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MEETING REVIEW

Cyclic Dinucleotide-Controlled Regulatory Pathways in *Streptomyces* Species

Natalia Tschowri

Institut für Biologie/Mikrobiologie, Humboldt-Universität zu Berlin, Berlin, Germany

The cyclic dinucleotides cyclic 3′,5′-diguanylate (c-di-GMP) and cyclic 3′,5′-diadenylate (c-di-AMP) have emerged as key components of bacterial signal transduction networks. These closely related second messengers follow the classical general principles of nucleotide signaling by integrating diverse signals into regulatory pathways that control cellular responses to changing environments. They impact distinct cellular processes, with c-di-GMP having an established role in promoting bacterial adhesion and inhibiting motility and c-di-AMP being involved in cell wall metabolism, potassium homeostasis, and DNA repair. The involvement of c-dinucleotides in the physiology of the filamentous, nonmotile streptomycetes remained obscure until recent discoveries showed that c-di-GMP controls the activity of the developmental master regulator BldD and that c-di-AMP determines the level of the resuscitation-promoting factor A(RpfA) cell wall-remodelling enzyme. Here, I summarize our current knowledge of c-dinucleotide signaling in *Streptomyces* species and highlight the important roles of c-di-GMP and c-di-AMP in the biology of these antibiotic-producing, multicellular bacteria.

Streptomyces, the largest genus of actinobacteria, consists of species of Gram-positive bacteria with high G+C content in their DNA. *Streptomyces* species inhabit diverse natural environments such as terrestrial and aquatic ecosystems but are mostly found in soil, where they recycle carbon and other nutrients trapped in insoluble organic debris from plants and fungi (1). These are multicellular bacteria with branched vegetative filaments consisting of long chains of joined single, multinucleoid cells. They are nonflagellated, sessile organisms during their mycelial growth phase but can spread to new habitats during reproductive growth via the dispersal of spores. Streptomycetes are the most abundant source of antibiotics and other bioactive molecules and are therefore among the most important taxa of clinical and industrial microorganisms. Morphological differentiation and antibiotic production are temporally and genetically coordinated in *Streptomyces*, and much progress has been made in uncovering the regulatory mechanisms that govern developmental processes in these bacteria (2, 3).

The first suggestion that the nucleotide second messenger cyclic 3′,5′-diguanylate (c-di-GMP) might be involved in the control of differentiation in *Streptomyces* came when several c-di-GMP metabolizing enzymes were found to be under developmental control (4). c-di-GMP was discovered in the late 1980s as an allosteric activator of cellulose synthase in *Gluconacetobacter xylinus* (5) and is now the best-known representative of the c-dinucleotide family. Three distinct protein domains are responsible for the synthesis and degradation of this second messenger: the GGDEF, EAL, and HD-GYP domains. These domain names are based on amino acid motifs that crucially contribute to the active sites of these enzymes. Homodimeric GGDEF domains with diguanylate cyclase (DGC) activity bind two GTP substrate molecules to their active site and catalyze the formation of c-di-GMP (6, 7). On the other hand, bacteria have evolved two domains with phosphodiesterase (PDE) activity for c-di-GMP degradation. EAL domains attack one ester bond of the circular dinucleotide, yielding the linear dinucleotide 5′-phosphoguanylyl-(3′-5′)-guanosine (pGpG) (8, 9), and the less frequent HD-GYP domains hydrolyze c-di-GMP into two GMP molecules (10). A unique and remarkable feature of c-di-GMP is its conformational flexibility, which enables the molecule to bind to different types of effectors but limits bioinformatic prediction of c-di-GMP binding sites. In complex with effector proteins, c-di-GMP has been shown to exist as a linear monomer (11), as an intercalated dimer (7), and as a tetramer (12). To date, 6 classes of c-di-GMP effectors have been described: degenerate GGDEF/EAL domains (13–16), PilZ domains (17), GIL domains (18), riboswitches (19), histidine kinases (20), and transcription factors. Of these, transcriptional regulators represent the most diverse class of c-di-GMP effectors, comprising members of the TetR (21), cyclic AMP (cAMP) receptor protein (CRP)/FNR (22–24), NtrC (25, 26), and FixJ/LuxR/CsgD (27) protein families. Through its ability to control the activity of such a broad range of effectors, c-di-GMP regulates diverse physiological processes, but its pivotal role in Gram-negative bacteria is to control transitions from motile-planktonic single cells to sedentary multicellular communities (28).

While c-di-GMP is highly distributed among the members of the bacterial kingdom, the more recently discovered c-dinucleotide c-di-AMP is predominantly found in Gram-positive genera, including *Streptomyces*. DAC (diadenylate cyclase) domains, bearing no amino acid or structural similarity to the GGDEF domain, synthesize c-di-AMP from two ATP molecules (29), and the DHH-DHHA1 domain containing the Asp-His-His motif or the HD domain with a catalytic His-Asp motif hydrolyzes the cyclic molecule to pApA (30, 31). Multiple studies have provided evidence that a well-balanced level of c-di-AMP is vital for bacterial physiology and that it is the first essential second messenger in bacteria, as shown for *Staphylococcus aureus*, *Bacillus subtilis*,

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Address correspondence to natalia.tschowri@hu-berlin.de.

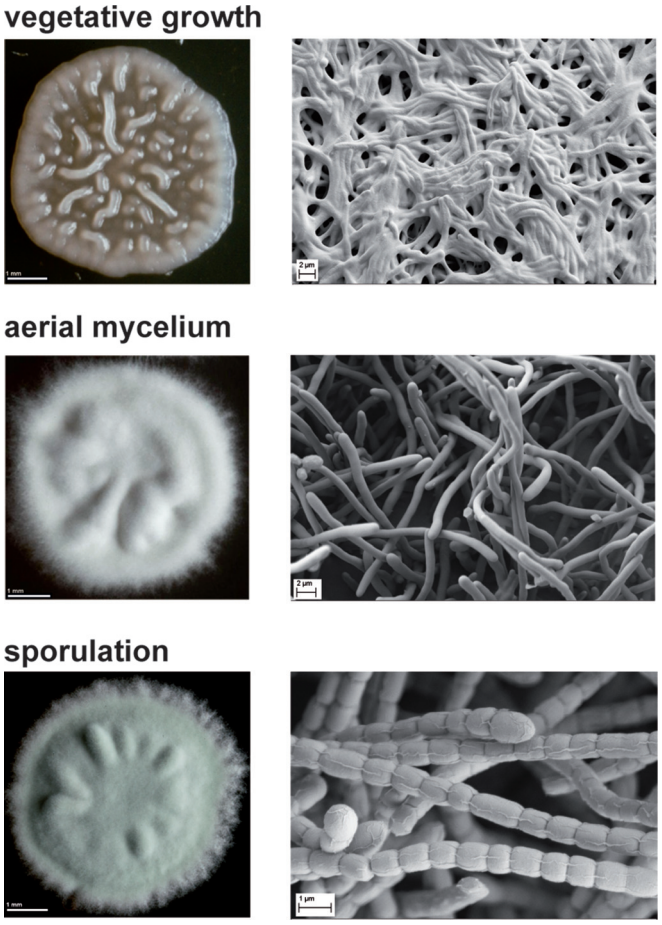

FIG 1 *S. venezuelae* developmental stages. Colony morphology (left) and scanning electron micrographs (right) are shown. During the vegetative growth phase, hyphae form a dense mat of filaments on and in the substrate and the colony appears bald and shiny. The emergence of aerial hyphae marks the beginning of morphological and physiological differentiation and results in a fuzzy and white colony phenotype. In the course of sporulation, multigenomic aerial hyphae differentiate into 50 to 100 unigenomic prespore compartments, which mature into dormant exospores. The synthesis of a polyketide pigment during spore maturation gives the final *S. venezuelae* colonies a characteristic green color.

Streptococcus pneumoniae, and *Listeria monocytogenes* (32–36). c-di-AMP signaling is mediated by a large variety of effectors, such as transcriptional regulators (37), potassium transporters, cation-proton antiporters, and histidine kinases (38), PII-like proteins (39), pyruvate carboxylases (40), and riboswitches (41), and is involved in the control of sporulation, DNA repair, cell wall and potassium homeostasis, virulence, and metabolic functions (42, 43).

In this review I summarize and discuss recent discoveries that have established important roles for c-dinucleotides in the control of key cellular processes in *Streptomyces*, placing c-di-GMP and c-di-AMP signaling in the context of multicellular differentiation.

A BACTERIAL LIFE CYCLE UNDER c-DI-GMP CONTROL

The *Streptomyces* life cycle is defined by a progression through several distinct developmental stages, each characterized by a particular morphology of the bacterial colony (Fig. 1). The vegetative phase of growth initiates with swelling of a free spore and the emergence of one or more germ tubes. Apical hyphal extension and branching directed by the coiled-coil protein DivIVA (44) lead to the formation of the vegetative (or substrate) mycelium, which is a dense network of filamentous vegetative hyphae (Fig. 1). The reproductive stage begins with the emergence of aerial hyphae when stress (e.g., nutrient limitation) is encountered. A fibrous sheath consisting of the amyloid chaplin proteins (45, 46), the rodlins (47), and, on some media, the lantibiotic-like surfactant peptide SapB (48) enables aerial hyphae to grow out of the aqueous environment of the substrate mycelium into the air to form the aerial mycelium, which gives the developing colonies their characteristic fuzzy appearance (Fig. 1). The onset of sporulation begins with the synchronous septation of each multigenomic aerial hypha into ca. 50 to 100 unigenomic prespore compartments and ends with spore maturation. Mature spores are pigmented, resulting in gray colonies in *S. coelicolor* or green colonies in *S. venezuelae* (Fig. 1).

Differentiation is controlled by the Bld and Whi developmental regulators. Mutations in *bld* loci block aerial mycelium formation and therefore cause a "bald" and shiny colony phenotype, whereas *whi* mutants make aerial hyphae but are blocked in spore formation, resulting in white colonies due to the absence of the polyketide pigment associated with mature spores (2).

A key recent study demonstrated that intracellular c-di-GMP levels dictate the speed at which the *Streptomyces* life cycle is completed (12). By overexpressing an active DGC in *S. venezuelae*, the authors showed that elevated levels of c-di-GMP arrest development in the vegetative growth phase. Conversely, low levels of the c-dinucleotide, generated by overexpressing a PDE, accelerate development, causing the vegetative hyphae to sporulate without the need to form an aerial mycelium.

SYNTHESIS AND DEGRADATION OF c-DI-GMP IN *STREPTOMYCES*

Although most streptomycetes contain overlapping sets of up to 85% identical proteins that make or break c-di-GMP, a few variations exist; for example, *S. venezuelae* encodes a GGDEF-EAL composite protein, SVEN_0451, no ortholog of which can be found in *S. coelicolor*. On the other hand, *S. coelicolor* has one additional conserved GGDEF protein, SCP1.113, a GGDDF domain protein, SCO1398, and an extra degenerate EAL protein, SCO1397, which have no equivalents in *S. venezuelae* (Fig. 2).

The GGDEF-EAL CdgA protein was the first c-di-GMP-metabolizing enzyme studied in *Streptomyces*. Aiming to characterize the function of CdgA, den Hengst et al. altered the levels of the protein and found that overexpression of *cdgA* blocks the formation of aerial hyphae and the synthesis of the blue antibiotic actinorhodin in *S. coelicolor*. These effects could be achieved only with a protein version containing an intact GGDEF motif and not with a mutagenized variant containing AADEF residues in the active site, strongly arguing that c-di-GMP was involved in the observed phenotype and thereby setting the stage for the participation of c-di-GMP in *Streptomyces* development and secondary metabolite production (4). Nevertheless, a direct biochemical demonstration of the DGC activity of CdgA is still missing and the function of the conserved C-terminal EAL domain remains to be determined.

One year later, in 2011, Tran et al. provided evidence that the GGDEF domain protein CdgB is another DGC involved in *S. coelicolor* development (49). By using high-performance liquid chro-

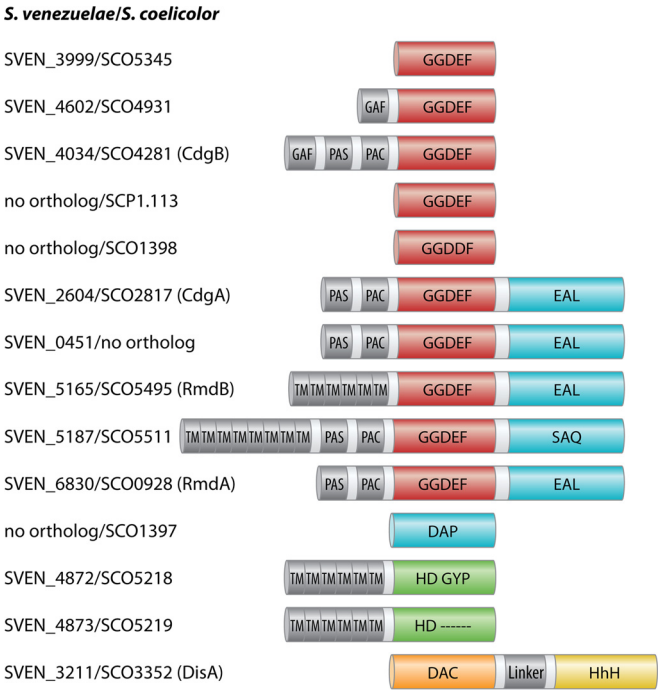

FIG 2 Domain organization of predicted c-di-GMP and c-di-AMP metabolizing proteins in *S. venezuelae* and *S. coelicolor*. *S. venezuelae* and *S. coelicolor* encode an overlapping set of GGDEF, EAL, HD-GYP, and DAC domain proteins, with minor modifications. SVEN_0451 is unique to *S. venezuelae*. SCP1.113, SCO1398, and SCO1397 orthologs are not present in *S. venezuelae*. Predicted signaling domains (GAF, PAS, and PAC) and transmembrane helices (TM) as well as linker regions are shown in gray. Missing residues in SVEN_4873 are shown as a dashed line, and the helix-hairpin-helix domain (HhH) of DisA is indicated.

matography (HPLC)-based c-di-GMP detection analysis, they demonstrated that purified CdgB synthesizes c-di-GMP *in vitro*. Depending on the media used, the overproduction of CdgB showed effects similar to those seen with overproduction of CdgA, i.e., inhibition of aerial mycelium formation and actinorhodin production.

Two GGDEF-EAL proteins, RmdA and RmdB, were shown to function as PDEs and to impact development in *S. coelicolor* (50). Deletion of either *rmdA* or *rmdB* causes a mild defect in development, delaying spore formation. In contrast, deletion of both genes has a dramatic effect, causing a total inhibition of aerial mycelium formation, which suggests functional redundancy. Aiming to identify the signals that activate the enzymatic function of RmdA, the authors found that hemin can bind to this protein. However, evidence that hemin specifically binds to the PAS-PAC sensory domain and that binding of the ligand has an effect on the PDE function is still lacking. In addition, the role of the conserved GGDEF domain in RmdA as well as in RmdB remains unknown.

BldD LINKS c-DI-GMP SIGNALING AND DEVELOPMENT IN *STREPTOMYCES*

BldD is a DNA binding protein that directly controls key developmental genes in *Streptomyces* (51–53). With the identification of the complete BldD regulon, comprising ca. 167 genes, including 42 targets encoding regulatory proteins, it became apparent that BldD has a central, pleiotropic role in *Streptomyces* development and acts as a master regulator that represses sporulation genes during vegetative growth (4). Nevertheless, how the BldD-dependent repression of sporulation genes is relieved during development remained a mystery.

This mystery was solved when BldD was shown to be a c-di-GMP effector protein (12). Tschowri et al. demonstrated that c-di-GMP binds to the RXD-X_8-RXXD motif located in the C-terminal domain (CTD) of BldD and enhances its activity as a transcriptional repressor (12). Notably, the crystal structure of the BldD CTD–c-di-GMP complex revealed that four molecules of c-di-GMP assemble into a tetramer that enables the two BldD CTDs to form a stable dimer and thus drive DNA binding. Uniquely, there are no protein-protein contacts between the two CTDs and it is the ligand only that connects the two protein domains (see Fig. 4A). Consequently, high levels of c-di-GMP drive BldD dimerization, leading to DNA binding through the N-terminal domains (NTDs), repression of the sporulation cascade, and a block in development (see Fig. 4A). On the other hand, when c-di-GMP levels are low, BldD is inactive, leading to activation of the developmental cascade and sporulation.

bldD is an ancient gene that was present in very early actinobacteria but that has been lost several times in the course of evolution of the phylum (54). Orthologs of *bldD* show high conservation and local synteny and are found in all sporulating actinomycetes (54). Moreover, the c-di-GMP binding signature is present in all sequenced *bldD* orthologs and c-di-GMP is predicted to be present in all sporulating actinomycetes. This suggests that the BldD–c-di-GMP complex controls developmental processes not only in streptomycetes but also in all sporulating actinobacteria.

c-DI-GMP TURNOVER ENZYMES ARE DIRECT TARGETS OF DEVELOPMENTAL REGULATORS

BldD not only senses c-di-GMP levels in the cell but also controls the expression of c-di-GMP-producing enzymes in response to ligand binding. Among the direct BldD targets in *S. venezuelae* are four genes encoding proven or putative DGCs: *cdgA*, *cdgB*, *sven4602*, and *sven5187* (4, 12, 49) (Fig. 3). Thus, 4 of 6 potential DGCs in *S. venezuelae* are transcriptionally controlled by a c-di-GMP-sensing regulator. This wiring might suggest that there is a crucial need for a carefully fine-tuned c-di-GMP level in the cell. However, evidence for the involvement of a negative feedback loop is lacking, and whether *bldD* and any of these 4 DGCs are expressed and active in the same time and place during development and do indeed constitute a common c-di-GMP signaling module needs to be clarified.

In addition to BldD, the WhiA transcriptional regulator is also involved in c-di-GMP signaling by directly controlling the expression of *cdgB*. WhiA is widespread in Gram-positive bacteria and is required for cessation of aerial growth and initiation of sporulation in *Streptomyces* species. This regulator is predominantly active at the onset of sporulation and moderately represses the transcription of *cdgB* (55) (Fig. 3). Since BldD and WhiA are active at distinct differentiation stages in *Streptomyces* species, it is unlikely that these two transcriptional regulators compete for binding to the *cdgB* promoter. However, the function of WhiA seems to be subject to posttranslational regulation (55) and it will be interesting to identify the conditions that lead to derepression of *cdgB* by WhiA.

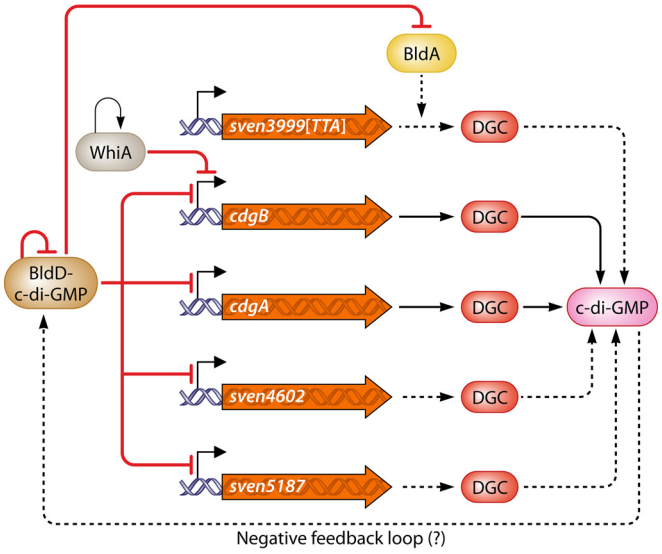

FIG 3 Developmental regulators control the levels of c-di-GMP synthesizing diguanylate cyclases (DGCs) in *S. venezuelae*. The BldD–c-di-GMP complex directly represses the expression of genes encoding proven (*cdgB* and *cdgA*) and predicted (*sven4602* and *sven5187*) DGCs (4, 12, 49). This suggests that a negative feedback loop might be involved whereby c-di-GMP made by these DGCs binds to BldD, leading to repression of transcription. At the onset of sporulation, *cdgB* is also subject to repression by the developmental regulator WhiA (55). *sven3999* encoding a putative DGC contains the rare TTA codon, suggesting that the BldA tRNA is required for its translation. Dashed lines indicate predicted wiring.

Further, the developmental regulatory BldA tRNA appears to participate in the c-di-GMP signaling cascade by providing a leu-tRNA^{UUA} for translation of TTA codons in *sven3999* and the orthologous gene *sco5345* (Fig. 3) as well as in *rmdB*. The TTA codon is rare in GC-rich streptomycetes, and *bldA* encodes the sole tRNA able to translate the UUA codon into the amino acid leucine (56). A *bldA* mutant is bald mainly because *adpA* and *amfR* contain a TTA codon in their DNA, and the presence of *bldA* is essential for their efficient translation (57). The AdpA transcriptional regulator not only controls the expression of sporulation genes but also governs chromosome replication by binding to the *oriC* region (58, 59). AmfR is required for the synthesis of the SapB surfactant peptide, which contributes to the hydrophobic sheath formation to allow aerial hyphae to escape the aqueous substrate environment during aerial mycelium formation. Similarly, *sco5345* in *S. coelicolor* and the orthologous *sven3999* gene in *S. venezuelae*, encoding a conserved GGDEF protein, contain a TTA codon (Fig. 3). Moreover, translation of the PDE RmdB in *S. coelicolor* also seems to depend on BldA due to the presence of a TTA codon in *rmdB* (54). The regulation of several c-di-GMP metabolizing enzymes by the developmental regulators BldD, WhiA, and BldA further emphasizes the central role of c-di-GMP signaling in *Streptomyces* differentiation.

BldD-MEDIATED ROLES OF c-DI-GMP IN ANTIBIOTIC PRODUCTION AND PATHOGENICITY

Antibiotic production in *Streptomyces* species is linked to morphological differentiation, and the developmental regulators that control aerial mycelium formation often also impact natural product synthesis, directly or indirectly (60). In the case of BldD, the BldD–c-di-GMP-controlled *adpA* and *bldA* genes are directly involved in the production of three different antibiotics in *S. coelicolor*: the blue actinorhodin (ACT), the red undecylprodigiosin (RED), and methylenomycin (MM) (60). The synthesis of actinorhodin is completely dependent on the pathway-specific regulator ActII-ORF4, which activates the expression of genes that encode biosynthetic enzymes within the *act* gene cluster (61). Among others, AdpA seems to directly bind to the *actII*-ORF4 promoter region, as shown by DNA-affinity capture assays (62). The same screen identified AdpA as a direct regulator of *redD*, which requires RedZ for expression and activates undecylprodigiosin biosynthetic genes (62, 63). However, how direct binding of AdpA to the *actII*-ORF4 as well as to the *redD* promoter regions affects gene expression awaits further investigation.

Strikingly, several genes involved in antibiotic synthesis in *S. coelicolor* contain a TTA codon and are therefore dependent on the BldA tRNA for translation. A TTA codon is present in *adpA* (64), *actII*-ORF4 (61), and *redZ* (65) as well as in *mmyB* and *mmfL*, encoding an activator and an enzyme involved in methylenomycin biosynthesis, respectively (66). In *S. venezuelae*, the indirect role of BldD–c-di-GMP extends to the regulation of chloramphenicol biosynthesis. Recently, it was shown that BldD–c-di-GMP-dependent response regulator BldM represses the expression of the chloramphenicol biosynthetic gene cluster (67), but the regulatory mechanism is not understood.

In *Saccharopolyspora erythraea*, BldD directly regulates the synthesis of the antibiotic erythromycin by binding to the promoters of the erythromycin biosynthetic gene cluster (68). *S. erythraea* encodes 13 GGDEF proteins, 11 GGDEF-EAL composite proteins, one GGDEF-HD-GYP fusion protein, and one EAL domain protein (28). Since the RXD-X_8-RXXD c-di-GMP binding motif is conserved in *S. erythraea* BldD, it is very likely that c-di-GMP signals through BldD to control erythromycin production in this actinobacterium. The involvement of BldD–c-di-GMP in the production of a commercially valuable secondary metabolite raises the possibility that the manipulation of c-di-GMP levels is a promising tool for the full exploitation of antibiotic synthesis in sporulating actinomycetes.

A recent study systematically investigated the influence of classical *bld* genes on the pathogenicity of the agronomically important plant pathogen *Streptomyces scabies*, and the researchers found that *bldD*, *bldC*, *bldA*, *adpA*, and *bldG* are required for the full virulence of the bacterium (69). *S. scabies* is one of the best-characterized plant-pathogenic species and is the causative agent of the scab disease of taproot crops such as potato, carrot, radish, turnip, and beet. The prime source of the ability of *S. scabies* and related pathogenic streptomycetes to cause the scab disease is the production of the virulence factor thaxtomin A, which is activated by the regulator TxtR and functions as a cellulose synthesis inhibitor (70). Bioinformatic analysis revealed that a putative BldD binding site is present in the upstream region of *txtR*, suggesting that BldD may directly control the expression of *txtR* (69). BldD from *S. scabies* is 98% identical to the BldD ortholog in *S. venezuelae*, and the c-di-GMP binding signature is well conserved. Moreover, *S. scabies* encodes 4 GGDEF and 4 GGDEF-EAL domain proteins (28), suggesting that c-di-GMP signaling plays a role in the biology of the organism and that c-di-GMP signals through BldD to influence the virulence of pathogenic streptomycetes.

THE EMERGING ROLE OF c-DI-AMP IN CELL WALL METABOLISM OF *STREPTOMYCES* SPECIES

Streptomyces species encode one DAC domain-containing protein, which is homologous to DNA integrity-scanning protein A (DisA) in *B. subtilis* and consists of a N-terminal DAC domain and a C-terminal helix-hairpin-helix domain separated by a short linker region (Fig. 2). The DGA and RHR motifs, crucial for enzymatic activity (29), are conserved in DisA from *Streptomyces* species, and the protein was recently shown to be an active DAC *in vivo* in *S. venezuelae* (71). Streptomycetes do not have any DHH-DHHA1 domain-containing proteins or HD-type PDEs able to cleave c-di-AMP, so the counterpart to DisA remains unknown.

The function of DisA has been thoroughly studied in *B. subtilis*, and it is generally accepted that DisA is involved in DNA repair mechanisms. It has been shown that DisA scans chromosomal DNA for integrity and reduces c-di-AMP synthesis when DNA lesions are encountered, resulting in a delayed entry into sporulation to allow repair of DNA damage (72, 73). Moreover, it has been reported that DisA is involved in the arrest of DNA replication during *B. subtilis* spore outgrowth, to ensure that germinating spores are free of damaged DNA (74). How DisA and c-di-AMP contribute to recruitment of DNA repair factors is not well understood, but the diadenylate cyclase has been shown to interact with the RadA recombination protein, which participates in processing of Holliday junctions (75, 76).

The role of c-di-AMP in *Streptomyces* species has yet to be investigated in any detail; however, in contrast to evidence from *B. subtilis* and other species (see the introduction), it is already clear that c-di-AMP is not essential in *S. venezuelae*, since a *disA* mutant lacks detectable c-di-AMP and is viable under standard growth conditions (71). The first relevant published study provided evidence that c-di-AMP made by DisA may be involved in cell wall remodelling during spore germination in *Streptomyces* species (71). In actinobacteria, the resuscitation-promoting factor (Rpf) proteins are a class of enzymes that hydrolyze the sugar backbone of peptidoglycan and play an important role in the resuscitation of dormant cells (77–79). A detailed analysis of *rpfA* regulation revealed that this locus functions as a regulatory node that integrates signals from at least three different second messengers: cAMP-CRP is required for transcription initiation, a c-di-AMP binding *ydaO*-like riboswitch is involved in transcript elongation, and a ppGpp-responsive protease controls RpfA stability (71).

The *ydaO* riboswitch is widespread in Gram-positive bacteria and is typically located in the 5′ untranslated (leader) region (UTR) of controlled genes (80). In *B. subtilis*, the *ydaO* riboswitch is present in the 5′ UTR of the *ydaO* gene, the function of which is unknown, and of the *ktrAB* operon, encoding the two subunits of a potassium transporter. Representing a so-called genetic OFF switch, binding of c-di-AMP to the *ydaO* switch results in premature transcription termination (41). Similarly, the *ydaO*-like riboswitch in *S. coelicolor* is located in the 5′ UTR of *rpfA* and leads to transcription attenuation upon c-di-AMP binding (Fig. 4B). The *ydaO* or *rpfA* riboswitch is conserved in actinobacteria and is mostly associated with genes involved in cell wall metabolism, suggesting that c-di-AMP controls cell wall remodelling in these bacteria (80). However, *rpfA* is very likely not the only c-di-AMP-regulated gene in *Streptomyces* since there are at least three additional genes encoding cell wall lytic enzymes that carry an *rpfA*-like riboswitch in their 5′ UTRs (71, 77). The identification of further c-di-AMP effector molecules in *Streptomyces* is an important goal for the future.

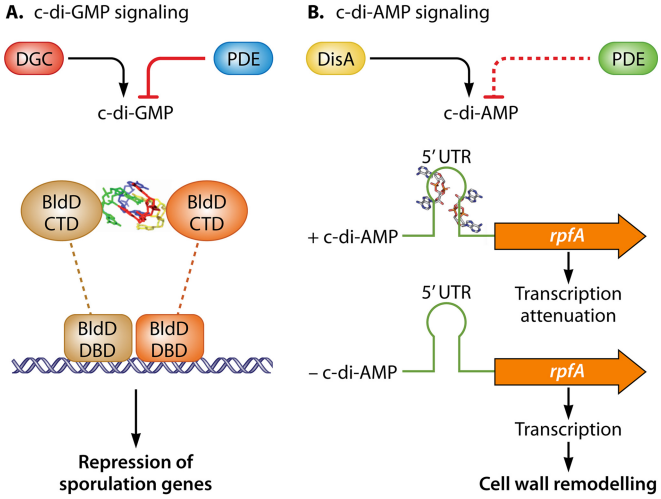

FIG 4 Graphical summary of the c-di-GMP and c-di-AMP signaling pathways. (A) GGDEF-type diguanylate cyclases (DGCs) and EAL- and HD-GYP-type phosphodiesterases (PDEs) make and break c-di-GMP. A tetrameric c-di-GMP binds to the developmental master regulator BldD and links the two otherwise separate C-terminal domains (CTDs), resulting in protein dimerization. This enables the transcriptional repressor BldD to effectively bind to target DNA via its N-terminal DNA binding domain (DBD), leading to repression of sporulation genes during vegetative growth (4, 12). (B) DisA is the sole DAC protein responsible for synthesis of c-di-AMP. A c-di-AMP degrading PDE has yet to be identified (indicated by a dashed line). Binding of 2 c-di-AMPs to the *ydaO*-like riboswitch (41) in the 5′ untranslated region (5′ UTR) upstream of *rpfA* (resuscitation-promoting factor A) results in transcriptional attenuation. The absence of c-di-AMP favors *rpfA* transcription and accumulation of the RpfA cell wall-remodelling enzyme during spore germination (71).

CONCLUSIONS

Given that c-di-GMP stimulates hyphal vegetative growth and inhibits sporulation in *Streptomyces* species, it remains faithful to its role as a lifestyle regulator. But, in contrast to its control of the "stick-or-swim" switch in Gram-negative species, it controls the hypha-to-spore transition in *Streptomyces* species. By controlling the activity of the BldD developmental master regulator, c-di-GMP determines the timing of sporulation. On the basis of recent findings, we now have a detailed understanding of the processes triggered by binding of c-di-GMP to BldD, but we have little knowledge of the signaling events that occur upstream of BldD. To get a more complete picture of c-di-GMP signaling in streptomycetes, it will be essential to identify the DGCs and PDEs that specifically control the c-di-GMP sensed by BldD and also to determine the internal and external stimuli that fine-tune the levels and activities of these enzymes.

To date, very little is known about c-di-AMP signaling in streptomycetes, but low levels seem to favor spore germination by stimulating the accumulation of enzymes involved in cell wall remodelling. Whether c-di-AMP synthesis is also connected to DNA integrity, as is the case in *B. subtilis*, remains to be determined. In the absence of DHH-DHHA1- and HD domain-containing proteins, how c-di-AMP is degraded in streptomycetes also awaits future investigations.

The most recently discovered c-dinucleotide in the collection

of the bacteria is the hybrid molecule c-AMP-GMP, which is synthesized from ATP and GTP by DncV in *Vibrio cholerae* (81). *Streptomyces* species do not have a DncV homolog, but it is tempting to speculate that, with c-di-GMP and c-di-AMP, the set of c-dinucleotides employed by these bacteria is not yet complete. Altogether, recent findings indicate that c-dinucleotides are important players in signal transduction networks in streptomycetes and represent an exciting research area for future studies.

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