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Natural Products – Mini-Review

WblA, a global regulator of antibiotic biosynthesis in *Streptomyces*

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Abstract: *Streptomyces* species are soil-dwelling bacteria that produce vast numbers of pharmaceutically valuable secondary metabolites (SMs), such as antibiotics, immunosuppressants, antiviral, and anticancer drugs. On the other hand, the biosynthesis of most SMs remains very low due to tightly controlled regulatory networks. Both global and pathway-specific regulators are involved in the regulation of a specific SM biosynthesis in various *Streptomyces* species. Over the past few decades, many of these regulators have been identified and new ones are still being discovered. Among them, a global regulator of SM biosynthesis named WblA was identified in several *Streptomyces* species. The identification and understanding of the WblAs have greatly contributed to increasing the productivity of several *Streptomyces* SMs. This review summarizes the characteristics and applications on WblAs reported to date, which were found in various *Streptomyces* species and other actinobacteria.

Keywords: *Streptomyces*, WhiB-like gene A (*wblA*), Secondary metabolite regulation

Introduction

Streptomyces are Gram-positive high G + C filamentous soil bacteria with superior characteristics in producing a variety of secondary metabolites (SMs), including many pharmaceutically valuable compounds, such as antibiotics, as well as anticancer, antiviral, and immunosuppressant agents (Bérdy, 2005; Kinghorn et al., 2009; Mann, 2001; Sivalingam et al., 2019) . *Streptomyces* SMs are synthesized by a group of enzymes encoded by their corresponding biosynthetic gene cluster (BGC). They are typically under tight and complicated regulation at the transcriptional level (Liu et al., 2013). The biosynthesis of *Streptomyces* SM is regulated through multiple regulatory pathways induced by both nutritional and environmental stimuli (Lee et al., 2005; Sun et al., 2017; van Wezel & McDowall, 2011). Although various global regulatory systems present in most *Streptomyces* species can control both morphological differentiation and SM production, the SM biosynthetic gene sets are also subject to pathway-specific regulation by linked regulatory genes (van der Heul et al., 2018). Most of these pathway-specific regulatory genes are transcriptionally regulated by a range of global regulatory networks in most *Streptomyces* species (Liu et al., 2013; Xia et al., 2020).

Among the global regulatory proteins, the WhiB-like (Wbl) family of proteins, which are only present in Actinobacteria, such as *Streptomyces, Corynebacteria,* and *Mycobacteria*, are a major class (Bush, 2018, refer to this reference for comprehensive review on Wbls). Following the initial characterization of WhiB in *S. coelicolor* (Chater, 1972; Davis & Chater, 1992), multiple paralogs were identified in many other *Streptomyces* species (Soliveri et al., 2000). Genome sequencing revealed the prevalence of Wbl paralogs throughout the phylum. Fourteen Wbl proteins were identified in *S. coelicolor*, with 11 encoded on the chromosome, and 3 encoded on the large linear plasmid, SCP1 (Bentley et al., 2002, 2004). The $WblA_{sco}$ is a WhiB4 ortholog, which is one of three Wbl-family members that regulate both differentiation and SM biosynthesis in *S. coelicolor*. In *S. coeilcolor*, a $wblA_{sco}$ mutant exhibits a defect in sporulation, with some aerial hyphae failing to sporulate and appearing thinner compared to the wild type (Aínsa et al., 2000; Fowler-Goldsworthy et al., 2011).

A Global Antibiotic Downregulator, $WblA_{sco}$ in *S. coelicolor*

The first biological function of WblA as an antibiotic downregulator was identified from somewhat unexpected experimental results. After the genome of *S. coelicolor* was sequenced nearly 20 years ago, *Streptomyces* interspecies DNA microarray analysis was applied to detect the global changes in mRNA abundance associated with the overproduction of the anticancer compound doxorubicin (DOX) in an *S. peucetius* overproducing industrial mutant (OIM) strain. *S. coelicolor* genome was the only available genome at the time to facilitate transcriptome analysis. The results showed that the $wblA_{sco}$ gene was a pleiotropic downregulator of antibiotic biosynthesis in *S. coelicolor* (Fowler-Goldsworthy et al., 2011). Comparative transcriptome analyses of cultures of the wild-type and OIM mutant strains of *S. peucetius* using *S. coelicolor* cDNA microarrays which was the only available genome at the time identified more than 100 *S. coelicolor* potential candidate genes that showed at least a twofold change in transcription between the wild-type and OIM mutant strains. After further analysis of the growth phase-dependent transcription profiles of these potential candidate genes, 20 genes exhibiting particularly large transcriptional changes between the two strains were selected and overexpressed individually in *S. coelicolor* (Kang et al., 2007).

Among them, SCO3579, which was previously proposed to be a whiB-like putative transcription factor gene, was identified and later named $wblA_{sco}$ in *S. coelicolor* (Soliveri et al., 2000). Although *whiB* is a developmental regulatory gene that was identified in *S. coelicolor* as being essential for the sporulation of aerial

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Table 1. *WblA* orthologs identified from various *Streptomyces* species

Gene	Strain	Size	Identity (with WblAsco)	Compounds	SM production in DIS mutant (fold)	References
$WblA_{sco}$	*S. coelicolor* M145	112 aa	100%	Actinorhodin	Increased (5.6-fold)	Lee et al. (2010)
$WblA_{spe}$	*S. peucetius* OIM-1	114 aa	95%	Doxorubicin, daunorubicin	Increased (1.7-fold)	Kang et al. (2007), Noh et al. (2010)
$WblA_{tmc}$	*Streptomyces* sp. CK4412	130 aa	96%	Tautomycetin	Increased (3.2-fold)	Nah et al. (2012)
$WblA_{gh}$	*S. ghanaensis* ATCC14672	128 aa	96%	Moenomycin A	Increased (2.3-fold) Increased (2.5-fold)	Rabyk et al. (2011) Yan et al. (2020)
$WblA_{sro}$	*S. roseosporus* NRRL15998	116 aa	90%	Daptomycin	Increased (1.5-fold)	Huang et al. (2017)
$WblA_{san}$	*S. ansochromogenes* 7100	112 aa	96%	Nikkomycin (major) Tylosin analogs (cryptic)	Abolished Activated	Lu et al. (2015)
$WblA_{so}$	*S. somaliensis* SCSIO ZH66	124 aa	85%	Violapyrone	Increased (NR)	Huang et al. (2016)
$WblA_{sve}$	*S. venezuelae* ATCC15439	115 aa	90%	Pikromycin	Increased (3.5-fold)	Yan et al. (2016)
$WblA_{scb}$	*Streptomyces* sp. CB03234	131 aa	93%	Tiancimycins	Increased (13.9-fold)	Nguyen et al. (2003)

SM, secondary metabolite; DIS, disruption; NR, not reported.

hyphae, the biological function of $wblA_{sco}$ in SM regulation was not determined (Soliveri et al., 2000). The overexpression of $wblA_{sco}$ inhibited the biosynthesis of actinorhodin (ACT), undecylprodigiosin (RED), and calcium-dependent antibiotic (CDA) in *S. coelicolor*. Moreover, transcripts encoded by pathway-specific activators of the three major *S. coelicolor* antibiotics (i.e. *actIIORF4* for ACT, *redD/Z* for RED, and *cdaR* for CDA) were reduced in the $wblA_{sco}$-overexpressing *S. coelicolor*, implying that $wblA_{sco}$ acts broadly to downregulate antibiotic biosynthesis in *S. coelicolor* (Kang et al., 2007).

During *Streptomyces* interspecies DNA microarray analysis, a previously-unidentified *tetR* family transcriptional regulatory gene (SCO1712), as well as a carbon flux regulating 6-phosphofructokinase gene (SCO5426), were also found to downregulate SM biosynthesis in the *S. coelicolor* wild-type strain as well as in a $wblA_{sco}$ deletion mutant (Kim et al., 2011; Lee et al., 2010). The *S. coelicolor* triple knockout mutant (Δ$wblA_{sco}$, ΔSCO1712, and ΔSCO5426) showed the highest level of ACT production compared to any of the single and double knockout mutants in *S. coelicolor*, suggesting that $wblA_{sco}$ along with other $wblA_{sco}$-independent regulatory and precursor pathway genes could be optimized synergistically for SM production in *Streptomyces* (Kim et al., 2011).

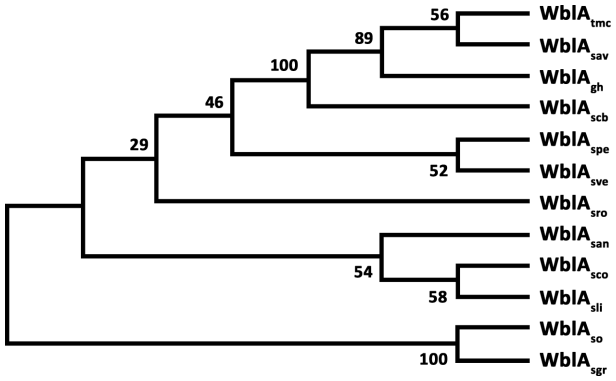

Fig. 1. WblAs phylogenetic tree among *Streptomyces* species. Phylogenetic tree was built using the Mega X software by neighbor joining test by Bootstrap. $WblA_{sco}$ from *S. coelicolor*; $wblA_{spe}$ from *S. peucetius*; $wblA_{tmc}$ from *Streptomyces* sp. CK4412; $wblA_{gh}$ from *S. ghanaensis*; $wblA_{sro}$ from *S. roseosporus*; $wblA_{san}$ from *S. ansochromogenes*; $wblA_{so}$ from *S. somaliensis*; $wblA_{sve}$ from *S. venezuelae*; $wblA_{scb}$ from *Streptomyces* sp. CB03234; $wblA_{sav}$ from *S. avermitilis*; and $wblA_{sgr}$ from *S. griseus*.

WblA Orthologs in Other *Streptomyces* Species

$WblA_{spe}$ in *S. peucetius*

Subsequently, a $wblA_{sco}$ ortholog named $wblA_{spe}$ from *S. peucetius* was identified through the construction of a total genomic DNA library of the above-mentioned *S. peucetius* OIM and the screening using a $wblA_{sco}$ gene probe. As expected, the production of both DXR and its precursor, daunorubicin (DNR) were improved through gene disruption of $wblA_{spe}$ from *S. peucetius* OIM (Table 1). Moreover, several putative $wblA_{spe}$-dependent genes were also identified using interspecies DNA microarray analysis between the *S. peucetius* OIM and $wblA_{spe}$-disrupted *S. peucetius* OIM. Among the putative $wblA_{spe}$-dependent genes tested, a conserved hypothetical protein (SCO4967) further stimulated the production of DXR/DNR/aklavinone in the $wblA_{spe}$-disrupted *S. peucetius* OIM. These results suggest that sequential genetic manipulation of the $wblA_{spe}$ and its dependent genes identified from comparative transcriptome analysis could provide an efficient and rational strategy for improving the titer of DXR/DNR in *S. peucetius* strain. This was the first example to improve the antibiotic-OIM strain using a microarray-driven reverse engineering approach in *Streptomyces* species (Noh et al., 2010).

$WblA_{tmc}$ in *Streptomyces* sp. CK4412

Tautomycetin (TMC) is a linear polyketide compound with a novel activated T cell-specific immunosuppressant and anticancer activities (Chae et al., 2004; Lee, Lee, et al., 2006; Niu et al., 2012). The whole-genome sequencing of the *Streptomyces* sp. CK4412 chromosome revealed the entire TMC (~80-kb) BGC as well as the $wblA_{sco}$ ortholog gene named $wblA_{tmc}$. The $wblA_{tmc}$ from *Streptomyces* sp. CK4412 showed 96% amino acid identity compared to a previously known $wblA_{sco}$. (Figs. 1 & 2). The targeted gene disruption of $wblA_{tmc}$ in *Streptomyces* sp. CK4412 caused an approximately threefold higher TMC production titer than that in the wild-type strain. Moreover, transcription analyses of the two TMC pathway-specific positive regulatory genes, *tmcN* and *tmcT*, located within its BGC showed that the only *tmcT* expression was strongly downregulated by $wblA_{tmc}$ in *Streptomyces* sp. CK4412 (Nah et al., 2012). The *tmcN* expression was not affected by either deletion or overexpression of $wblA_{tmc}$ in

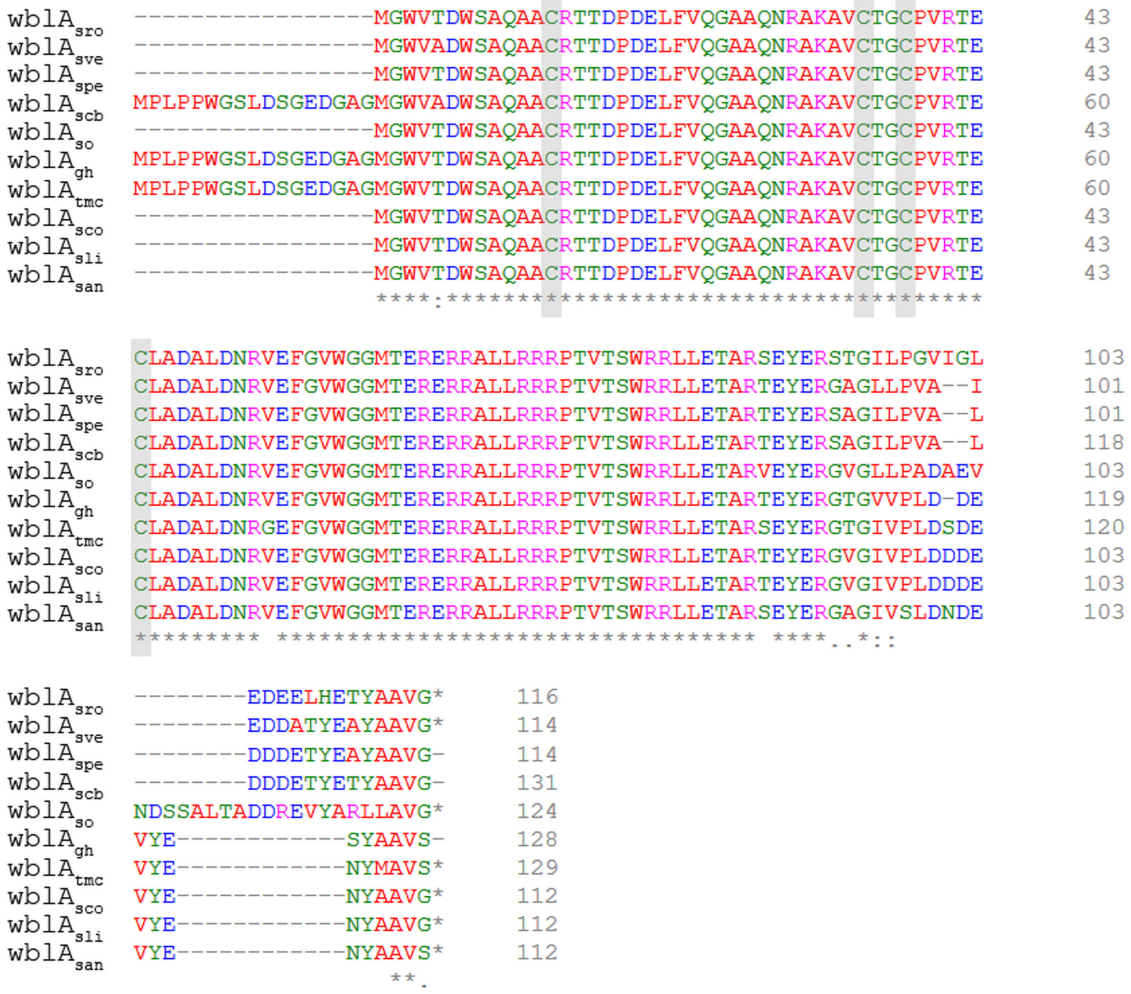

Fig. 2. Sequence alignments among the *Streptomyces* WblAs. Conserved (asterisk) and related (colon) amino acids were marked underneath. Gray box; cysteine conserved region.

Streptomyces sp. CK4412 (Nah et al., 2012). These results suggest that the TMC BGC regulatory network is controlled by two pathway-specific positive regulators, $WblA_{tmc}$-dependent TmcT and $WblA_{tmc}$-independent TmcN, in *Streptomyces* sp. CK4412 (Nah et al., 2012).

$WblA_{gh}$ in *S. ghanaensis*

Another $wblA_{sco}$ ortholog named $wblA_{gh}$ was identified from the whole-genome sequencing of the moenomycin producer, *S. ghanaensis* ATCC14672. A deletion of $wblA_{gh}$ stimulated a more than twofold increase in moenomycins production along with inhibition of aerial mycelium sporulation. Moreover, the $wblA_{gh}$ overexpression in *S. ghanaensis* ATCC14672 decreased the moenomycin production by 50%, implying that $WblA_{gh}$ is a global antibiotic downregulator in *S. ghanaensis* ATCC14672. Since the moenomycin BGC in *S. ghanaensis* ATCC14672 did not contain any pathway-specific regulatory genes, the downstream target of $WblA_{gh}$ is not known. Although the regulation of putative *Streptomyces* subtilisin inhibitor (SSI) named SSFG_01 620 was proposed to be linked to the $WblA_{gh}$ deletion in *S. ghanaensis* ATCC14672, the detailed mechanism between $WblA_{gh}$ and SSFG_01 620 needs to be further pursued (Rabyk et al., 2011).

$WblA_{sro}$ in *S. roseosporus*

The $wblA_{sco}$ ortholog gene named $wblA_{sro}$ was also identified in the whole genome sequencing of the daptomycin producer *S. roseosporus*. Three types of strains, the $wblA_{sro}$ disruption strain, the complemented strain, and the overexpression strains, were generated to determine if it could affect the production of SMs as well as morphogenesis. The disruption mutant of $wblA_{sro}$ enhanced daptomycin production by more than 50% as well as blocked aerial mycelium sporulation. In contrast, overexpression of $wblA_{sro}$ resulted in a significant decrease in the daptomycin production titer. As expected, the transcription of the key daptomycin positive regulatory genes *atrA*, *dptR2*, and *dptR3*, and the structural gene, *dptE*, were increased remarkably in the $wblA_{sro}$ disruption mutant. These results suggest that $wblA_{sro}$ plays a key downregulatory role in controlling daptomycin biosynthesis (Huang et al., 2017).

$WblA_{san}$ in *S. ansochromogenes*

Another $wblA_{sco}$ ortholog named $wblA_{san}$ was found in *S. ansochromogenes* 7100 sequencing analysis. The $wblA_{san}$ disruption mutant of *S. ansochromogenes* 7100 failed to sporulate as well as to produce nikkomycin, a major SM produced by *S. ansochromogenes* 7100 during fermentation. Moreover, two novel 16-membered

tylosin-like macrolides were observed only in a fermentation broth of Δ*wblA*$_{san}$ strain. These two compounds, which had different functional groups at the C6 position comparing with tylosin, exhibited similar antibacterial activities against several Gram-positive bacteria including *Staphylococcus aureus* and *Bacillus cereus*. Interestingly, however, these two compounds displayed much higher activity against *S. pneumoniae* than tylosin, suggesting that *wblA* ortholog disruption approach could activate cryptic compounds hidden in *Streptomyces* species, and the compounds identified by the Δ*wblA*$_{san}$ in *S. ansochromogenes* might be used to broaden the application of tylosin (Lu et al., 2015).

WblA$_{so}$ in *S. somaliensis*

A *wblA*$_{sco}$ ortholog named *wblA*$_{so}$ was found in deep sea-derived *S. somaliensis* SCSIO ZH66 through the whole-genome sequencing analysis. To activate cryptic BGCs in *S. somaliensis* SCSIO ZH66, the *wblA*$_{so}$ from *S. somaliensis* SCSIO ZH66 was inactivated. Noticeable changes in SM production from the *S. somaliensis* Δ*wblA*$_{so}$ mutant were observed and the α-pyrone compound named violapyrone B (VLP B) was isolated. The VLP BGC consisted of a type III polyketide synthase (PKS) gene *vioA* and a pathway-specific positive regulatory gene *vioB*. The inactivation of *vioB* further led to the isolation of another four VLPs analogs, one novel SM and two improved anti-MRSA (methicillin-resistant *S. aureus*, MRSA) SMs compared to VLP B. Transcriptional analysis showed that *wblA*$_{so}$ seemed to regulate the expression levels of *whi* genes and *wbl* genes by different degrees, suggesting an intertwined regulatory mechanism of *wblA*$_{so}$ in SM biosynthesis as well as in morphological differentiation from *S. somaliensis* SCSIO ZH66. The *wblA*$_{so}$ inactivation-driven VLPs identification results imply that the *wblA*$_{so}$ orthologs would be effective targets for the activation of cryptic BGCs in marine-derived *Streptomyces* strains (Huang et al., 2016).

WblA$_{sve}$ in *S. venezuelae*

S. venezuelae ATCC15439 is a versatile producer for various macrolide antibiotics. The 12-membered and 14-membered ring macrolides are biosynthesized by pikromycin BGC (Lee, Park, et al., 2006; Oh & Kang, 2012; Xue et al., 1998; Zhang & Sherman, 2001). Because of low levels of pikromycin production, genetic engineering for titer improvement have been developed (Maharjan et al., 2008; Pyeon et al., 2017; Yi et al., 2018). The *wblA* ortholog gene named *wblA*$_{sve}$ was also found with a high degree of amino acid identity (90% with WblA$_{sco}$) from *S. venezuelae* ATCC15439. Sporulation was blocked by a disruption of *wblA*$_{sve}$ in *S. venezuelae* ATCC15439. The production of pikromycin was increased by 3.5-fold in *S. venezuelae*Δ*wblA*$_{sve}$ and decreased by 2.5-fold in the *wblA*$_{sve}$ overexpressing strain (Woo et al., 2014), suggesting that the WblA$_{sve}$ controls both morphological differentiation and pikromycin production in *S. venezuelae*.

WblA$_{scb}$ in *Streptomyces* sp. CB03234

Streptomyces sp. CB03234 is a native producer of both ten-membered enediyne tiancimycins (TNMs) and diterpernoid tiancilactones (TNLs) (Dong et al., 2018; Yan et al., 2016). Among the TNMs discovered, both TNM-A and TNM-D showed superior cytotoxic activities against several cancer cell lines (Yan et al., 2016, 2018). Comparative transcription analysis between a wild-type and a streptomycin-induced ribosome engineered TNMs high-producer CB03234-S exhibited that the *wblA*$_{scb}$ transcription level was relatively higher than those of other *wbl* genes (Zhang et al., 2020). To overcome the low titer issue of these compounds, the *wblA*$_{sco}$ ortholog named *wblA*$_{scb}$ (tentatively named here) was disrupted via homologous recombination, resulting in 13.9- and 1.7-fold increases of TNM-A in CB3234S and CB3234-S, respectively (Zhang et al., 2020). The production of TNLs, a group of main fermentation metabolites in CB03234, was also affected by the deletion of *wblA*$_{scb}$. Although the *wblA*$_{scb}$ in CB03234 deletion could improve the titers of TNMs significantly, the sporeless bald phenotypes could lead to the CB03234Δ*wblA*$_{scb}$ unsuitable for the scaled-up TNMs production. Since the existence of *wblA*$_{scb}$ is necessary for a spore formation but undesirable for TNMs overproduction, the NitR-ε-caprolactam (CPL) based inducible expression system for *wblA*$_{scb}$ was constructed in the CB03234Δ*wblA*$_{scb}$. This cell factory system successfully maintained the overproduction of TNMs without any additional processes and recovered the normal development of sporulation upon induction (Zhang et al., 2020).

WblA Orthologs as SM Upregulators

Although most WblAs stated above negatively regulate SM biosynthesis in *Streptomyces* species, a couple of WblA orthologs such as WblA$_{ch}$ in *S. chattanoogensis* L10 and WblA$_{sxi}$ in *S. xiamenensis* 318 have been reported to act as SM upregulators in their hosts (Bu et al., 2019; Yu et al., 2014). The *wblA*$_{sxi}$ overexpression under the *Streptomyces* constitutive promoter *ermE*p* stimulated the production of two tetramate macrolactams, ikarugamycin and capsimycin B in *S. xiamenensis* 318 (Bu et al., 2020). Similarly, the WblA$_{ch}$ was proved to function as an activator of natamycin biosynthesis in *S. chattanoogensis* L10. The *wblA*$_{ch}$ disruption downregulated natamycin production, while the *wblA*$_{ch}$ overexpressed stimulated the natamycin yield by ~30% (Yu et al., 2014). These findings suggest that some *wblA* orthologs are positively involved in the regulation of SM biosynthesis in some *Streptomyces* species.

Putative Regulatory Mechanism of WblA in *Streptomyces*

AdpA Binding to the *wblA* Promoter Region

Although many reports support that WblA is a general downregulator of SM biosynthesis in *Streptomyces* species, its regulatory target and mechanism are unclear. Although WblA has been proposed to be a transcriptional factor binding to a specific promoter of the target gene, there is no experimental evidence showing direct binding between WblA and the target promoter sequence (Bush, 2018). On the other hand, some studies on how the *wblA* gene expression is controlled have been reported. The upstream region of *wblA* in *S. coelicolor* was predicted to contain several putative AdpA binding motifs (Lee et al., 2013; Nguyen et al., 2003; Wolanski et al., 2011). AdpA is a key regulator controlling various processes involved in *Streptomyces* morphological differentiation and SM biosynthesis (Higo et al., 2012; Ohnishi et al., 2005). AdpA$_{sc}$ was shown to bind specifically the *wblA*$_{sco}$ upstream binding motifs through an electrophoretic mobility shift assay (EMSA), even though AdpA$_{sc}$ failed to bind to the mutated *wblA* upstream motif (Lee et al., 2013). Moreover, an AdpA$_{sco}$ disruption mutant showed increased *wblA*$_{sc}$ transcription in *S. coelicolor*, suggesting that AdpA$_{sc}$ negatively regulates *wblA*$_{sco}$ transcription in *S. coelicolor* (Fig. 3, Lee et al., 2013).

BldD$_{sgh}$ Binding to the *wblA*$_{gh}$ Promoter Region

Cyclic dimeric 3′–5′ guanosine monophosphate, c-di-GMP, is a ubiquitous second messenger controlling diverse cellular processes in bacteria (Römling et al., 2013). In *Streptomyces*, c-di-GMP plays a crucial role in a complex morphological

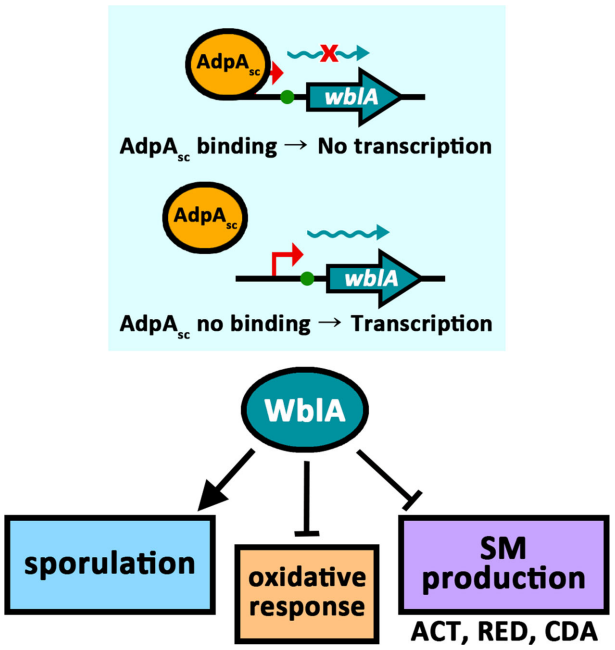

Fig. 3. Regulatory network of WblA controlled by AdpA$_{sc}$ in *S. coelicolor*. ACT, actinorhodin; RED, undecylprodigiocin; CDA, calcium-dependent antibiotic.

differentiation by modulating the activity of the pleiotropic regulator BldD (Tschowri et al., 2014). Previous work on the transcriptional analysis of the BldD-targeted genes in non-*Streptomyces* actinobacterium *Actinoplanes missouriensis* showed that BldD repressed 12 genes, including a *wblA* ortholog (Mouri et al., 2017). The deletion of *bldD*$_{gh}$ led to an increase in *wblA*$_{gh}$ expression. While the *bldD*$_{gh}$-deleted mutant of *S. ghanaensis* showed strongly reduced moenomycin A (MmA) production, the double mutant *S. ghanaensis* Δ*bldD*$_{gh}$Δ*wblA*$_{gh}$ restored the capacity to produce MmA. This suggests that WblA$_{gh}$ plays a crucial role in the regulation of MmA biosynthesis and that BldD$_{gh}$ controls the expression of *wblA*$_{gh}$. EMSA proved the direct control of *wblA*$_{gh}$ transcription by BldD$_{gh}$. Increasing concentrations of BldD$_{gh}$ resulted in the formation of one nucleoprotein complex with the *wblA*$_{gh}$ promoter through EMSA. Moreover, binding of BldD$_{gh}$ to the *wblA*$_{gh}$ promoter was improved significantly in the presence of increasing concentrations of c-di-GMP. The specificity of this interaction is shown by the ability of the unlabeled *wblA*$_{gh}$ promoter to compete with the radiolabeled one for BldD$_{gh}$ (Makitrynskyy et al., 2020). This suggests that the high expression of *wblA* is mediated by the deletion of *bldD*, which leads to the repression of antibiotic synthesis (Yan et al., 2020).

Oxidative Stress Response by WblA

In addition to the *Streptomyces* SM regulation, WblA was also suggested to be involved in the oxidative stress response (Kim et al., 2012). Since WhcA, a WblA ortholog in *C. glutamicum*, was found to play a negative role in the oxidative stress response, *wblA*$_{sco}$ was speculated to have a similar role in *S. coelicolor*. A *wblA*$_{sco}$-deletion mutant showed a less sensitive response to oxidative stress induced by the diamide present in the solid plate culture. Comparative real-time qRT-PCR analysis showed that the transcription levels of oxidative stress-related genes, including *sodF*, *sodF2*, *sodN*, *trxB*, and *trxB2*, were higher in the *wblA*$_{sco}$-deletion mutant than the wild type, both in the absence and presence of oxidative stress. Moreover, expression of these target genes in the *S. coelicolor* wild type was stimulated only in the presence of oxidative stress, suggesting that WblA$_{sco}$ might play a negative role in the oxidative stress response of *S. coelicolor*, similar to that found in *C. glutamicum* WhcA (Kim et al., 2012).

Because WhcA was confirmed to interact with dioxygenase-encoding SpiA (stress protein interacting with WhcA) in *C. glutamicum*, a SpiA ortholog in *S. coelicolor* SCO2553 protein (named SpiA$_{sco}$) was also proposed to interact with WblA in *S. coelicolor*. Using heterologous expression in *Escherichia coli* and *in vitro* pull-down assays, WblA$_{sco}$ was confirmed to bind to the SpiA$_{sco}$, which was influenced by oxidants, such as diamide. These observations suggest that the interaction between WblA$_{sco}$ and SpiA$_{sco}$ is not only specific but also modulated by the redox status of the cell. Moreover, a *spiA*$_{sco}$-disruption mutant exhibited a less sensitive response to the oxidative stress induced by the diamide present in solid plate culture. Real-time qRT-PCR analysis also showed that the transcription levels of oxidative stress response genes were higher in the *spiA*$_{sco}$-deletion mutant than in wild-type *S. coelicolor*. These results show that SpiA$_{sco}$ negatively regulates WblA during the oxidative stress responses in *S. coelicolor* (Kim et al., 2013).

WblA Orthologs in Other Actinobacteria

The Gram-positive rare actinomycete *Pseudonocardia* autotrophica KCTC9441 was previously identified in the novel di-sugar-containing polyene compound producer, which was called NPP (Nystatin-like *Pseudonocardia* Polyene) (Kim et al., 2009). By whole-genome sequencing, a WblA ortholog was isolated and identified from *P. autotrophica*. WblA$_{pau}$ showed 49% amino acid identity with various *Streptomyces* WblAs and 39% amino acid identity with a WblA ortholog, WhcA from *C. glutamicum* (Kim et al., 2014). Although no significant differences in NPP production were observed in the heterologous expression of *wblA*$_{sco}$, a disruption of *wblA*$_{pau}$ resulted in an approximately 80% increase in NPP production (Kim et al., 2014). These results suggest that the biological significance of *wblA*$_{pau}$ might be similar to a previously known *wblA* from various *Streptomyces* strains, even though the amino acid identity was relatively low.

Corynebacterium rarely produces antibiotics and other SMs, there are no reports on WhcA-driven SM regulation. On the other hand, WhcA and other WhiB-like proteins appear to play key roles in the regulation of stress-related processes. WhcA appears to regulate negatively the genes involved in response to oxidative stress (Choi et al., 2009). WhcA was reported to interact directly with its partner, SpiA (Stress protein encoding a dioxygenase/oxidoreductase interacting with WhcA). This WhcA-SpiA interaction was confirmed experimentally to be disrupted in the presence of the oxidant diamide (Park et al., 2012). As stated above, this mechanism appears to be conserved in *S. coelicolor*, in which a SpiA$_{sco}$ was found to bind to WblA$_{sco}$ and downregulate the WblA-dependent oxidative stress response (Kim et al., 2013).

There is also a WblA ortholog named WhiB4$_{mtb}$ in *Mycobacterium tuberculosis* (Bush, 2018). The genome of *M. smegmatis* MC^2 155, a model strain to study *M. tuberculosis*, has been sequenced (Mohan et al., 2015). The *M. smegmatis* MC^2 155 and *M. tuberculosis* share significant similarities in their genome. The *in silico* analysis using antiSMASH 5.0 predicted presence of 18 SM BGCs in MC^2 155. However, little is known on relation between SM regulation and WhiB4mtb. A major role of WhiB4$_{mtb}$ in Mycobacteria is believed to regulate redox-sensing and its homeostasis. A deletion of *whiB4*$_{mtb}$ leads to the hyper-induction of antioxidants, increased resistance to oxidative stress *in vitro*, and enhanced survival in macrophages (Alam et al., 2007; Chawla et al., 2012). WhiB4$_{mtb}$

also contains an O_2- and NO-sensitive [4Fe–4S] cluster and the $WhiB4_{mtb}$ [4Fe–4S] cluster appears to be more sensitive to O_2 than that other reported Wbls (Crack et al., 2009; Kudhair et al., 2017; Singh et al., 2007).

Concluding remarks

WblAs are highly homologous global SM regulators present in most *Streptomyces* species as well as its closely related actinobacteria, of which the detailed mechanism needs to be further elucidated. Although WblAs typically regulate the SM BGC expression at the transcriptional level by controlling the target pathway-specific regulatory gene expression, there was no direct evidence showing direct binding between WblA and the target promoter sequence. The WblA is believed to be controlled by another global regulator such as AdpA and also involved in morphological differentiation and oxidative stress response through its iron-sulfur cluster. Considering the prevalence of WblAs and its conserved regulatory roles, the strategy for selective manipulation of WblAs should provide an efficient approach to improve the SM titer and discover cryptic SMs in actinobacteria.

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Conflict of Interest

The authors declare no conflict of interest.

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