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. 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. Following the initial characterization of WhiB in S. coelicolor (Chater, 1972; Davis & Chater, 1992), multiple paralogs were identified in many other Streptomyces species. 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 WblAsco is a WhiB4 ortholog, which is one of three Wbl-family members that regulate both differentiation and SM biosynthesis in S. coelicolor. In S. coelicolor, a wblAsco 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, WblAsco 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 wblAsco 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 wblAsco 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...
Table 1. WblA orthologs identified from various Streptomyces species

| Gene       | Strain             | Size | Identity (with WblAsco) | Compounds                        | SM production in DIS mutant (fold) | References         |
|------------|--------------------|------|-------------------------|----------------------------------|-----------------------------------|------------------|
| WblAsco    | S. coelicolor M145 | 112 aa | 100%                    | Actinorhodin                      | Increased (5.6-fold)               | Lee et al., 2010 |
| WblAspe    | S. peucetius OIM-1 | 114 aa | 95%                     | Doxorubicin, daunorubicin         | Increased (1.7-fold)               | Kang et al., 2007, Noh et al., 2010 |
| WblAtmc    | Streptomyces sp. CK4412 | 130 aa | 96%                     | Tautomycin                     | Increased (3.2-fold)               | Nah et al., 2012  |
| WblAtgh    | S. griseus ATCC14672 | 128 aa | 96%                     | Mnoenomycin A                   | Increased (2.3-fold)               | Rabyk et al., 2011 |
| WblAtgr    | S. roseopersus NRRL15998 | 116 aa | 90%                     | Daptomycin                      | Increased (2.5-fold)               | Yan et al., 2020  |
| WblAtgan   | S. ansochromogenes 7100 | 112 aa | 96%                     | Nikkomycin (major)              | Increased (1.5-fold)               | Huang et al., 2017 |
| WblAtso    | S. somaliensis ATCC15439 | 115 aa | 90%                     | Nilkmycin                       | Abolished                         | Lu et al., 2015   |
| WblAtsr    | S. venezuelae ATCC15439 | 124 aa | 85%                     | Violapyrone                     | Increased (NR)                    | Huang et al., 2016 |
| WblAtsb    | Streptomyces sp. CB03234 | 131 aa | 93%                     | Pikromycin                      | Increased (3.5-fold)               | Yan et al., 2016  |
| WblAtsa    | S. aveawitilis sp. CB03234 | 129 aa | 85%                     | Tiamicynics                     | Increased (13.9-fold)              | Nguyen et al., 2003 |

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

hyphae, the biological function of wblAsco in SM regulation was not determined (Soliveri et al., 2000). The overexpression of wblAsco inhibited the biosynthesis of actinorhodin (ACT), unde-
cyloprediosin (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. actII ORF4 for ACT, redD/Z for RED, and cdaR for CDA) were reduced in the wblAsco-overexpressing S. coelicolor, implying that wblAsco acts broadly to downregulate antibiotic biosynthesis in S. coelicolor (Kang et al., 2007).

During Streptomyces interspecies DNA microarray analysis, a previously-unidentified tet family transcriptional regulatory gene (SCO1712), as well as a carbon flux regulating 6-phosphofructokinase gene (SCOS5426), were also found to downregulate SM biosynthesis in the S. coelicolor wild-type strain as well as in a wblAsco deletion mutant (Kim et al., 2011; Lee et al., 2010). The S. coelicolor triple knockout mutant (ΔwblAsco, ΔSCO1712, and ΔSCOS5426) showed the highest level of ACT production compared to any of the single and double knockout mutants in S. coelicolor, suggesting that wblAsco along with other wblA-dependent regulat-
ory and precursor pathways were critical for SM production in Streptomyces (Kim et al., 2011).

WblA Orthologs in Other Streptomyces Species

WblAspe in S. peucetius

Subsequently, a wblAspe ortholog named wblAspe 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 wblAspe gene probe. As expected, the production of both DXR and its precursor, daunorubicin (DNR) were improved through gene disruption of wblAspe from S. peucetius OIM (Table 1). Moreover, several putative wblAspe-dependent genes were also identified using interspecies DNA microarray analysis between the S. peucetius OIM and the wblAspe-disrupted S. peucetius OIM. Among the putative wblAspe-dependent genes tested, a con-
served hypothetical protein (SCO4967) further stimulated the production of DXR/DNR/aklavinone in the wblAspe-disrupted S. peucetius OIM. These results suggest that sequential genetic man-
ipulation of the wblAspe 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 engineer-
ing approach in Streptomyces species (Noh et al., 2010).

WblAtmc in Streptomyces sp. CK4412

Tautomycin (TMC) is a linear polyketide compound with a novel activated T cell-specific immunosuppressant and anti-
cancer 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 wblAtmc ortholog gene named wblAtmc. The wblAtmc from Streptomyces sp. CK4412 showed 96% amino acid identity compared to a previously known wblAtmc. (Figs. 1 & 2). The tar-
gested gene disruption of wblAtmc 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 tmCN expression was strongly downregulated by wblAtmc in Strepto-
myces sp. CK4412 (Nah et al., 2012). The tmCN expression was not affected by either deletion or overexpression of wblAtmc in

Fig. 1. WblAs phylogenetic tree among Streptomyces species. Phylogenetic tree was built using the Mega X software by neighbor joining test by Bootstrap. WblAtmc from S. coelicolor, wblAspe from S. peucetius, wblAtmh from Streptomyces sp. CK4412; wblAtgh from S. ghanaensis; wblAtso from S. roseopersus; wblAtgan from S. ansochromogenes; wblAtsr from S. somaliensis; wblAtgr from S. venezuelae; wblAtsb from Streptomyces sp. CB03234; wblAtsa from S. avermitilis; and wblAtsr from S. griseus.
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_{utmc}-dependent TmcT and WblA_{utmc}-independent TmcN, in Streptomyces sp. CK4412 (Nah et al., 2012).

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

Another wblA_{esco} 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_{so} in S. roseosporus**

The wblA_{esco} ortholog gene named wblA_{so} was also identified in the whole genome sequencing of the daptomycin producer *S. roseosporus*. Three types of strains, the wblA_{so} 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_{so} enhanced daptomycin production by more than 50% as well as blocked aerial mycelium sporulation. In contrast, overexpression of wblA_{so} 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_{so} disruption mutant. These results suggest that wblA_{so} plays a key downregulatory role in controlling daptomycin biosynthesis (Huang et al., 2017).

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

Another wblA_{esco} 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 ΔwblAsc 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 ΔwblAsc in S. ansachromogenes might be used to broaden the application of tylosin (Lu et al., 2015).

**WblAsc in S. somaliensis**
A wblAsc ortholog named wblAsc 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 wblAsc from S. somaliensis ZH66 was inactivated. Noticeable changes in SM production from the S. somaliensis ΔwblAsc mutant were observed and the α-pyrone compound named violapryrone 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 (methylcillin-resistant S. aureus, MRSA) SMs compared to VLP B. Transcriptional analysis showed that wblAsc seemed to regulate the expression levels of urch genes and urch genes by different degrees, suggesting an intertwined regulatory mechanism of wblAsc in SM biosynthesis as well as in morphological differentiation from S. somaliensis SCSIO ZH66. The wblAsc inactivation-driven VLPs identification results imply that the wblAsc orthologs would be effective targets for the activation of cryptic BGCs in marine-derived Streptomyces strains (Huang et al., 2016).

**WblAscve 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 wblAscve was also found with high degree of amino acid identity (90% with WblAsc) from S. venezuelae ATCC15439. Sporulation was blocked by the disruption of wblAscve in S. venezuelae ATCC15439. The production of pikromycin was increased by 3.5-fold in S. venezuelaeΔwblAscve and decreased by 2.5-fold in the wblAscve overexpressing strain (Woo et al., 2014), suggesting that the WblAscve controls both morphological differentiation and pikromycin production in S. venezuelae.

**WblAscve in Streptomyces sp. CB03234**
Streptomyces sp. CB03234 is a native producer of both ten-membered enediney tiamycins (TNMs) and diterpenoid tianclactones (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 wblAscve transcription level was relatively higher than those of other urch genes (Zhang et al., 2020). To overcome the low titer issue of these compounds, the wblAscve ortholog named wblAscve (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 wblAscve. Although the wblAscve deletion in CB3234 deletion could improve the titers of TNMs significantly, the spineless bald phenotypes could lead to the CB3234AwblAscve unsuitable for the scaled-up TNMs production. Since the existence of wblAscve is necessary for a sporulation but undesirable for TNMs overproduction, the NitR- and caprolactam (CPL) based inducible expression system for wblAscve was constructed in the CB03234AwblAscve. 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).

**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). AdpAscve was shown to bind specifically the wblAscve upstream binding motifs through an electrophoretic mobility shift assay (EMSA), even though AdpAscve failed to bind to the mutated wblA upstream motif (Lee et al., 2013). Moreover, an AdpAscve disruption mutant showed increased wblAscve transcription in S. coelicolor, suggesting that AdpAscve negatively regulates wblAscve transcription in S. coelicolor (Fig. 3, Lee et al., 2013).

**BldDsgh Binding to the wblA Promoter Region**
Cyclic dimeric 3′–5′ guanosine monophosphate, c-di-GMP, is a ubiquitous second messenger controlling diverse cellular processes in bacteria (Romling et al., 2013). In Streptomyces, c-di-GMP plays a crucial role in a complex morphological
While the elements of oxidative stress-related genes, including trxB, showed a less sensitive response to oxidative stress in the mutant showed a less sensitive response to oxidative stress in the wildtype, both in the absence and presence of oxidative stress. Moreover, expression of these target genes in the wildtype, both in the absence and presence of oxidative stress.

Regulatory network of WblA controlled by AdpA

Fig. 3. Regulatory network of WblA controlled by AdpAn. ACT, actinorhodin; RED, undecylenoylprodigicin; CDA, calcium-dependent antibiotic.

**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, wblAsco was speculated to have a similar role in S. coelicolor. A wblAsco-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 wblAsco-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 WblAsco 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 SpiAsco) was also proposed to interact with WblA in S. coelicolor. Using heterologous expression in Escherichia coli and in vitro pull-down assays, WblAsco was confirmed to bind to the SpiAsco, which was influenced by oxidants, such as diamide. These observations suggest that the interaction between WblAsco and SpiAsco is not only specific but also modulated by the redox status of the cell. Moreover, a spiAsco-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 spiAsco-deletion mutant than in wild-type S. coelicolor. These results show that SpiAsco 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*. WblApau 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 wblAsco, a disruption of wblAsco resulted in an approximately 80% increase in NPP production (Kim et al., 2014). These results suggest that the biological significance of wblAsco might be similar to a previously known wblA from various *Streptomyces* strains, even though the amino acid identity was relatively low.

*Corinebacterium* 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 (Cho 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 SpiAsco was found to bind to WblAsco and downregulate the WblA-dependent oxidative stress response (Kim et al., 2013).

There is also a WblA ortholog named WhiB4mtb in *Mycobacterium tuberculosis* (Bush, 2018). The genome of *M. smegmatis* MC2 155, a model strain to study *M. tuberculosis*, has been sequenced (Mohan et al., 2015). The *M. smegmatis* MC2 155 and *M. tuberculosis* share significant similarities in their genome. In *in silico* analysis using antiSMASH 5.0 predicted presence of 18 SM BGCs in MC2 155, a model strain to study *M. tuberculosis* (Bush, 2018). The genome of *M. smegmatis* MC2 155, a model strain to study *M. tuberculosis*, has been sequenced (Mohan et al., 2015). The *M. smegmatis* MC2 155 and *M. tuberculosis* share significant similarities in their genome. In *in silico* analysis using antiSMASH 5.0 predicted presence of 18 SM BGCs in MC2 155. However, little is known on relation between SM regulation and WhiB4mtb. A major role of WhiB4mtb in *Mycobacteria* is believed to regulate redox-sensing and its homeostasis. A deletion of whiB4mtb 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). WhiB4mtb
also contains an O2- and NO-sensitive [4Fe–4S] cluster and the WhiB4$_{trans}$ [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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