Positive Feedback Regulation of *stgR* Expression for Secondary Metabolism in *Streptomyces coelicolor*

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LysR-type transcriptional regulators (LTTRs) compose a large family and are responsible for various physiological functions in bacteria, while little is understood about their regulatory mechanism on secondary metabolism in *Streptomyces*. Here we reported that StgR, a typical LTTR in *Streptomyces coelicolor*, was a negative regulator of undecylprodigiosin (Red) and γ -actinorhodin (Act) production in the early developmental phase of secondary metabolism by suppressing the expression of two pathway-specific regulator genes, *redD* and *actII-orf4*, respectively. Meanwhile, *stgR* expression was downregulated during secondary metabolism to remove its repressive effects on antibiotic production. Moreover, *stgR* expression was positively autoregulated by direct binding of StgR to its own promoter (*stgRp*), and the binding site adjacent to translation start codon was determined by a DNase I footprinting assay. Furthermore, the StgR-*stgRp* interaction could be destroyed by the antibiotic γ -actinorhodin produced from *S. coelicolor*. Thus, our results suggested a positive feedback regulatory mechanism of *stgR* expression and antibiotic production for the rapid and irreversible development of secondary metabolism in *Streptomyces*.

LysR-type transcriptional regulators (LTTRs), first named after LysR, a transcriptional activator of *lysA* in *Escherichia coli* (1), have expanded considerably to the largest family after 3 decades and spread ubiquitously in bacteria. They have been structurally well characterized to have a conserved helix-turn-helix (HTH) motif at the N terminus for DNA binding and a regulatory domain for substrate or inducer binding at the C terminus (2). Consistent with their wide distributions and great quantities, they have diverse and conserved regulatory functions in bacteria for primary metabolism (3, 4), secondary metabolism (5, 6), stress responses (7), cell division (8), virulence (9, 10), protection (11), etc. Typically, most LTTRs function as the global transcriptional regulators by directly binding to the promoters of their regulons. Upon signaling, the assimilated extracellular ligands or produced intracellular metabolites can act as substrates or inducers to interact with the C-terminal domain to cause conformation changes to influence the DNA-binding affinity of LTTRs (2, 12).

Species of *Streptomyces*, the soil-dwelling Gram-positive bacteria, are well-known for their complex morphogenesis and secondary metabolism. Among their abundant secondary metabolites, antibiotics are produced with patent clinical or commercial applications (13). The onset of secondary metabolism is triggered by environmental stimuli, and subsequent intracellular signaling pathways are equally required for felicitous development of secondary metabolism (14). Nutrients, including *N*-acetylglucosamine, and autoregulatory factors, such as γ -butyrolactones, can regulate antibiotic production through DasR- and AdpA-mediated signaling pathways, respectively (14, 15). Other global regulatory systems, including the two-component system (TCS), ppGpp, alternative sigma factors, etc., all play essential roles in antibiotic production (16–19). Most of the signaling pathways cross talk and converge on the promoters of synthesis gene clusters or the pathway-specific regulator genes for proper production of antibiotics (20).

LysR-type transcriptional regulators (LTTRs) also distribute widely in *Streptomyces*. Whole-genome sequencing revealed about 40 LTTRs in *Streptomyces coelicolor* (21), followed by *Streptomyces*

avermitilis with 33 and *Streptomyces venezuelae* with 31, suggesting a potentially complex interplay of these regulators in the complicated life cycles of *Streptomyces*. However, only several pathway-specific LTTRs have been reported as transcriptional activators for the biosynthesis of antibiotics in *Streptomyces*, such as FkbR from *Streptomyces tsukubaensis* for tacrolimus (5), ThnI from *Streptomyces cattleya* for thienamycin (6), and AbaB from *Streptomyces antibioticus* for actinorhodin and undecylprodigiosin, while ClaR from *Streptomyces clavuligerus* functions as a repressor for cephamycin production (22). Other numerous LTTRs in *Streptomyces*, especially those located outside gene clusters and potentially having more global effects on antibiotic biosynthesis, have not been functionally examined, and their regulatory mechanisms on secondary metabolism are poorly understood.

In this report, we present evidence that StgR is a LysR-type transcriptional repressor in the early step of secondary metabolism and that *stgR* expression is regulated in a positive feedback manner for the proper development of secondary metabolism in *S. coelicolor*, providing for the first time a regulatory mechanism of a LysR-type transcriptional regulator for the development of *Streptomyces*.

MATERIALS AND METHODS

Strains and media. *Escherichia coli* strains were cultured in LB medium. Liquid 3% Trypticase soy broth (TSB) plus 5% polyethylene glycol 6000 (PEG 6000) was used for vegetative mycelium preparation in primary metabolism. Solid R2YE and liquid YEME media were used for cell differentiation of *S. coelicolor*, and MS medium was used for spore preparation (13, 23).

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TABLE 1 Plasmids and cosmids used in this study

Plasmid or cosmid	Description	Source or references
pSET152	Integrative shuttle vector	32
pL220	<i>stgR</i> in pSET152	This study
pTA2	T vector	Toyobo, Japan
pL221	<i>stgR</i> promoter full length (<i>stgRpFL</i>) in pTA2	This study
pL222	130 bp of <i>SCO2965</i> promoter (<i>SCO2965p</i>) in pTA2	This study
pL223	148 bp of <i>stgRp</i> in pTA2	This study
pIJ8660	Promoter-probing plasmid	24
pL224	278 bp of <i>stgRp</i> in pIJ8660	This study
pET32a	<i>E. coli</i> expression vector	Novagen
pL225	<i>stgR</i> in pET32a	This study
N6-68	Cosmid containing <i>stgR</i>	Zhong-Jun Qin, personal communication
pL226	<i>stgR</i> disruption cosmid, N6-68 containing <i>stgR::FRT-aadA-FRT</i>	This study
pL227	<i>stgR</i> disruption cosmid, N6-68 containing <i>stgR::FRT</i>	This study
pLM26	Kanamycin resistance gene and <i>ermEp*</i> in pIJ8630	18
pL228	<i>stgR</i> in pLM26	This study

Plasmid construction. All plasmids and primers are listed in Table 1 and Table 2, respectively. Primers 1 and 2 were used to amplify *stgR* with a promoter and terminator, which was digested with BglII/EcoRI and ligated into BamHI/EcoRI site of pSET152 to give rise to the complementation plasmid pL220. Primer pairs 1 and 3, 1 and 4, and 3 and 5 were used for amplification of promoters *stgRpFL*, *SCO2965p*, and *stgRp*, respectively. After dA addition with *Taq* polymerase (TaKaRa), all promoters were ligated into pTA2 to create plasmids pL221, pL222, and pL223, respectively. The *stgRpFL* fragment from pL221 digested with BglII was linked to pIJ8660 (24) digested with BglII for plasmid pL224. The *stgR* was amplified with primers 6 and 7, digested with BamHI/XhoI, and inserted into the BamHI/XhoI site of pET32a to generate pL225. Primers 19 and 20 were used for *stgR* open reading frame (ORF) amplification. *stgR* was ligated into pTA2 and digested with NdeI/NotI inserted into pLM26 for plasmid pL228. KOD plus *neo* (Toyobo) was used for all PCRs, and DNA fragments were verified by DNA sequencing.

TABLE 2 Primers used in this study

Primer no.	Sequence (5' to 3')
1	ACTAAGATCTGGGCAGCCGGCGGTGAGATTC
2	ATAGAATTCGCCCCACCAGGTTTCGAGCG
3	ACTAAGATCTCGCGCACTTACAACGGCGGTG
4	CGCGACACCCTGGTCGC
5	GTGACGGCCAGGAGGGG
6	ATTAGGATCCATGCCCGCACCAGCCACC
7	ATTACTCGAGTCACCTGTGGACGGACATCAC
8	TTGTAAGTTTCGCCGGATGCCCGCACCAGCCACCTC GACATTCCGGGGATCCGTCGACC
9	ATGTAGCGCACGCGGAGGATGTGTGTTGCCGCGTG TCATGTAGGCTGGAGCTGCTTC
10	CCATCCGCTCATGGGAGTG
11	TACAGGCTGGGTCCGTGGTC
12	CCTGGTGCTGCTGCTCCTCA
13	CGTCTGCAGCGTCGTCATG
14	CGCGGGCTTCGTGCTGTCC
15	TTGCCGATCTGCTTGAGGTAGTCC
16	Biotin-GCCAGGGTTTCCCAGTCACGA
17	GAGCGGATAACAATTTACACAGG
18	6-FAM-GTTGTAAAACGACGGC
19	CATATGCCCGCACCAGCCACC
20	GCGGCCGCTCACTGTGGACGGACATCACAGC

Strain construction. The *Streptomyces coelicolor* strains used in this study are listed in Table 3. *stgR* was disrupted by a PCR-targeting strategy (25). The *stgR::FRT-aadA-FRT* disruption cassette was amplified with primers 8 and 9 from the EcoRI/HindIII fragment in pIJ779 and introduced into *E. coli* BW25113/pIJ790 with cosmid N6-68 for cosmid pL226. ("FRT" is the FLP recombination target.) The in-frame deletion cosmid pL227 for insertion inactivation of *stgR* was created by passage of cosmid pL226 through *E. coli* BT340 cells grown at 42°C. Disruption cosmid pL227 was conjugated by *E. coli* ET12567/pUZ8002 into wild-type strain M145 to in-frame knock out *stgR* for strain L188. The genotypes of all strains were verified by PCR and Southern blotting (our unpublished data).

Quantification and preparation of secondary metabolites. Quantitative measurements of actinorhodin (Act) or undecylprodigiosin (Red) were described previously (18). Preparation of crude extract of γ -actinorhodin and its intermediates was described previously (26). For extracellular metabolite preparation, wild-type M145 cells were cultured in liquid R5—medium (23) for 6 days, and supernatant was acidified to pH 3.0 with HCl, extracted with 2 volumes of ethyl acetate three times, and vacuum evaporated. The mycelia were lysed by ultrasonication, and the supernatant was extracted with 2 volumes of ethyl acetate three times and vacuum evaporated for preparation of intracellular Act and intermediates. The residues were resuspended in dimethyl sulfoxide (DMSO) at 100 μ g/ μ l. The extracellular γ -actinorhodin was purified by high-performance liquid chromatography (HPLC) as described previously (27) and resuspended in DMSO at 10 μ M.

RNA preparation, reverse transcription, and qRT-PCR. RNA from mycelia of wild-type (M145) or Δ *stgR* (L188) in YEME medium at different stages of secondary metabolism was prepared by ultrasonication and acid-phenol extraction as described previously (18). Genomic DNA was removed by RNase-free DNase I (TaKaRa), and cDNA was prepared with Moloney murine leukemia virus (MMLV) reverse transcriptase as described by the manufacturer (TaKaRa). Quantitative real-time PCR (qRT-PCR) was performed in two independent experiments with SYBR Premix Ex Taq II (TaKaRa) with primer pair 10 and 11 for *redD*, primer pair 12 and 13 for *actII-orf4*, and primer pair 14 and 15 for *hrdB*. The housekeeping gene *hrdB* was used as an internal control. Fold changes of

TABLE 3 *Streptomyces coelicolor* strains in this study

Strain	Description or genotype	Reference
M145	Wild type	13
L188	<i>stgR</i> in-frame deletion, Δ <i>stgR::FRT</i>	This study

FIG 1 StgR negatively regulates the early developmental phase of secondary metabolism. (A) Wild-type strain M145, the $\Delta stgR$ mutant (L188), and the complementation strain L188/pL220 ($\Delta stgR$ + *stgR*) were streaked on the R2YE plate simultaneously, incubated at 30°C for the indicated time, and photographed. (B and C) Quantitative assay of antibiotic production. M145 and $\Delta stgR$ mutant (L188) mycelia were collected from YEME medium at various stages of secondary metabolism. Undecylprodigiosin (Red) (B) and actinorhodin (Act) (C) were quantitatively measured by absorbance (optical density [OD]) at the 530- or 640-nm wavelength, respectively. The ratios of absorbance to wet weight were calculated, and the numbers in the graphs are the means of three independent experiments. Standard deviations (SD) are shown as error bars. (D and E) Quantitative assay of gene expression of pathway-specific regulators for antibiotic production. RNA was prepared from M145 and $\Delta stgR$ mutant (L188) mycelia from YEME medium and reverse transcribed. Fold changes were shown as expression ratio of *redD* to *hrdB* (D) or *actII-orf4* to *hrdB* (E) as measured by qPCR in two independent experiments. (F) Spores of wild-type strain M145 (WT) and wild-type cells with *stgR* overexpression under *ermEp** (WT + *ermEp*-stgR*) were streaked on R2YE medium for the time indicated and photographed.

redD or *actII-orf4* expression were quantified as $2^{-\Delta\Delta CT}$ according to the TaKaRa protocol.

EMSA. For the electrophoretic mobility shift assay (EMSA), BL21(DE3) cells containing expression vector pET32a or pL225 (pET32a-*stgR*) were induced to express soluble 6-His-tagged TrxA or 6-His-TrxA-StgR with 0.1 mM IPTG (isopropyl- β -D-thiogalactopyranoside) at 18°C overnight. Both proteins were purified with Ni^{2+} -nitrilotriacetic acid (NTA) as described by the manufacturer (Novagen, Merck). All promoter regions were cloned in pTA2, and 5'-biotin-labeled probes were amplified by PCR with 5'-biotin-labeled universal forward primer 16 and reverse primer 17. About 1 ng of probe was incubated with 50 or 100 ng of purified proteins at 25°C in buffer (10 mM Tris, 100 mM Na_2HPO_4 [pH 8.0], 50 μ g/ml sheared sperm DNA) for 30 min and loaded on 5% native polyacrylamide gel for separation in 0.5 $\times$ Tris-borate-EDTA (TBE) running buffer. DNA was then electroblotted to the nylon membrane, UV fixed, and detected with streptavidin-horseradish peroxidase (HRP) and BeyoECL Plus (Beyotime, China). For competition assays, the crude extracts or antibiotics were incubated with 100 ng of purified StgR for 30 min,

followed by addition of biotin-*stgR* probe and further incubation for 30 min.

DNase I footprinting assay. Purified StgR protein was ultrafiltered with YM-10 (Millipore) for the 10-kDa cutoff and buffer exchanged with 20 mM Tris-HCl (pH 7.5). 5'-6-Carboxyfluorescein (5'-FAM)-labeled *stgR* probe was amplified with universal primers 18 and 17 from plasmid pL223 and gel purified. About 50 ng of probe was incubated with 3 μ g of StgR or without StgR in 20 mM Tris-HCl (pH 7.5)-5% glycerol at room temperature for 30 min, and 0.03 U of DNase I (Promega) was added in the presence of 10 mM $MgCl_2$ and 1 mM $CaCl_2$. After partial digestion for exactly 1 min at room temperature, reactions were stopped by an equal volume of 100 mM EDTA (pH 8.0), immediately followed by phenol-chloroform extraction, precipitation with 0.75 M NH_4Ac , 40 μ g of glycogen, 70% ethanol, and washing with 70% ethanol. DNA mixed with Liz-500 DNA marker (MCLAB) was loaded into an ABI 3130 sequencer, and electropherograms were analyzed with Genemapper v4.0 software (Applied Biosystems) to align and determine the protected region. The DNA sequencing ladder was prepared with 5'-FAM-labeled universal primer 18

FIG 2 Expression profile of *stgR* during secondary metabolism. Mycelia of M145/pL224 (wild type + *stgRp-egfp*) and L188/pL224 (Δ *stgR* + *stgRp-egfp*) were collected from YEME for the indicated time. Protein samples were extracted by sonication, and about 20 μ g of total protein was loaded for Western blotting with anti-GFP (α -GFP) antibody or Coomassie brilliant blue staining as the loading control.

according to the instructions of the Thermo Sequenase dye primer manual cycle sequencing kit (USB).

RESULTS AND DISCUSSION

StgR, a LysR-type transcriptional regulator, negatively regulated the early development of secondary metabolism. *SCO2964*, encoding a protein with a helix-turn-helix (HTH) motif at the N terminus and type 2 periplasmic binding proteins (PBP2) at the C

terminus by BLAST and with highest identity (36.6%) to *Streptomyces lipmanii* LysR-type transcriptional regulator StgR (<http://streptomyces.org.uk/>), was therefore named *stgR* in *Streptomyces coelicolor*.

stgR was not located in a gene cluster, suggesting a possible global role in developmental programs of *S. coelicolor*. On solid R2YE medium, *S. coelicolor* could produce the red antibiotic undecylprodigiosin and the blue antibiotic actinorhodin during cell differentiation (13). After growth for 22 h on R2YE medium, the Δ *stgR* mutant displayed a red appearance much earlier than the wild type, and 38 h later, blue pigments were observed in the Δ *stgR* mutant but not in wild-type cells (Fig. 1A), suggesting deletion of *stgR* resulted in earlier development of secondary metabolism. However, no significant difference was observed between the wild type and Δ *stgR* mutant after 2 days or later. Complementation with wild-type *stgR* under its native promoter could restore the phenotypes of earlier secondary metabolism development of the Δ *stgR* mutant to a level similar to that of the wild type (Fig. 1A). Consistent with these observations, quantitative measurement of undecylprodigiosin (Red) and γ -actinorhodin (Act) also showed that the Δ *stgR* mutant produced both antibiotics much earlier and at higher levels than the wild type, but the difference began to narrow 60 h later, and similar production levels of Red and Act

FIG 3 StgR binds to the *stgR* promoter (*stgRp*). (A) Intergenic region organization of *stgR* (*SCO2964*) and *SCO2965*. The lengths of various probes for EMSA in panels B and C are shown. (B) StgR bound to the whole intergenic region. StgR was expressed in pET32a and purified through Ni²⁺-NTA. 5'-Biotin-labeled EMSA probes from pTA2 (V) or pL221 (V-*stgRpFL*) were used for binding assays with 0, 50, or 100 ng of purified StgR. The shifted bands are the protein-DNA complex. (C) StgR bound to the *stgR* promoter (*stgRp*). 5'-Biotin-labeled EMSA probes amplified from pL222 (V-*SCO2965p*) or pL223 (V-*stgRp*) were used for binding assays with 100 ng of purified StgR. (D) Protein control of StgR-*stgRp* interaction. One hundred nanograms of purified 6-His-TrxA (32a) expressed from pET32a or 6-His-TrxA-StgR (32a-StgR) expressed from pL225 was incubated with 5'-biotin-labeled *stgRp* probe in the EMSA.

FIG 4 DNase I footprinting assay for StgR binding site determination. (A) 5'-FAM-labeled *stgRp* probe was used in the DNase I footprinting assay with or without purified StgR. The protected region is underlined and italic and has been annotated with the DNA sequence. (B) The promoter region of *stgR*. The StgR binding site deduced from the DNase I footprinting assay is underlined and italic, and the translation start codon is boxed.

were observed after 3 or 5 days, respectively (Fig. 1B and C). Furthermore, quantitative real-time PCR showed that expression of *redD* and *actII-orf4*, encoding two pathway-specific transcription activators for Red and Act production, respectively (28, 29), was upregulated in the Δ *stgR* mutant concomitant with accelerated development of secondary metabolism (Fig. 1D and E). However, we did not observe StgR bound to the promoter of *redD* or *actII-orf4* (our unpublished data), suggesting an indirect effect of StgR on gene expression. Nevertheless, these data suggested a repressive effect of *stgR* on *redD* and *actII-orf4* expression for Red and Act production, respectively, in the early developmental phase of sec-

ondary metabolism. Meanwhile, overexpression of *stgR* under a strong constitutive promoter, *ermEp** (30), also caused a delayed production of Red and Act in wild-type cells after 28 and 50 h, respectively (Fig. 1F), further supporting the idea that StgR functioned as a negative regulator of secondary metabolism. Deletion of *stgR* did not result in a significant morphological difference (our unpublished data). However, when wild-type cells differentiated into aerial mycelia or spores with a white or gray appearance, respectively, the wild-type strain with *stgR* overexpression still remained in the substrate mycelia and aerial mycelia 24 and 56 h later, respectively (Fig. 1F), suggesting overexpression of *stgR* could delay morphological development.

Downregulation and positive autoregulation of *stgR* expression during secondary metabolism. Since StgR acted as a transcription repressor on secondary metabolism, we next checked the expression profile of *stgR* during secondary metabolism. With a green fluorescent protein (GFP) reporter assay, where the *stgR* promoter (*stgRp*) was placed just in front of the *gfp* gene in a promoter-probing plasmid, pIJ8660 (24), we found a continuous decreased protein level of GFP when cells produced Red and Act both in the wild type and in the Δ *stgR* mutant (Fig. 2), suggesting *stgR* expression was downregulated during secondary metabolism. Meanwhile, it was also observed that the GFP protein level in the Δ *stgR* mutant was much lower than that in the wild type at all developmental phases (Fig. 2), suggesting that *stgR* was positively autoregulated. These results suggested that the downregulated *stgR* expression could remove the repressive effects of StgR for the appropriate development of secondary metabolism, and positive autoregulation might contribute to the fast downregulation of *stgR* expression during secondary metabolism, which was also consistent with the observations that overexpression of *stgR* under a strong constitutive promoter could cause delayed cell differentiation. We also speculated that some aspect of secondary metab-

FIG 5 Actinorhodin from *S. coelicolor* can disrupt StgR-*stgRp* interaction. Intracellular (A) or extracellular (B) crude extract with ethyl acetate (EA) from wild-type cells or HPLC-purified γ -actinorhodin (C) was incubated with StgR in a concentration gradient before addition of biotin-labeled *stgRp* probe. DMSO was the solution control. (D) The antibiotics ampicillin (Amp), kanamycin (Km), apramycin (Apra), hygromycin (Hygro), and streptomycin (Strep) were used at 5 μ g/ μ l for the EMSA binding competition assay as in panel C. A 5- μ g/ μ l concentration of extracellular ethyl acetate extracts (EA extract) was the positive control for the binding competition.

olism, or perhaps some products of secondary metabolism, might reduce *stgR* expression during secondary metabolism.

StgR binds to upstream of *stgR*. Next, we explored whether StgR regulated its own expression by binding to its promoter. The ORFs of *stgR* and its adjacent gene, *SCO2965*, are in the opposite orientation (Fig. 3A). EMSA showed that purified StgR could bind to the whole intergenic region of *stgR* and *SCO2965* (*stgRpFL*) (Fig. 3B). The 278 bp of *stgRpFL* region contained both *stgR* promoter (*stgRp*) and *SCO2965* promoter (*SCO2965p*). To narrow down the binding region and to exclude the possibility of StgR binding to *SCO2965p*, the 278-bp region was divided into two parts, 130 bp and 148 bp, which approximately corresponded to *SCO2965p* and *stgRp*, respectively (Fig. 3A). EMSA showed that StgR could bind to *stgRp*, but not to *SCO2965p* (Fig. 3C). Moreover, the protein control TrxA expressed from pET32a did not bind to *stgRp* (Fig. 3D), further confirming the binding specificity of StgR to its own promoter. Meanwhile, it was found that the binding site of StgR on *stgRp* was very close to the translation start codon as determined by a DNase I footprinting assay (Fig. 4). These results strongly suggested a direct interaction of StgR to its promoter. Combined with previous gene expression profile analysis (Fig. 2), our results also suggested that StgR bound to its promoter to positively regulate its expression.

Secondary metabolite could prevent StgR-*stgRp* interaction. Next we investigated whether some products in secondary metabolism could affect *stgR* expression. We observed that the intracellular and extracellular ethyl acetate (EA) extracts from wild-type cells in secondary metabolism could obviously destroy the binding of StgR to *stgRp*, respectively (Fig. 5A and B). Thus, it was speculated that some compounds produced in secondary metabolism could inhibit binding of StgR to its promoter. The intracellular ethyl acetate extract contains γ -actinorhodin and its intermediates, while the extracellular ethyl acetate has γ -actinorhodin, which is exported outside after its synthesis is completed inside (26, 29). The extracellular γ -actinorhodin was purified by HPLC (27), and it could inhibit StgR-*stgRp* binding at a low concentration (10^{-7} M) (Fig. 5C). However, the antibiotics from fungi (ampicillin) or other *Streptomyces* spp. (kanamycin, apramycin, hygromycin, and streptomycin) even at a high concentration ($5 \mu\text{g}/\mu\text{l}$ or $>10^{-3}$ M) had no effects on binding of StgR to *stgRp* (Fig. 5D), supporting the hypothesis that only the secondary metabolites from *S. coelicolor* could have their roles in disruption of the StgR-*stgRp* interaction.

Streptomyces spp. are typically environmental bacteria, which have evolved to adapt multiple measures to accommodate variable surroundings, such as being in the dormant spore form under hazardous conditions but having active vegetative mycelia under favorable circumstances during their complex morphological development (31). Meanwhile, the secondary metabolites produced during secondary metabolism to inhibit the growth of their surrounding species, especially after nutrition depletion, are ecologically essential for their competitive survival (13). Therefore, it will be very important to adjust rapidly, especially to secondary metabolism after environmental stresses. The existence of abundant LysR-type transcriptional regulators (LTTRs) in *Streptomyces* is reminiscent of their potentially diverse roles in regulation of morphological development and secondary metabolism. Here, using *S. coelicolor* as an example, we reported that an LTTR StgR was a transcriptional repressor in the early phase of secondary metabolism. Our results also revealed that secondary metabolites could

FIG 6 A proposed model of positive feedback regulation of *stgR* expression for secondary metabolism.

also act as the regulators of their own fast production, since a small amount of secondary metabolites produced after onset of secondary metabolism accumulated and competitively interfered with binding of StgR to its own promoter, *stgRp*, thus resulting in disassociation of StgR from *stgRp*, decreased expression of *stgR*, and increased production of secondary metabolites. This positive feedback regulation of *stgR* expression is economical, though very simple, but leads to the rapid repressed expression of *stgR* and the irreversible development of secondary metabolism (Fig. 6).

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