expression of the activator genes (Fig. 5C and D). The caveat is that for many families of transcriptional regulators it is not possible to identify whether the gene is a repressor or an activator based on sequence information alone. The diversity of microbial regulatory proteins is expansive [149], and only two systems are described here to demonstrate the functional differences.

The TetR family repressors consist of a helix-turn-helix DNA binding domain and a receptor domain that responds to the presence of a small molecule ligand [150]. In the absence of the specific signaling molecule, TetR dimers bind to DNA and prevent transcription of downstream genes. Binding of the ligand induces a conformational shift that repositions the helix-turn-helix motifs of the dimer far apart in such a way that the proteins lose their ability to bind DNA [151]. Interestingly, some TetR proteins have been proven to respond to γ-butyrolactone

autoinducers during the onset of secondary metabolism [152], while others bind directly to both endogenous and exogenous SMs [107,153]. Inactivation of TetR family repressors have led to the activation of jadomycin [153,154], kinamycin [155], auricin [156] and ceoelimycin biosynthesis [157,158].

Another predominant class of regulators is homologous to LuxR from *Vibrio fischeri*, which is a transcription factor involved in quorum sensing [159]. The protein consists of an α/β/α sandwich domain that binds the activator ligand and a DNA binding response domain. In contrast to the TerR family, most characterized LuxR proteins are transcriptional activators, where ligand binding induces homodimerization, which enables binding of the protein to DNA in so-called *tra* box sequences and initiate transcription [160]. An important subfamily of LuxR regulators in Actinobacteria are LAL–proteins (Large ATP-binding regulators of the LuxR family) [161], originally discovered in the regulon for maltose utilization, that contains an additional ATP-binding domain at the N-terminus responsible for the interaction with the inducers, maltotriose and ATP [162]. In a recent example, LuxR-type activators were over-expressed and TetR-type repressors were inactivated to initiate production of totopotensamides [163].

4.2.3. Heterologous expression in surrogate hosts

Many microbes that have the capability to produce natural products, such as strains of Cyanobacteria and filamentous fungi, are genetically intractable [164]. Heterologous expression provides an opportunity to access the genomic potential these organisms harbor in a genetically compliant host. The capture of target BGCs was classically done through screening of cosmid gene libraries, which was both time-consuming and could not accurately clone the desired DNA fragment, but advances in direct cloning strategies and DNA assembly tools have provided attractive alternatives. These methodologies are described in more detail in Section 4.2.4., since changing the expression host may require manipulation of control elements such as promoter sequences to ensure sufficient transcription levels (Fig. 5E).

The choice of expression host is far from trivial and many factors need to be considered. The genetically malleable *Escherichia coli* and *Saccharomyces cerevisiae* provide attractive hosts for production of bacterial and fungal SMs, respectively, but since neither is naturally equipped for production of complex chemicals, issues with metabolic flux, codon usage, self-resistance and activation of biosynthetic acyl/peptidyl carrier proteins need to be resolved. *E. coli* seems to be well-suited for expression of pathways originating from Cyanobacteria that are challenging to modify genetically, with production of microcystin [165] and lyngbyatoxin reported [166]. Several fungal terpene synthases have also been expressed in *E. coli*, but multi-gene clusters are much more challenging due to the requirement to remove introns and modify promoter regions. *E. coli* has had less success with BGCs originating from high-GC Actinobacteria, even though the entire erythromycin pathways has been expressed in this host [167–169]. The titres of SMs produced in *E. coli* have been generally low. Other promising heterologous hosts for production of prokaryotic SMs include *Myxococcus xanthus*, *Pseudomonas putida* [170] and *Bacillus subtilis* [171].

In many instances, it would appear that hosts that are evolutionarily closely linked to the organism harbouring the target pathway may be best suited for heterologous expression. Several well-characterized *Streptomyces* sp. are easily amenable and are efficient surrogate hosts for the heterologous characterization of different genes from Actinobacteria. In particular, *S. albus* J1074 [172], *S. lividans* TK24 [173], *S. venezuelae* ATCC10712 and *S. coelicolor* A3(2) have been widely used for the expression of cryptic pathways. *S. coelicolor* is genetically the most studied actinomycete and a large array of tools are available for manipulation of the organism [174], whereas the closely related *S. lividans* TK24 has particularly excelled at expression of proteins [175]. *S. venezuelae*, is well-suited for the heterologous expression of the PKs and NRPS due to the presence of large number of innate PPTase genes [176]. Finally, cloning genes in *S. albus* is facilitated by a defective restriction modification system and the naturally minimized genome may help increasing product titres [177]. In filamentous fungi, *Aspergillus* species have been widely used as heterologous hosts. An early example includes the transfer of the entire penicillin gene cluster to *A. nidulans*, while more recently geodin has been heterologously produced in this host [178–180]. *A. oryzae* has gained interest for heterologous expression, as it has traditionally been used in the food industry and has been given the status of GRAS (generally regarded as safe) organism. Selected recent examples of SMs produced in this host include paxilline [181], alfatrem [182] and pleuromutilin [183]. The heterologous expression of *A. terreus* asperfuranone cluster in the model ascomycete, *A. nidulans*, resulted in awakening of the asperfuranone biosynthetic pathway when several non-reducing polyketide synthase genes were placed under the control of *alcA*p [184].

An ideal host for heterologous expression would provide sufficient metabolic building blocks for the synthesis of SMs, but would not have native pathways that could interfere with the biosynthesis or generate background. To this end, several strains have been genome minimized to function better as expression chasses. Four gene clusters have been deleted from the genomes of *S. coelicolor* M1152 and M1154, which have been further improved for metabolite production by ribosome engineering [185]. Extensive manipulation, including deletion of a 1.5 Mb subtelomeric region and 78% of putative transposase genes of wild type *S. avermitilis* led to high success rates and product yields in the expression of exogenous biosynthetic pathways [186]. These ongoing efforts are likely to result in a handful of “superhosts” for the heterologous expression of diverse metabolic pathways.

4.2.4. Refactoring of gene clusters

The emergence of new metabolic engineering methods and the continually decreasing costs of DNA synthesis have enabled complete refactoring of entire BGCs, which typically includes three distinct stages. The first step involves the architectural design of the synthetic gene cluster from biological part libraries to obtain desired promoter, gene and terminator sequences, which may be ordered synthetically or amplified using PCR. In the next step, the pathway is assembled together with advanced cloning methods such as recombineering or TAR-cloning typically in *E. coli* or *Sacc. cerevisiae*. TAR cloning takes advantage of the high efficiency natural homologous recombination in yeast that allows for both direct capture of BGCs and simultaneous cloning of multiple DNA fragments [187]. In contrast, recombineering utilizes recombination systems from phages to enable homologous recombination to occur in *E. coli* [188]. In addition, the combination of long-amplicon PCR and DNA recombination (DiPaC) has recently been utilized for the direct cloning of the anabaenopeptins and erythromycin pathways [189]. The ultimate step involves the transfer of the artificial BGC to the expression host selected for the production of SMs (Fig. 5F).

Reports on fully synthetic refactoring of BGCs have still been scarce due to the significant costs associated with the method. Even though this approach would allow for unprecedented freedom in pathway design, the complexity of metabolic pathways required for the production of even simple SMs makes the task of designing functional BGC *in silico* far from trivial. For these reasons, the methodology appears to be best suited for improvements in the production titres of known value-added metabolites and not for the activation of unknown pathways. Examples of fully synthetic refactored gene clusters include rebeccamycin and pyrrolnitrin [190].

A more widely used approach in refactoring has been to modify regulatory elements or promoter regions of natural gene clusters prior to heterologous expression. Expression of the unmodified taromycin gene cluster directly in *S. coelicolor* M1146 did not lead to the production of the lipopeptide, but activation of the pathway was achieved by deletion of pathway –specific repressors using *in vivo* yeast recombination-mediated PCR targeting [191]. Conversely, refactoring of a strong constitutive promoter in front of a cluster situated activator

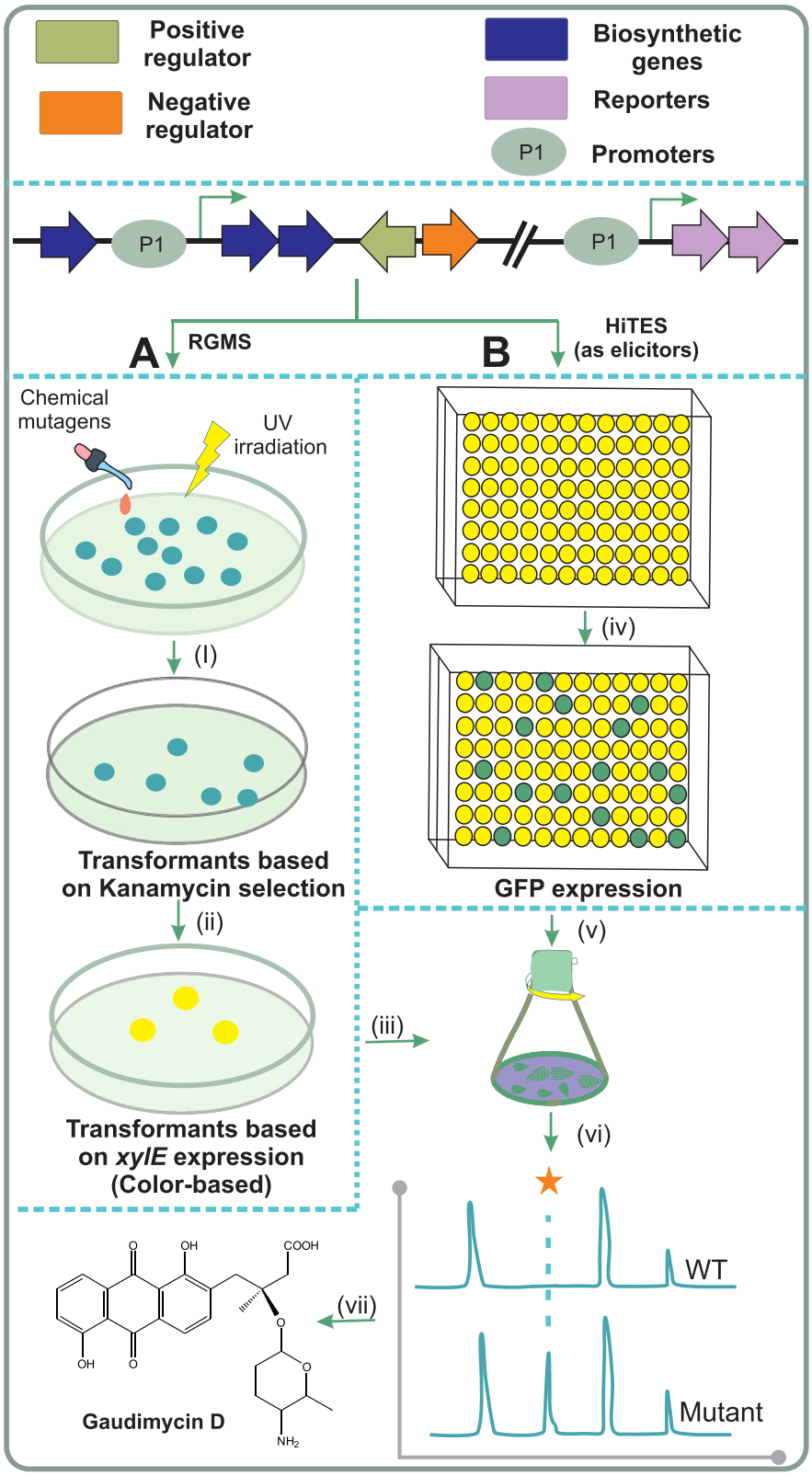

Fig. 6. Targeted genome-wide approaches for the activation of the silent gene clusters. A) In Reporter Guided Mutant selection, promoters identified from the target pathway are utilized to report increased transcription from the BGC. The selected BGC is activated (i) either by random chemical mutagens or UV irradiation to generate mutants where the pathway has been upregulated. (ii) The double-reporter system (*xylE-neo*) allows for selection of mutants in the presence of kanamycin and screening of the mutant library for highest expression levels of *xylE*. Abbreviation: RGMS, Reporter-guided mutant selection. B) In high-throughput elicitor screening, activation of the gene clusters is achieved by finding a small-molecular weight compound that triggers expression. (iv) Elicitors from a chemical library are added to individual wells for performing the elicitation response, which is (v) followed by monitoring transcription levels from a promoter probe construct expressing GFP. (vi) Metabolic profiling can be used to detect novel SMs from cultivations of wild type and biosynthesis activated strains, followed by (vii) structural elucidation of the target metabolite. Abbreviations: GFP, Green fluorescence protein; HiTES, High-throughput elicitor screening; WT, wild-type strain.

enabled the heterologous production of pacidamycin D [190]. In a more arduous approach, individual genes or short operons from BGCs may also be amplified by PCR and placed in front of active promoters. After reassembly of full length pathways, polycyclic tetramate macrolactams [192,193] and spectinabilin [194] have been produced in heterologous hosts. The PCR –amplification step was circumvented in the refactoring of the novobiocin gene cluster, where a cosmid harbouring the BGC was modified using recombineering to facilitate the generation of plug-and-play expression modules [195]. Through a plug-and-play promoter insertion strategy, introduction of three novel constitutive promoters of *S. coelicolor*, upstream of *jadJ* in the cryptic jadomycin B gene cluster in *S. venezuelae*, led to cluster activation. Importantly, expression of *jadJ*

from these three promoters led to higher transcription levels than when the widely used *ermE*p* was utilized [196]. The benefit of strong constitutive promoters is that they may lead to increased transcription levels and product yields, as has been shown in the engineering of polycyclic tetramate macrolactams [193].

4.3. Targeted genome-wide methodologies

To date, only few approaches have been developed that are able to focus on the desired cryptic BGC detected by genome mining, which are able to utilize pleiotropic methods to widely probe conditions that lead to activation of the pathway. These targeted genome-wide methodologies (Fig. 6) typically take advantage of a biosensor to report increased transcription or other activity from the desired BGC, but differ in the ways of generating alterations to the global transcriptome.

4.3.1. Reporter guided systems

A pioneering Reporter-Guided Mutant Selection (RGMS) method developed in the group of Prof. Keqian Yang provides a widely applicable approach for targeted activation of silent gene clusters in the native organism [197]. RGMS combines two effective yet simple techniques: cloning of key promoter sequences from the gene cluster of interest in front of a double *xylE-neo* reporter system and the use of genome-wide mutagenesis to generate a library of mutants that contain tremendous genomic diversity. The core rationale relies on randomly modulating the complex regulatory cascades that are controlling secondary metabolism and converting expression of the target pathway into a signal that enables selection of a mutant with the desired phenotype. The reporter cassette allows initial selection by kanamycin resistance (*neo*), followed by colour-based detection of catechol oxidase (*xylE*) activity, which allows for distinction between spontaneous kanamycin-resistant and genuine target-activated mutants (Fig. 6A).

RGMS was initially developed for industrial strain improvement purposes by taking advantage of the correlation between increased transcription levels from the target BGC and product yields. The method has been used to improve production of lovastatin [198], clavulanic acid [199] and natamycin [200] in *Aspergillus terreus*, *S. clavuligerus* and *S. gilvosporeus*, respectively. In addition, the methodology has also been successfully carried out in combination with ribosome engineering, through exposure to streptomycin, to achieve over-production of daptomycin in *S. roseosporus* NRRL11379 [201]. Recently, BGC situated TetR –family transcriptional repressors have been developed as alternative biosensors to aid screening of mutant libraries. These reporter proteins bind end products of the pathways and are therefore able to report increased production of the target metabolites directly, which reduces the number of false positives encountered with reporters that only monitor transcription levels. The method was utilized for increasing the production of panamycins in *S. alboniger* [202].

Many of the cryptic gene clusters denoted “silent” may, in effect, be transcribed at a low basal level, but in such low quantities that the corresponding metabolite remains undetected. Therefore RGMS is also suitable for the activation of BGCs and has been successfully utilized on the jadomycin and gaudimycin pathways in *S. venezuelae* ISP5230 and *Streptomyces* sp. PGA64, respectively [197]. The jadomycin BGC is silent under typical culture conditions unless special environmental cues such as heat-shock are applied [203]. To establish proof of concept, jadomycin biosynthesis could be re-activated by using RGMS strategy, whereas work on the cryptic gaudimycin pathway led to the isolation of two novel anthraquinone aminoglycosides [197]. The indigoidine synthetase gene *bpsA*, which is responsible for synthesis of a blue pigment ‘indigoidine’, has also been utilized as a reporter in the activation of the cryptic coelimycin gene cluster in *S. lividans*, where the activation was achieved through over-expression of a pathway-specific regulatory gene [204].

4.3.2. High-throughput elicitor screening

Many SMs are produced in response to various chemical signals, which may either be communication molecules or chemical weapons made by other organisms cohabiting the same environment. If the environmental signal can be identified in the laboratory using small molecule libraries, expression of silent BGCs can be achieved (Fig. 6B). The chemogenetic HiTES (high-throughput elicitor screening) method does this by coupling the screening to a promoter-probe system, where either lacZ [205] or eGFP [206] is utilized to report increased transcription from the target BGC. The approach has been successfully applied to two gene clusters in the Gram-negative pathogenic model bacterium *Burkholderia thailandensis* E264, where a silent virulence factor, namely malleilactone (*mal*) and a poorly expressed burkholdac (*bhc*) cluster were targeted [205]. The strategy has also been implemented in *S. albus* J1074, where the nonribosomal peptide surugamide (*sur*) cluster was awakened [207]. Interestingly, the cytotoxins etoposide and ivermectin were found to be effective inducers resulting the occurrence of 14 novel compounds in the culture media.

5. Concluding thoughts and future perspectives

The abundance of silent and cryptic pathways in microbial genomes provides an exciting opportunity for the discovery of new chemical entities with high therapeutic potential. The diverse strategies developed for awakening these pathways offer an excellent starting point, but to date no single superior methodology has been developed. This is not unexpected, since the BGCs reside in vastly diverse micro-organisms and in many cases are strictly regulated to be activated only under highly specific conditions. The availability of the numerous methods described above, which all have their strengths and weaknesses, can therefore be considered as a significant advantage and it will remain the responsibility of the researcher to choose the most appropriate one for any given project.

Acknowledgements

The authors would like to dedicate this article to the memory of Prof. Keqian Yang, who made significant contributions to our understanding of secondary metabolic pathways in *Streptomyces*. The authors would like to thank the Jane and Aatos Erkko Foundation for their financial support.

References

[1] Singh SB. Confronting the challenges of discovery of novel antibacterial agents. Bioorg Med Chem Lett 2014;24:3683–9. https://doi.org/10.1016/j.bmcl.2014.06.053.
[2] Awan AR, Shaw WM, Ellis T. Biosynthesis of therapeutic natural products using synthetic biology. Adv Drug Deliv Rev 2016;105:96–106. https://doi.org/10.1016/j.addr.2016.04.010.
[3] Rutledge PJ, Challis GL. Discovery of microbial natural products by activation of silent biosynthetic gene clusters. Nat Rev Microbiol 2015;13:509–23. https://doi.org/10.1038/nrmicro3496.
[4] Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007;70:461–77. https://doi.org/10.1021/np068054v.
[5] Challinor VL, Bode HB. Bioactive natural products from novel microbial sources. Ann N Y Acad Sci 2015;1354:82–97. https://doi.org/10.1111/nyas.12954.
[6] Schäberle TF, Lohr F, Schmitz A, König GM. Antibiotics from myxobacteria. Nat Prod Rep 2014;31:953. https://doi.org/10.1039/c4np00011k.
[7] Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus *Streptomyces*? Arch Microbiol 2001;176:386–90. https://doi.org/10.1007/s002030100345.
[8] Bérdy J. Bioactive microbial metabolites. J Antibiot (Tokyo) 2005;58:1–26. https://doi.org/10.1038/ja.2005.1.
[9] Hatanaka H, Kino T, Miyata S, Inamura N, Kuroda A, Goto T, et al. FR-900520 and FR-900523, novel immunosuppressants isolated from a *Streptomyces*. II. Fermentaion, isolation and physio-chemical and biological characteristics. J Antibiot (Tokyo) 1988;41:1592–601. https://doi.org/10.7164/antibiotics.41.1592.
[10] Hu J, Wunderlich D, Thiericke R, Dahse H-M, Grabley S, Feng X-Z, et al. Jenamidines A to C: unusual alkaloids from *Streptomyces* sp. with specific antiproliferative properties obtained by chemical screening. J Antibiot (Tokyo)

2003;56:747–54. https://doi.org/10.7164/antibiotics.56.747.
[11] Tapiolas DM, Roman M, Fenical W, Stout TJ, Clardy J. Octalactins A and B: cytotoxic eight-membered-ring lactones from a marine bacterium, *Streptomyces* sp. J Am Chem Soc 1991;113:4682–3. https://doi.org/10.1021/ja00012a048.
[12] Patel RN. Biocatalytic synthesis of chiral intermediates for antiviral and antihypertensive drugs. J Am Oil Chem Soc 1999;76:1275–81. https://doi.org/10.1007/s11746-999-0139-7.
[13] Maullu C, Lampis G, Deidda D, Petruzzelli S, Pompei R. A rapid method for screening large numbers of environmental microorganisms for antiviral activity. Appl Environ Microbiol 1998;64:1161–2.
[14] Tong J, Trapido-Rosenthal H, Wang J, Wang Y, Li QX, Lu Y. Antiviral activities and putative identification of compounds in microbial extracts from the Hawaiian coastal waters. Mar Drugs 2012;10:521–38. https://doi.org/10.3390/md10030521.
[15] Imada C. Enzyme inhibitors and other bioactive compounds from marine actinomycetes. Antonie Leeuwenhoek 2005;87:59–63. https://doi.org/10.1007/s10482-004-6544-x.
[16] Newman DJ. Developing natural product drugs: supply problems and how they have been overcome. Pharmacol Ther 2016;162:1–9. https://doi.org/10.1016/j.pharmthera.2015.12.002.
[17] Lewis K. Platforms for antibiotic discovery. Nat Rev Drug Discov 2013;12:371–87. https://doi.org/10.1038/nrd3975.
[18] Anderson AC. The process of structure-based drug design. Chem Biol 2003;10:787–97. https://doi.org/10.1016/j.chembiol.2003.09.002.
[19] Antel J. Integration of combinatorial chemistry and structure-based drug design. Curr Opin Drug Discov Dev 1999;2:224–33.
[20] Piddock LJV. The crisis of no new antibiotics-what is the way forward? Lancet Infect Dis 2012;12:249–53. https://doi.org/10.1016/S1473-3099(11)70316-4.
[21] Wright GD. Something old, something new: revisiting natural products in antibiotic drug discovery. Can J Microbiol 2014;60:147–54. https://doi.org/10.1139/cjm-2014-0063.
[22] Georgiev MI. Natural products utilization. Phytochem Rev 2014;13:339–41. https://doi.org/10.1007/s11101-014-9363-3.
[23] Zhang Z, Pan H-X, Tang G-L. New insights into bacterial type II polyketide biosynthesis. F1000Research 2017;6:172. https://doi.org/10.12688/f1000research.10466.1.
[24] Robbins T, Liu YC, Cane DE, Khosla C. Structure and mechanism of assembly line polyketide synthases. Curr Opin Struct Biol 2016;41:10–8. https://doi.org/10.1016/j.sbi.2016.05.009.
[25] Payne JAE, Schoppet M, Hansen MH, Cryle MJ. Diversity of nature's assembly lines-recent discoveries in non-ribosomal peptide synthesis. Mol Biosyst 2017;13:9–22. https://doi.org/10.1039/c6mb00675b.
[26] Hudson GA, Mitchell DA. RiPP antibiotics: biosynthesis and engineering potential. Curr Opin Microbiol 2018;45:61–9. https://doi.org/10.1016/j.mib.2018.02.010.
[27] Bentley SD, Chater KF, Cerdeño-Tárraga A-M, Challis GL, Thomson NR, James KD, et al. Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 2002;417:141–7. https://doi.org/10.1038/417141a.
[28] Machida M, Asai K, Sano M, Tanaka T, Kumagai T, Terai G, et al. Genome sequencing and analysis of *Aspergillus oryzae*. Nature 2005;438:1157–61. https://doi.org/10.1038/nature04300.
[29] Omura S, Ikeda H, Ishikawa J, Hanamoto A, Takahashi C, Shinose M, et al. Genome sequence of an industrial microorganism Streptomyces avermitilis: deducing the ability of producing secondary metabolites. Proc Natl Acad Sci 2001;98:12215–20. https://doi.org/10.1073/pnas.211433198.
[30] Yaegashi J, Oakley BR, Wang CCC. Recent advances in genome mining of secondary metabolite biosynthetic gene clusters and the development of heterologous expression systems in *Aspergillus nidulans*. J Ind Microbiol Biotechnol 2014;41:433–42. https://doi.org/10.1007/s10295-013-1386-z.
[31] Pidot SJ, Coyne S, Kloss F, Hertweck C. Antibiotics from neglected bacterial sources. Int J Med Microbiol 2014;304:14–22. https://doi.org/10.1016/j.ijmm.2013.08.011.
[32] Gross H, Loper JE. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Prod Rep 2009;26:1408–46. https://doi.org/10.1039/b817075b.
[33] Mousa WK, Athar B, Merwin NJ, Magarvey NA. Antibiotics and specialized metabolites from the human microbiota. Nat Prod Rep 2017;34:1302–31. https://doi.org/10.1039/c7np00021a.
[34] Milshteyn A, Schneider JS, Brady SF. Mining the metabiome: identifying novel natural products from microbial communities. Chem Biol 2014;21. https://doi.org/10.1016/j.chembiol.2014.08.006.
[35] Burkhart BJ, Hudson GA, Dunbar KL, Mitchell DA. A prevalent peptide-binding domain guides ribosomal natural product biosynthesis. Nat Chem Biol 2015;11:564–70. https://doi.org/10.1038/nchembio.1856.
[36] Sardar D, Lin Z, Schmidt EW. Modularity of RiPP enzymes enables designed synthesis of decorated peptides. Chem Biol 2015;22:907–16. https://doi.org/10.1016/j.chembiol.2015.06.014.
[37] Piel J, Hui D, Wen G, Butzke D, Platzer M, Fusetani N, et al. Antitumor polyketide biosynthesis by an uncultivated bacterial symbiont of the marine sponge *Theonella swinhoei*. Proc Natl Acad Sci U S A 2004;101:16222–7. https://doi.org/10.1073/pnas.0405976101.
[38] Hover BM, Kim SH, Katz M, Charlop-Powers Z, Owen JG, Ternei MA, et al. Culture-independent discovery of the malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens. Nat Microbiol 2018;3:415–22. https://doi.org/10.1038/s41564-018-0110-1.
[39] Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, et al. A new antibiotic kills pathogens without detectable resistance. Nature 2015;517:455–9. https://doi.org/10.1038/nature14098.
[40] Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, et al. antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 2015;43:W237–43. https://doi.org/10.1093/nar/gkv437.
[41] Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, et al. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 2011;39:W339–46. https://doi.org/10.1093/nar/gkr466.
[42] Khaldi N, Seifuddin FT, Turner G, Haft D, Nierman WC, Wolfe KH, et al. SMURF: genomic mapping of fungal secondary metabolite clusters. Fungal Genet Biol 2010;47:736–41. https://doi.org/10.1016/j.fgb.2010.06.003.
[43] van Heel AJ, de Jong A, Montalbán-López M, Kok J, Kuipers OP. BAGEL3: automated identification of genes encoding bacteriocins and (non-) bactericidal post-translationally modified peptides. Nucleic Acids Res 2013;41:448–53. https://doi.org/10.1093/nar/gkt391.
[44] Skinnider MA, Johnston CW, Edgar RE, Dejong CA, Merwin NJ, Rees PN, et al. Genomic charting of ribosomally synthesized natural product chemical space facilitates targeted mining. Proc Natl Acad Sci U S A 2016;113:E6343–51. https://doi.org/10.1073/pnas.1609014113.
[45] Skinnider MA, Dejong CA, Rees PN, Johnston CW, Li H, Webster ALH, et al. Genomes to natural products PRediction informatics for secondary metabolomes (PRISM). Nucleic Acids Res 2015;43:9645–62. https://doi.org/10.1093/nar/gkv1012.
[46] Medema MH, Kottmann R, Yilmaz P, Cummings M, Biggins JB, Blin K, et al. Minimum information about a biosynthetic gene cluster. Nat Chem Biol 2015;11:625–31. https://doi.org/10.1038/nchembio.1890.
[47] Bibb MJ. Regulation of secondary metabolism in streptomycetes. Curr Opin Microbiol 2005;8:208–15. https://doi.org/10.1016/j.mib.2005.02.016.
[48] Cimermancic P, Medema MH, Claesen J, Kurita K, Wieland Brown LC, Mavrommatis K, et al. Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. Cell 2014;158:412–21. https://doi.org/10.1016/j.cell.2014.06.034.
[49] Wisecaver JH, Slot JC, Rokas A. The evolution of fungal metabolic pathways. PLoS Genet 2014;10. https://doi.org/10.1371/journal.pgen.1004816.
[50] Siitonen V, Claesson M, Patrikainen P, Aromaa M, Mäntsälä P, Schneider G, et al. Identification of late-stage glycosylation steps in the biosynthetic pathway of the anthracycline nogalamycin. Chembiochem 2012;13:120–8. https://doi.org/10.1002/cbic.201100637.
[51] Kelly WL, Pan L, Li C. Thiostrepton biosynthesis: prototype for a new family of bacteriocins. J Am Chem Soc 2009;131:4327–34. https://doi.org/10.1021/ja807890a.
[52] Miao V, Coëffet-LeGal MF, Brian P, Brost R, Penn J, Whiting A, et al. Daptomycin biosynthesis in *Streptomyces roseosporus*: cloning and analysis of the gene cluster and revision of peptide stereochemistry. Microbiology 2005;151:1507–23. https://doi.org/10.1099/mic.0.27757-0.
[53] Mast Y, Weber T, Gölz M, Ort-Winklbauer R, Gondran A, Wohlleben W, et al. Characterization of the "pristinamycin supercluster" of *Streptomyces pristinaespiralis*. Microb Biotechnol 2011;4:192–206. https://doi.org/10.1111/j.1751-7915.2010.00213.x.
[54] Nieselt K, Battke F, Herbig A, Bruheim P, Wentzel A, Jakobsen ØM, et al. The dynamic architecture of the metabolic switch in *Streptomyces coelicolor*. BMC Genom 2010;11:1–9. https://doi.org/10.1186/1471-2164-11-10.
[55] van der Heul HU, Bilyk BL, McDowall KJ, Seipke RF, van Wezel GP. Regulation of antibiotic production in Actinobacteria: new perspectives from the post-genomic era. Nat Prod Rep 2018;00:1–30. https://doi.org/10.1039/C8NP00012C.
[56] Rigali S, Titgemeyer F, Barends S, Mulder S, Thomae AW, Hopwood DA, et al. Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by Streptomyces. EMBO Rep 2008;9:670–5. https://doi.org/10.1038/embor.2008.83.
[57] Martín JF. Phosphate control of the biosynthesis of antibiotics and other secondary metabolites is mediated by the PhoR-PhoP system: an unfinished story. J Bacteriol 2004;186:5197–201. https://doi.org/10.1128/JB.186.16.5197-5201.2004.
[58] Martín JF, Liras P. Engineering of regulatory cascades and networks controlling antibiotic biosynthesis in *Streptomyces*. Curr Opin Microbiol 2010;13:263–73. https://doi.org/10.1016/j.mib.2010.02.008.
[59] Martín JF, Casqueiro J, Liras P. Secretion systems for secondary metabolites: how producer cells send out messages of intercellular communication. Curr Opin Microbiol 2005;8:282–93. https://doi.org/10.1016/j.mib.2005.04.009.
[60] Ikeno S, Yamane Y, Ohishi Y, Kinoshita N, Hamada M, Tsuchiya KS, et al. ABC transporter genes, *kasKLM*, responsible for self-resistance of a kasugamycin producer strain. J Antibiot (Tokyo) 2000;53:373–84. https://doi.org/10.7164/antibiotics.53.373.
[61] Chen J, Liu M, Liu X, Miao J, Fu C, Gao H, et al. Interrogation of *Streptomyces avermitilis* for efficient production of avermectins. Synth Syst Biotechnol 2016;1:7–16. https://doi.org/10.1016/j.synbio.2016.03.002.
[62] Ochi K. Metabolic initiation of differentiation and secondary metabolism by Streptomyces griseus: significance of the stringent response (ppGpp) and GTP content in relation to A factor. J Bacteriol 1987;169:3608–16. https://doi.org/10.1128/jb.169.8.3608-3616.1987.
[63] Ochi K. From microbial differentiation to ribosome engineering. Biosci Biotechnol Biochem 2007;71:1373–86. https://doi.org/10.1271/bbb.70007.
[64] Artsimovitch I, Patlan V, Sekine S-I, Vassylyeva MN, Hosaka T, Ochi K, et al. Structural basis for transcription regulation by alarmone ppGpp. Cell 2004;117:299–310. https://doi.org/10.1016/S0092-8674(04)00401-5.
[65] Ross W, Vrentas CE, Sanchez-Vazquez P, Gaal T, Gourse RL. The magic spot: a ppGpp binding site on *E. coli* RNA polymerase responsible for regulation of transcription initiation. Mol Cell 2013;50:420–9. https://doi.org/10.1016/j.molcel.

2013.03.021.
[66] Liu K, Bittner AN, Wang JD. Diversity in (p)ppGpp metabolism and effectors. Curr Opin Microbiol 2015;24:72–9. https://doi.org/10.1016/j.mib.2015.01.012.
[67] Ochi K. Cryptic pathways and implications for novel drug discovery. Microb. Resour., Elsevier; 2017. p. 189–203. https://doi.org/10.1016/B978-0-12-804765-1.00009-6.
[68] Martinez-Costa OH, Arias P, Romero NM, Parro V, Mellado RP, Malpartida F. A *relA/spoT* homologous gene from *Streptomyces coelicolor* A3(2) controls antibiotic biosynthetic genes. J Biochem Chem 1996;271:10627–34. https://doi.org/10.1074/jbc.271.18.10627 May 3,1996.
[69] Chakraburtty R, Bibb M. The ppGpp synthetase gene (*relA*) of *Streptomyces coelicolor* A3(2) plays a conditional role in antibiotic production and morphological differentiation. J Bacteriol 1997;179:5854–61. https://doi.org/10.1128/jb.179.18.5854-5861.1997.
[70] Gomez-Escribano JP, Liras P, Pisabarro A, Martín JF. An *rplK*$^{\Delta 29\text{-}PALG\text{-}32}$ mutation leads to reduced expression of the regulatory genes *ccaR* and *claR* and very low transcription of the *ceaS2* gene for clavulanic acid biosynthesis in *Streptomyces clavuligerus*. Mol Microbiol 2006;61:758–70. https://doi.org/10.1111/j.1365-2958.2006.05266.x.
[71] Lai C, Xu J, Tozawa Y, Okamoto-Hosoya Y, Yao X, Ochi K. Genetic and physiological characterization of *rpoB* mutations that activate antibiotic production in *Streptomyces lividans*. Microbiology 2002;148:3365–73. https://doi.org/10.1099/00221287-148-11-3365.
[72] Xu J, Tozawa Y, Lai C, Hayashi H, Ochi K. A rifampicin resistance mutation in the *rpoB* gene confers ppGpp-independent antibiotic production in *Streptomyces coelicolor* A3(2). Mol Genet Genom 2002;268:179–89. https://doi.org/10.1007/s00438-002-0730-1.
[73] Tanaka Y, Kasahara K, Hirose Y, Murakami K, Kugimiya R, Ochi K. Activation and products of the cryptic secondary metabolite biosynthetic gene clusters by rifampin resistance (*rpoB*) mutations in actinomycetes. J Bacteriol 2013;195:2959–70. https://doi.org/10.1128/JB.00147-13.
[74] Shima J, Hesketh A, Okamoto S, Kawamoto S, Ochi K. Induction of actinorhodin production by *rpsL* (encoding ribosomal protein S12) mutations that confer streptomycin resistance in *Streptomyces lividans* and *Streptomyces coelicolor* A3(2). J Bacteriol 1996;178:7276–84. https://doi.org/10.1128/JB.178.24.7276-7284.1996.
[75] Ochi K, Zhang D, Kawamoto S, Hesketh A. Molecular and functional analysis of the ribosomal L11 and S12 protein genes (*rplK* and *rpsL*) of *Streptomyces coelicolor* A3(2). Mol Gen Genet 1997;256:488–98. https://doi.org/10.1007/s004380050593.
[76] Wang G, Hosaka T, Ochi K. Dramatic activation of antibiotic production in Streptomyces coelicolor by cumulative drug resistance mutations. Appl Environ Microbiol 2008;74:2834–40. https://doi.org/10.1128/AEM.02800-07.
[77] Hosoya Y, Okamoto S, Muramatsu H, Ochi K. Acquisition of certain streptomycin-resistant (*str*) mutations enhances antibiotic production in bacteria. Antimicrob Agents Chemother 1998;42:2041–7.
[78] Tanaka Y, Hosaka T, Ochi K. Rare earth elements activate the secondary metabolite-biosynthetic gene clusters in *Streptomyces coelicolor* A3(2). J Antibiot (Tokyo) 2010;63:477–81. https://doi.org/10.1038/ja.2010.53.
[79] Hosaka T, Ohnishi-Kameyama M, Muramatsu H, Murakami K, Tsurumi Y, Kodani S, et al. Antibacterial discovery in actinomycetes strains with mutations in RNA polymerase or ribosomal protein S12. Nat Biotechnol 2009;27:462–4. https://doi.org/10.1038/nbt.1538.
[80] Whitehouse I, Rando OJ, Delrow J, Tsukiyama T. Chromatin remodelling at promoters suppresses antisense transcription. Nature 2007;450:1031–5. https://doi.org/10.1038/nature06391.
[81] Bednar J, Horowitz RA, Grigoryev SA, Carruthers LM, Hansen JC, Koster AJ, et al. Nucleosomes, linker DNA, and linker histone form a unique structural motif that directs the higher-order folding and compaction of chromatin. Proc Natl Acad Sci U S A 1998;95:14173–8. https://doi.org/10.1073/pnas.95.24.14173.
[82] Saha A, Wittmeyer J, Cairns BR. Chromatin remodelling: the industrial revolution of DNA around histones. Nat Rev Mol Cell Biol 2006;7:437–47. https://doi.org/10.1038/nrm1945.
[83] Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009;10:295–304. https://doi.org/10.1038/nrg2540.
[84] Jenuwein T, Allis C. Translating the histone code. Science 2001;293:1074–80. https://doi.org/10.1126/science.1063127.
[85] Marks PA, Richon VM, Breslow R, Rifkind RA. Histone deacetylase inhibitors as new cancer drugs. Curr Opin Oncol 2001;13:477–83.
[86] Yu J-H, Keller N. Regulation of secondary metabolism in filamentous fungi. Annu Rev Phytopathol 2005;43:437–58. https://doi.org/10.1146/annurev.phyto.43.040204.140214.
[87] Gacek A, Strauss J. The chromatin code of fungal secondary metabolite gene clusters. Appl Microbiol Biotechnol 2012;95:1389–404. https://doi.org/10.1007/s00253-012-4208-8.
[88] Shwab EK, Jin WB, Tribus M, Galehr J, Graessle S, Keller NP. Histone deacetylase activity regulates chemical diversity in *Aspergillus*. Eukaryot Cell 2007;6:1656–64. https://doi.org/10.1128/EC.00186-07.
[89] Bok JW, Chiang YM, Szewczyk E, Reyes-Domingez Y, Davidson AD, Sanchez JF, et al. Chromatin-level regulation of biosynthetic gene clusters. Nat Chem Biol 2009;5:462–4. https://doi.org/10.1038/nchembio.177.
[90] Williams RB, Henrikson JC, Hoover AR, Lee AE, Cichewicz RH. Epigenetic remodeling of the fungal secondary metabolome. Org Biomol Chem 2008;6:1895. https://doi.org/10.1039/b804701d.
[91] Reyes-Dominguez Y, Bok JW, Berger H, Shwab EK, Basheer A, Gallmetzer A, et al. Heterochromatic marks are associated with the repression of secondary metabolism clusters in *Aspergillus nidulans*. Mol Microbiol 2010;76:1376–86. https://doi.org/10.1111/j.1365-2958.2010.07051.x.
[92] Lombardi PM, Angell HD, Whittington DA, Flynn EF, Rajashankar KR, Christianson DW. Structure of prokaryotic polyamine deacetylase reveals evolutionary functional relationships with eukaryotic histone deacetylases. Biochemistry 2011;50:1808–17. https://doi.org/10.1021/bi101859k.
[93] Williams RM, Rimsky S. Molecular aspects of the E. coli nucleoid protein, H-NS: a central controller of gene regulatory networks. FEMS Microbiol Lett 1997;156:175–85. https://doi.org/10.1016/S0378-1097(97)00397-2.
[94] Moore JM, Bradshaw E, Seipke RF, Hutchings MI, McArthur M. Use and discovery of chemical elicitors that stimulate biosynthetic gene clusters in *Streptomyces* bacteria. Hopwood DA, editor. Methods enzymol, vol. 517. Elsevier Inc.; 2012. p. 367–85. https://doi.org/10.1016/B978-0-12-404634-4.00018-8.
[95] Aldridge M, Facey P, Francis L, Bayliss S, Del Sol R, Dyson P. A novel bifunctional histone protein in *Streptomyces*: a candidate for structural coupling between DNA conformation and transcription during development and stress? Nucleic Acids Res 2013;41:4813–24. https://doi.org/10.1093/nar/gkt180.
[96] Scherlach K, Hertweck C. Triggering cryptic natural product biosynthesis in microorganisms. Org Biomol Chem 2009;7:1753. https://doi.org/10.1039/b821578b.
[97] Bode HB, Bethe B, Höfs R, Zeeck A. Big effects from small changes: possible ways to explore nature's chemical diversity. Chembiochem 2002;3:619. https://doi.org/10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9.
[98] Reen FJ, Romano S, Dobson ADW, O'Gara F. The sound of silence: activating silent biosynthetic gene clusters in marine microorganisms. Mar Drugs 2015;13:4754–83. https://doi.org/10.3390/md13084754.
[99] Paranagama PA, Wijeratne EMK, Gunatilaka AAL. Uncovering biosynthetic potential of plant-associated fungi: effect of culture conditions on metabolite production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*. J Nat Prod 2007;70:1939–45. https://doi.org/10.1021/np070504b.
[100] Doull JL, Singh AK, Hoare M, Ayer SW. Conditions for the production of jadomycin B by *Streptomyces venezuelae* ISP5230: effects of heat shock, ethanol treatment and phage infection. J Ind Microbiol 1994;13:120–5. https://doi.org/10.1007/BF01584109.
[101] Liao Y, Wei ZH, Bai L, Deng Z, Zhong JJ. Effect of fermentation temperature on validamycin A production by *Streptomyces hygroscopicus* 5008. J Biotechnol 2009;142:271–4. https://doi.org/10.1016/j.jbiotec.2009.04.015.
[102] Hayes A, Hobbs G, Smith CP, Oliver SG, Butler PR. Environmental signals triggering methylenomycin production by *Streptomyces coelicolor* A3(2). J Bacteriol 1997;179:5511–5. https://doi.org/10.1007/s00203-014-1000-4.
[103] Rigali S, Titgemeyer F, Barends S, Mulder S, Thomae AW, Hopwood DA, et al. Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces*. EMBO Rep 2008;9:670–5. https://doi.org/10.1038/embor.2008.83.
[104] Craney A, Ozimok C, Pimentel-Elardo SM, Capretta A, Nodwell JR. Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism. Chem Biol 2012;19:1020–7. https://doi.org/10.1016/j.chembiol.2012.06.013.
[105] Onaka H, Tabata H, Igarashi Y, Sato Y, Furumai T. Goadsporin, a chemical substance which promotes secondary metabolism and morphogenesis in *Streptomycetes*. I. Purification and Characterization. J Antibiot (Tokyo) 2001;54:1036–44. https://doi.org/10.7164/antibiotics.54.1036.
[106] Onaka H. Novel antibiotic screening methods to awaken silent or cryptic secondary metabolic pathways in actinomycetes. J Antibiot (Tokyo) 2017:1–6. https://doi.org/10.1038/ja.2017.51.
[107] Wang W, Ji J, Li X, Wang J, Li S, Pan G, et al. Angucyclines as signals modulate the behaviors of *Streptomyces coelicolor*. Proc Natl Acad Sci U S A 2014;111:5688–93. https://doi.org/10.1073/pnas.1324253111.
[108] Amano SI, Sakurai T, Endo K, Takano H, Beppu T, Furihata K, et al. A cryptic antibiotic triggered by monensin. J Antibiot (Tokyo) 2011;64:703. https://doi.org/10.1038/ja.2011.69.
[109] Kawai K, Wang G, Okamoto S, Ochi K. The rare earth, scandium, causes antibiotic overproduction in *Streptomyces* spp. FEMS Microbiol Lett 2007;274:311–5. https://doi.org/10.1111/j.1574-6968.2007.00846.x.
[110] Davies J, Spiegelman GB, Yim G. The world of subinhibitory antibiotic concentrations. Curr Opin Microbiol 2006;9:445–53. https://doi.org/10.1016/j.mib.2006.08.006.
[111] Davies J. Are antibiotics naturally antibiotics? J Ind Microbiol Biotechnol 2006;33:496–9. https://doi.org/10.1007/s10295-006-0112-5.
[112] Wakefield J, Hassan HM, Jaspars M, Ebel R, Rateb ME. Dual induction of new microbial secondary metabolites by fungal bacterial co-cultivation. Front Microbiol 2017;8:1–10. https://doi.org/10.3389/fmicb.2017.01284.
[113] Oh DC, Kauffman CA, Jensen PR, Fenical W. Induced production of emericellamides A and B from the marine-derived fungus *Emericella* sp. in competing co-culture. J Nat Prod 2007;70:515–20. https://doi.org/10.1021/np060381f.
[114] Amano SI, Morota T, Kano YK, Narita H, Hashidzume T, Yamamoto S, et al. Promomycin, a polyether promoting antibiotic production in *Streptomyces* spp. J Antibiot (Tokyo) 2010;63:486–91. https://doi.org/10.1038/ja.2010.68.
[115] Schroeckh V, Scherlach K, Nutzmann H-W, Shelest E, Schmidt-Heck W, Schuemann J, et al. Intimate bacterial-fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*. Proc Natl Acad Sci U S A 2009;106:14558–63. https://doi.org/10.1073/pnas.0901870106.
[116] König CC, Scherlach K, Schroeckh V, Horn F, Nietzsche S, Brakhage AA, et al. Bacterium induces cryptic meroterpenoid pathway in the pathogenic fungus *Aspergillus fumigatus*. Chembiochem 2013;14:938–42. https://doi.org/10.1002/

cbic.201300070.
[117] Beld J, Sonnenschein EC, Vickery CR, Noel JP, Burkart MD. The phosphopantetheinyl transferases: catalysis of a post-translational modification crucial for life. Nat Prod Rep 2014;31:61–108. https://doi.org/10.1039/C3NP70054B.
[118] Bunet R, Riclea R, Laureti L, Hôtel L, Paris C, Girardet JM, et al. A single Sfp-type phosphopantetheinyl transferase plays a major role in the biosynthesis of PKS and NRPS derived metabolites in *Streptomyces ambofaciens* ATCC23877. PLoS One 2014;9:1–12. https://doi.org/10.1371/journal.pone.0087607.
[119] Gunnarsson N, Eliasson A, Nielsen J. Control of fluxes towards antibiotics and the role of primary metabolism in production of antibiotics. Adv Biochem Eng Biotechnol 2004;88:137–78. https://doi.org/10.1007/b99260.
[120] Zhang B, Tian W, Wang S, Yan X, Jia X, Pierens GK, et al. Activation of natural products biosynthetic pathways via a protein modification level regulation. ACS Chem Biol 2017;12:1732–6. https://doi.org/10.1021/acschembio.7b00225.
[121] Yan X, Zhang B, Tian W, Dai Q, Zheng X, Hu K, et al. Puromycin A, B and C, cryptic nucleosides identified from *Streptomyces alboniger* NRRL B-1832 by PPtase-based activation. Synth Syst Biotechnol 2018;3:76–80. https://doi.org/10.1016/j.synbio.2018.02.001.
[122] Tilburn J, Sarkar S, Widdick DA, Espeso EA, Orejas M, Mungroo J, et al. The *Aspergillus* PacC zinc finger transcription factor mediates regulation of both acid- and alkaline-expressed genes by ambient pH. EMBO J 1995;14:779–90. https://doi.org/10.1002/j.1460-2075.1995.tb07056.x.
[123] Brakhage AA. Regulation of fungal secondary metabolism. Nat Rev Microbiol 2013;11:21–32. https://doi.org/10.1038/nrmicro2916.
[124] Makitrynskyy R, Ostash B, Tsypik O, Rebets Y, Doud E, Meredith T, et al. Pleiotropic regulatory genes *bldA, adpA* and *absB* are implicated in production of phosphoglycolipid antibiotic moenomycin. Open Biol 2013;3:130121https://doi.org/10.1098/rsob.130121.
[125] Ohnishi Y, Yamazaki H, Kato J, Tomono A, Horinouchi S. *AdpA*, a central transcriptional regulator in the A-Factor regulatory cascade that leads to morphological development and secondary metabolism in *Streptomyces griseus*. Biosci Biotechnol Biochem 2005;69:431–9. https://doi.org/10.1271/bbb.69.431.
[126] Xu J, Zhang J, Zhuo J, Li Y, Tian Y, Tan H. Activation and mechanism of a cryptic oviedomycin gene cluster via the disruption of a global regulatory gene, *adpA*, in *Streptomyces ansochromogenes*. J Biol Chem 2017;292:19708–20. https://doi.org/10.1074/jbc.M117.809145.
[127] Becerril A, Álvarez S, Braña AF, Rico S, Díaz M, Santamaría RI, et al. Uncovering production of specialized metabolites by *Streptomyces argillaceus*: activation of cryptic biosynthesis gene clusters using nutritional and genetic approaches. PLoS One 2018;13:e0198145https://doi.org/10.1371/journal.pone.0198145.
[128] Fowler-Goldsworthy K, Gust B, Mouz S, Chandra G, Findlay KC, Chater KF. The actinobacteria-specific gene *wblA* controls major developmental transitions in *Streptomyces coelicolor* A3(2). Microbiology 2011;157:1312–28. https://doi.org/10.1099/mic.0.047555-0.
[129] Huang H, Hou L, Li H, Qiu Y, Ju J, Li W. Activation of a plasmid-situated type III PKS gene cluster by deletion of a *wbl* gene in deepsea-derived *Streptomyces somaliensis* SCSIO ZH66. Microb Cell Factories 2016;15:116. https://doi.org/10.1186/s12934-016-0515-6.
[130] Li H, Huang H, Hou L, Ju J, Li W. Discovery of antimycin-type depsipeptides from a *wbl* gene mutant strain of deepsea-derived *Streptomyces somaliensis* SCSIO ZH66 and their effects on pro-inflammatory cytokine production. Front Microbiol 2017;8:1–8. https://doi.org/10.3389/fmicb.2017.00678.
[131] Gruber TM, Gross CA. Multiple sigma subunits and the partitioning of bacterial transcription space. Annu Rev Microbiol 2003;57:441–66. https://doi.org/10.1146/annurev.micro.57.030502.090913.
[132] Paget MS, Helmann JD. The σ^{70} family of sigma factors. Genome Biol 2003;4:203. https://doi.org/10.1186/gb-2003-4-1-203.
[133] Jiang C, Wang H, Kang Q, Liu J, Bai L. Cloning and characterization of the polyether salinomycin biosynthesis gene cluster of *Streptomyces albus* XM211. Appl Environ Microbiol 2012;78:994–1003. https://doi.org/10.1128/AEM.06701-11.
[134] Shelest E. Transcription factors in fungi: TFome dynamics, three major families, and dual-specificity TFs. Front Genet 2017;8:53. https://doi.org/10.3389/fgene.2017.00053.
[135] Hu Y, Qin Y, Liu G. Collection and curation of transcriptional regulatory interactions in *Aspergillus nidulans* and *Neurospora crassa* reveal structural and evolutionary features of the regulatory networks. Front Microbiol 2018;9:1–8. https://doi.org/10.3389/fmicb.2018.00027.
[136] Jnawali HN, Liou K, Sohng JK. Role of σ-factor (orf21) in clavulanic acid production in *Streptomyces clavuligerus* NRRL3585. Microbiol Res 2011;166:369–79. https://doi.org/10.1016/j.micres.2010.07.005.
[137] Lee S, Yang SH, Kang C, Mo S, Suh J. Overexpression of the putative extracytoplasmic function sigma (σ) factor FujE enhances FK506 production in *Streptomyces* sp. strain KCCM 11116P. Can J Microbiol 2014;60:363–9. https://doi.org/10.1139/cjm-2014-0166.
[138] Taniguchi H, Henke NA, Heider SAE, Wendisch VF. Overexpression of the primary sigma factor gene *sigA* improved carotenoid production by *Corynebacterium glutamicum*: application to production of β-carotene and the non-native linear C50 carotenoid bisanhydrobacterioruberin. Metab Eng Commun 2017;4:1–11. https://doi.org/10.1016/j.meteno.2017.01.001.
[139] Stevens DC, Conway KR, Pearce N, Villegas-Peñaranda LR, Garza AG, Boddy CN. Alternative sigma factor over-expression enables heterologous expression of a Type II polyketide biosynthetic pathway in *Escherichia coli*. PLoS One 2013;8:e64858https://doi.org/10.1371/journal.pone.0064858.
[140] Jiang L, Liu Y, Wang P, Wen Y, Song Y, Chen Z, et al. Inactivation of the extracytoplasmic function sigma factor Sig6 stimulates avermectin production in *Streptomyces avermitilis*. Biotechnol Lett 2011;33:1955–61. https://doi.org/10.1007/s10529-011-0673-x.
[141] Zhuo Y, Zhang W, Chen D, Gao H, Tao J, Liu M, et al. Reverse biological engineering of *hrdB* to enhance the production of avermectins in an industrial strain of *Streptomyces avermitilis*. Proc Natl Acad Sci U S A 2010;107:11250–4. https://doi.org/10.1073/pnas.1006085107.
[142] Olano C, García I, González A, Rodriguez M, Rozas D, Rubio J, et al. Activation and identification of five clusters for secondary metabolites in *Streptomyces albus* J1074. Microb Biotechnol 2014;7. https://doi.org/10.1111/1751-7915.12116.
[143] Bai C, Zhang Y, Zhao X, Hu Y, Xiang S, Miao J, et al. Exploiting a precise design of universal synthetic modular regulatory elements to unlock the microbial natural products in *Streptomyces*. Proc Natl Acad Sci U S A 2015;112:12181–6. https://doi.org/10.1073/pnas.1511027112.
[144] Zhang MM, Wong FT, Wang Y, Luo S, Lim YH, Heng E, et al. CRISPR–Cas9 strategy for activation of silent Streptomyces biosynthetic gene clusters. Nat Chem Biol 2017;13:607–9. https://doi.org/10.1038/nchembio.2341.
[145] Kennedy J, Turner G. δ-(L-α-Aminoadipyl)-L-cysteinyl-D-valine synthetase is a rate limiting enzyme for penicillin production in *Aspergillus nidulans*. Mol Gen Genet MGG 1996;253:189–97. https://doi.org/10.1007/s004380050312.
[146] Yeh H-H, Ahuja M, Chiang Y-M, Oakley CE, Moore S, Yoon O, et al. Resistance gene-guided genome mining: serial promoter exchanges in *Aspergillus nidulans* reveal the biosynthetic pathway for Fellutamide B, a proteasome inhibitor. ACS Chem Biol 2016;11:2275–84. https://doi.org/10.1021/acschembio.6b00213.
[147] Huang J, Shi J, Molle V, Sohlberg B, Weaver D, Bibb MJ, et al. Cross-regulation among disparate antibiotic biosynthetic pathways of *Streptomyces coelicolor*. Mol Microbiol 2005;58:1276–87. https://doi.org/10.1111/j.1365-2958.2005.04879.x.
[148] Santos-Beneit F, Rodríguez-García A, Sola-Landa A, Martín JF. Cross-talk between two global regulators in *Streptomyces*: PhoP and AfsR interact in the control of *afsS, pstS* and *phoRP* transcription. Mol Microbiol 2009;72:53–68. https://doi.org/10.1111/j.1365-2958.2009.06624.x.
[149] Romero-Rodríguez A, Robledo-Casados I, Sánchez S. An overview on transcriptional regulators in *Streptomyces*. Biochim Biophys Acta Gene Regul Mech 2015;1849:1017–39. https://doi.org/10.1016/j.bbagrm.2015.06.007.
[150] Bhukya H, Anand R. TetR regulators: a structural and functional perspective. J Indian Inst Sci 2017;97:245–59. https://doi.org/10.1007/s41745-017-0025-5.
[151] Orth P, Schnappinger D, Hillen W, Saenger W, Hinrichs W. Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system. Nat Struct Biol 2000;7:215–9. https://doi.org/10.1038/73324.
[152] Onaka H, Horinouchi S. DNA-binding activity of the A-factor receptor protein and its recognition DNA sequences. Mol Microbiol 1997;24:991–1000. https://doi.org/10.1046/j.1365-2958.1997.4081772.x.
[153] Xu G, Wang J, Wang L, Tian X, Yang H, Fan K, et al. "Pseudo" γ-butyrolactone receptors respond to antibiotic signals to coordinate antibiotic biosynthesis. J Biol Chem 2010;285:27440–8. https://doi.org/10.1074/jbc.M110.143081.
[154] Zhang YY, Zou ZZ, Niu GQ, Tan HR. *jadR** and *jadR2* act synergistically to repress jadomycin biosynthesis. Sci China Life Sci 2013;56:584–90. https://doi.org/10.1007/s11427-013-4508-y.
[155] Bunet R, Song L, Mendes MV, Corre C, Hotel L, Rouhier N, et al. Characterization and manipulation of the pathway-specific late regulator AlpW reveals *Streptomyces ambofaciens* as a new producer of kinamycins. J Bacteriol 2011;193:1142–53. https://doi.org/10.1128/JB.01269-10.
[156] Lee HN, Huang J, Im JH, Kim SH, Noh JH, Cohen SN, et al. Putative TetR family transcriptional regulator SCO1712 encodes an antibiotic downregulator in *Streptomyces coelicolor*. Appl Environ Microbiol 2010;76:3039–43. https://doi.org/10.1128/AEM.02426-09.
[157] Takano E, Kinoshita H, Mersinias V, Bucca G, Hotchkiss G, Nihira T, et al. A bacterial hormone (the SCB1) directly controls the expression of a pathway-specific regulatory gene in the cryptic type I polyketide biosynthetic gene cluster of *Streptomyces coelicolor*. Mol Microbiol 2005;56:465–79. https://doi.org/10.1111/j.1365-2958.2005.04543.x.
[158] Gomez-Escribano JP, Song L, Fox DJ, Yeo V, Bibb MJ, Challis GL. Structure and biosynthesis of the unusual polyketide alkaloid coelimycin P1, a metabolic product of the *cpk* gene cluster of *Streptomyces coelicolor* M145. Chem Sci 2012;3:2716. https://doi.org/10.1039/c2sc20410j.
[159] Zhang R, Pappas KM, Brace JL, Miller PC, Oulmassov T, Molyneaux JM, et al. Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA. Nature 2002;417:971–4. https://doi.org/10.1038/nature00833.
[160] White CE, Winans SC. Identification of amino acid residues of the *Agrobacterium tumefaciens* quorum-sensing regulator TraR that are critical for positive control of transcription. Mol Microbiol 2005;55:1473–86. https://doi.org/10.1111/j.1365-2958.2004.04482.x.
[161] Schrijver AD, Mot RD. A subfamily of MalT-related ATP-dependent regulators in the LuxR family. Microbiology 1999;145:1287–8. https://doi.org/10.1099/13500872-145-6-1287.
[162] Danot O. A complex signaling module governs the activity of MalT, the prototype of an emerging transactivator family. Proc Natl Acad Sci U S A 2001;98:435–40. https://doi.org/10.1073/pnas.98.2.435.
[163] Chen R, Zhang Q, Tan B, Zheng L, Li H, Zhu Y, et al. Genome mining and activation of a silent PKS/NRPS gene cluster direct the production of Totopotensamides. Org Lett 2017;19:5697–700. https://doi.org/10.1021/acs.orglett.7b02878.
[164] Wilkinson B, Micklefield J. Mining and engineering natural-product biosynthetic pathways. Nat Chem Biol 2007;3:379–86. https://doi.org/10.1038/nchembio.2007.7.
[165] Liu T, Mazmouz R, Ongley SE, Chau R, Pickford R, Woodhouse JN, et al. Directing the heterologous production of specific cyanobacterial toxin variants. ACS Chem

Biol 2017;12:2021–9. https://doi.org/10.1021/acschembio.7b00181.
[166] Ongley SE, Bian X, Zhang Y, Chau R, Gerwick WH, Müller R, et al. High-titer heterologous production in *E. coli* of lyngbyatoxin, a protein kinase C activator from an uncultured marine cyanobacterium. ACS Chem Biol 2013;8:1888–93. https://doi.org/10.1021/cb400189j.
[167] Gustafsson C, Govindarajan S, Minshull J. Codon bias and heterologous protein expression. Trends Biotechnol 2004;22:346–53. https://doi.org/10.1016/j.tibtech.2004.04.006.
[168] Zhang Z, Smart TJ, Choi H, Hardy F, Lohans CT, Abboud MI, et al. Structural and stereoelectronic insights into oxygenase-catalyzed formation of ethylene from 2-oxoglutarate. Proc Natl Acad Sci 2017;114:4667–72. https://doi.org/10.1073/pnas.1617760114.
[169] Peiru S, Menzella HG, Rodriguez E, Carney J, Gramajo H. Production of the potent antibacterial polyketide Erythromycin C in *Escherichia coli*. Appl Environ Microbiol 2005;71:2539–47. https://doi.org/10.1128/AEM.71.5.2539-2547.2005.
[170] Choi KR, Cho JS, Cho IJ, Park D, Lee SY. Markerless gene knockout and integration to express heterologous biosynthetic gene clusters in *Pseudomonas putida*. Metab Eng 2018;47:463–74. https://doi.org/10.1016/j.ymben.2018.05.003.
[171] Liu Q, Shen Q, Bian X, Chen H, Fu J, Wang H, et al. Simple and rapid direct cloning and heterologous expression of natural product biosynthetic gene cluster in *Bacillus subtilis* via Red/ET recombineering. Sci Rep 2016;6:1–10. https://doi.org/10.1038/srep34623.
[172] Wendt-Pienkowski E, Huang Y, Zhang J, Li B, Jiang H, Kwon H, et al. Cloning, sequencing, analysis, and heterologous expression of the fredericamycin biosynthetic gene cluster from *Streptomyces griseus*. J Am Chem Soc 2005;127:16442–52. https://doi.org/10.1021/ja054376u.
[173] Young TS, Walsh CT. Identification of the thiazolyl peptide GE37468 gene cluster from *Streptomyces* ATCC 55365 and heterologous expression in *Streptomyces lividans*. Proc Natl Acad Sci U S A 2011;108:13053–8. https://doi.org/10.1073/pnas.1110435108.
[174] Gomez-Escribano JP, Bibb MJ. Heterologous expression of natural product biosynthetic gene clusters in *Streptomyces coelicolor*: from genome mining to manipulation of biosynthetic pathways. J Ind Microbiol Biotechnol 2014;41:425–31. https://doi.org/10.1007/s10295-013-1348-5.
[175] Rückert C, Albersmeier A, Busche T, Jaenicke S, Winkler A, Fridjónsson ÓH, et al. Complete genome sequence of *Streptomyces lividans* TK24. J Biotechnol 2015;199:21–2. https://doi.org/10.1016/j.jbiotec.2015.02.004.
[176] Baltz RH. Genetic manipulation of secondary metabolite biosynthesis for improved production in Streptomyces and other actinomycetes. J Ind Microbiol Biotechnol 2016;43:343–70. https://doi.org/10.1007/s10295-015-1682-x.
[177] Zaburannyi N, Rabyk M, Ostash B, Fedorenko V, Luzhetskyy A. Insights into naturally minimised *Streptomyces albus* J1074 genome. BMC Genom 2014;15:1–11. https://doi.org/10.1186/1471-2164-15-97.
[178] Nielsen MT, Nielsen JB, Anyaogu DC, Holm DK, Nielsen KF, Larsen TO, et al. Heterologous reconstitution of the intact Geodin gene cluster in *Aspergillus nidulans* through a simple and versatile PCR based approach. PLoS One 2013;8. https://doi.org/10.1371/journal.pone.0072871.
[179] Alberti F, Foster GD, Bailey AM. Natural products from filamentous fungi and production by heterologous expression. Appl Microbiol Biotechnol 2017;101:493–500. https://doi.org/10.1007/s00253-016-8034-2.
[180] Smith DJ, Burnham MKR, Edwards J, J EA, Turner G. Cloning and heterologous expression of the Penicillin biosynthetic gene cluster from *Penicillium chrysogenum*. Biotechnology 1990;8:39–41. https://doi.org/10.1038/nbt0190-39.
[181] Tagami K, Liu C, Minami A, Noike M, Isaka T, Fueki S, et al. Reconstitution of biosynthetic machinery for indole-diterpene paxilline in *Aspergillus oryzae*. J Am Chem Soc 2013;135:1260–3. https://doi.org/10.1021/ja3116636.
[182] Tagami K, Minami A, Fujii R, Liu C, Tanaka M, Gomi K, et al. Rapid reconstitution of biosynthetic machinery for fungal metabolites in *Aspergillus oryzae*: total biosynthesis of aflatrem. Chembiochem 2014;16:2076–80. https://doi.org/10.1002/cbic.201402195.
[183] Alberti F, Khairudin K, Venegas ER, Davies JA, Hayes PM, Willis CL, et al. Heterologous expression reveals the biosynthesis of the antibiotic pleuromutilin and generates bioactive semi-synthetic derivatives. Nat Commun 2017;8:1–9. https://doi.org/10.1038/s41467-017-01659-1.
[184] Chiang Y-M, Oakley CE, Ahuja M, Entwistle R, Schultz A, Chang S-L, et al. An efficient system for heterologous expression of secondary metabolite genes in *Aspergillus nidulans*. J Am Chem Soc 2013;135:7720–31. https://doi.org/10.1021/ja401945a.
[185] Gomez-Escribano JP, Bibb MJ. Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters. Microb Biotechnol 2011;4:207–15. https://doi.org/10.1111/j.1751-7915.2010.00219.x.
[186] Komatsu M, Komatsu K, Koiwai H, Yamada Y, Kozone I, Izumikawa M, et al. Engineered *Streptomyces avermitilis* host for heterologous expression of biosynthetic gene cluster for secondary metabolites. ACS Synth Biol 2013;2:384–96. https://doi.org/10.1021/sb3001003.
[187] Kouprina N, Larionov V. Transformation-associated recombination (TAR) cloning for genomics studies and synthetic biology. Chromosoma 2016;125:621–32. https://doi.org/10.1007/s00412-016-0588-3.
[188] Wang H, Li Z, Jia R, Hou Y, Yin J, Bian X, et al. RecET direct cloning and Redαβ recombineering of biosynthetic gene clusters, large operons or single genes for heterologous expression. Nat Protoc 2016;11:1175–90. https://doi.org/10.1038/nprot.2016.054.
[189] Greunke C, Duell ER, D'Agostino PM, Glöckle A, Lamm K, Gulder TAM. Direct Pathway Cloning (DiPaC) to unlock natural product biosynthetic potential. Metab Eng 2018;47:334–45. https://doi.org/10.1016/j.ymben.2018.03.010.
[190] Casini A, Chang FY, Eluere R, King AM, Young EM, Dudley QM, et al. A pressure test to make 10 molecules in 90 days: external evaluation of methods to engineer biology. J Am Chem Soc 2018;140:4302–16. https://doi.org/10.1021/jacs.7b13292.
[191] Yamanaka K, Reynolds KA, Kersten RD, Ryan KS, Gonzalez DJ, Nizet V, et al. Direct cloning and refactoring of a silent lipopeptide biosynthetic gene cluster yields the antibiotic taromycin A. Proc Natl Acad Sci U S A 2014;111:1957–62. https://doi.org/10.1073/pnas.1319584111.
[192] Luo Y, Zhang L, Barton KW, Zhao H. Systematic identification of a panel of strong constitutive promoters from *Streptomyces albus*. ACS Synth Biol 2015;4:1001–10. https://doi.org/10.1021/acssynbio.5b00016.
[193] Luo Y, Huang H, Liang J, Wang M, Lu L, Shao Z, et al. Activation and characterization of a cryptic polycyclic tetramate macrolactam biosynthetic gene cluster. Nat Commun 2013;4:2894. https://doi.org/10.1038/ncomms3894.
[194] Shao Z, Rao G, Li C, Abil Z, Luo Y, Zhao H. Refactoring the silent spectinabilin gene cluster using a plug-and-play scaffold. ACS Synth Biol 2013;2:662–9. https://doi.org/10.1021/sb400058n.
[195] Basitta P, Westrich L, Rösch M, Kulik A, Gust B, Apel AK. AGOS: a plug-and-play method for the assembly of artificial gene operons into functional biosynthetic gene clusters. ACS Synth Biol 2017;6:817–25. https://doi.org/10.1021/acssynbio.6b00319.
[196] Li S, Wang J, Li X, Yin S, Wang W, Yang K. Genome-wide identification and evaluation of constitutive promoters in streptomycetes. Microb Cell Factories 2015;14:172. https://doi.org/10.1186/s12934-015-0351-0.
[197] Guo F, Xiang S, Li L, Wang B, Rajasärkkä J, Gröndahl-Yli-Hannuksela K, et al. Targeted activation of silent natural product biosynthesis pathways by reporter-guided mutant selection. Metab Eng 2015;28:134–42. https://doi.org/10.1016/j.ymben.2014.12.006.
[198] Askenazi M, Driggers EM, Holtzman DA, Norman TC, Iverson S, Zimmer DP, et al. Integrating transcriptional and metabolite profiles to direct the engineering of lovastatin-producing fungal strains. Nat Biotechnol 2003;21:150–6. https://doi.org/10.1038/nbt781.
[199] Xiang S-H, Li J, Yin H, Zheng J-T, Yang X, Wang H-B, et al. Application of a double-reporter-guided mutant selection method to improve clavulanic acid production in *Streptomyces clavuligerus*. Metab Eng 2009;11:310–8. https://doi.org/10.1016/j.ymben.2009.06.003.
[200] Wang Y, Tao Z, Zheng H, Zhang F, Long Q, Deng Z, et al. Iteratively improving natamycin production in *Streptomyces gilvosporeus* by a large operon-reporter based strategy. Metab Eng 2016;38:418–26. https://doi.org/10.1016/j.ymben.2016.10.005.
[201] Wang L, Zhao Y, Liu Q, Huang Y, Hu C, Liao G. Improvement of A21978C production in *Streptomyces roseosporus* by reporter-guided *rpsL* mutation selection. J Appl Microbiol 2012;112:1095–101. https://doi.org/10.1111/j.1365-2672.2012.05302.x.
[202] Rebets Y, Schmelz S, Gromyko O, Tistechok S, Petzke L, Scrima A, et al. Design, development and application of whole-cell based antibiotic-specific biosensor. Metab Eng 2018;47:263–70. https://doi.org/10.1016/j.ymben.2018.03.019.
[203] Yang K, Han L, He J, Wang L, Vining LC. A repressor-response regulator gene pair controlling jadomycin B production in *Streptomyces venezuelae* ISP5230. Gene 2001;279:165–73. https://doi.org/10.1016/S0378-1119(01)00723-5.
[204] Sun Y-Q, Busche T, Rückert C, Paulus C, Rebets Y, Novakova R, et al. Development of a biosensor concept to detect the production of cluster-specific secondary metabolites. ACS Synth Biol 2017;6:1026–33. https://doi.org/10.1021/acssynbio.6b00353.
[205] Seyedsayamdost MR. High-throughput platform for the discovery of elicitors of silent bacterial gene clusters. Proc Natl Acad Sci U S A 2014;111. https://doi.org/10.1073/pnas.1400019111.
[206] Rosen PC, Seyedsayamdost MR. Though much is taken, much abides: finding new antibiotics using old ones. Biochemistry 2017;56:4925–6. https://doi.org/10.1021/acs.biochem.7b00782.
[207] Xu F, Nazari B, Moon K, Bushin LB, Seyedsayamdost MR. Discovery of a cryptic antifungal compound from *Streptomyces albus* J1074 using high-throughput elicitor screens. J Am Chem Soc 2017;139:9203–12. https://doi.org/10.1021/jacs.7b02716.