Supporting Information

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SI Materials and Methods

HPLC Analysis of Undecylprodigiosin Production. The cultures of *Streptomyces coelicolor* in supplemented minimal medium (SMM) were centrifuged at 16,200 × *g* for 5 min. The cell pellets were resuspended in Tris·HCl buffer (pH 7.2), sonicated for 10 min, and extracted by chloroform. The extracts were dried, redissolved in methanol, and filtered through a 0.22-μm membrane. Then, the samples were subjected to HPLC analysis on a Shimadzu Prominence HPLC system with dual λ UV detector and a YMC polymer-C18 column (4.6 × 250 mm). Separation was performed at the following conditions: 0–15 min, acetonitrile (85–100%); 15–20 min, acetonitrile (100%); and 20–26 min, acetonitrile (100–85%) for a total run time of 26 min. The retention time of undecylprodigiosin (Red) at these conditions was 10.6 min; the corresponding peak areas detected at 542 nm were used to calculate the concentration of Red.

LC-MS Analysis of Red. The peaks matched Red standard was collected and further identified by LC-MS. Mass detection was performed on Agilent Technologies 6460 Triple Quad LC/MS ion-trap equipped with an electrospray interface. Optimized MS parameters were as follows: ion spray voltage, 3.5 kV; ion source temperature, 350 °C.

Gel Mobility Shift Assays (EMSAs). The DNA probe (~6 ng) was incubated with purified ScbR2 (0.3 μM) and different concentration of antibiotics at 25 °C for 30 min in a buffer containing 20 mM Tris-base (pH 7.5), 2 mM DTT, 5 mM $MgCl_2$, 0.5 μg/μL calf BSA, and 5% glycerol in a total volume of 20 μL. After incubation and electrophoresis, the nondenaturing 4.5% polyacrylamide gels were stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen) for 30 min in TBE buffer (89 mM Tris-base, 89 mM boric acid, 1 mM EDTA, pH 8.0). The staining images were photographed under a UV transilluminator by using Bio-Rad GelDoc XR.

Construction of *gusA* Reporter Plasmids and Reporter Plasmid-Containing Strains. To construct the plasmid pLCgus, *gusA* was amplified with primers gusAF and gusAR from pGusT-ermE-E*, and the vector fragments of pLC803 were amplified with primers LCF and LCR from pLC803. Then, the two fragments were ligated by the enzymatic assembly method described by Gibson et al. (1) to generate pLCgus. To construct the plasmids pLCgus-adpAp and pLCgus-redDp, the promoters of *adpA* (*adpA*p) and *redD* (*redD*p) were amplified with primer pairs adpApF/adpApR and redDpF/redDpR, respectively. The fragments of pLCgus were amplified with primers pLCgusF and pLCgusR. Then, the pLCgus fragments were enzymatic assembled with the promoters of *adpA* and *redD*, respectively. The plasmids pLCgus-adpAp and pLCgus-redDp were transformed into *S. coelicolor* M145, ΔscbR2, and M145::scbR2 by conjugation, and the transformants were used to detect the activities of the corresponding promoters.

β-Glucuronidase Activity Assay. To measure the glucuronidase activity of GusA, a spectrophotometric method was used (2). The transformants were inoculated into liquid SMM and grown for 48 h at 28 °C and 220 rpm. Mycelia (of 2-mL culture) were harvested by centrifugation, washed once with distilled water, and resuspended in 0.5 mL of lysis buffer (2). Lysis was performed at 37 °C for 15 min. Then, 5 μL of 0.2 M 4-nitrophenyl β-D-glucuronide was added to the samples. When the reaction appeared yellow, 0.5 mL of 1 M Na_2CO_3 was added to terminate the reactions (3). Absorption at 415 nm was measured and divided by the wet weight of used mycelia and the reaction time to obtain Gus units (GU) [A415/(g·min)]. The assays were performed in triplicate. Data are presented as the means ± SD.

ChIP-Quantitative PCR. Samples of *S. coelicolor* M145 grown in SMM for different times were treated with 1% formaldehyde for 30 min at room temperature before glycine was added to a final concentration of 0.125 M to halt cross-linking. Cells were collected, washed with cold 1× PBS twice, and resuspended in lysis buffer (50 mM Hepes, pH 7.5, 137 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholate Na, 0.1% SDS, 1 mM PMSF, 1 μg/mL leupepatin). The sample was sonicated for 20 min at 2 s/8 s at 300 W to shear chromosomal DNA into fragments ranging from 200 to 500 bp on average. After centrifugation at 13,400 × *g* for 10 min at 4 °C to remove debris, 1 mL of supernatants diluted with the same volume of lysis buffer were precleared with 50 μL of salmon sperm (SS) DNA/Protein A Sepharose for 60 min at 4 °C. After centrifugation at 1,100 × *g* for 1 min, we transferred the supernatant to a new tube. ScbR2 monoclonal antibody was prepared by CoWin Biotech Co. Ltd. by using the method of Saito et al. (4). ScbR2 antibody was added to a final concentration of 1 μg/mL, and the samples were incubated overnight at 4 °C on a rotating wheel. Then, 50 μL of SS DNA/Protein A Sepharose was added, and incubation was continued for 8 h at 4 °C .The Sepharose beads were then washed with 1 mL of each of the following buffers: lysis buffer plus 0.5 M NaCl, 1× LNDET (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris, pH 8) and 50 mM Tris·HCl (pH 8.0) and 1 mM EDTA. Immunoprecipitated complexes were then eluted from the beads with freshly made Elute buffer (1% SDS, 0.1 M $NaHCO_3$, 10 mM EDTA, pH 8.0). Then, 0.2 M NaCl was added to each tube, and cross-linking was reversed by incubation at 65 °C for 10 h. Residual proteins were degraded by the addition of 200 μg/mL Protein K (in 10 mM EDTA and 40 mM Tris, pH 8.0) and incubation at 65 °C for 2 h, followed by phenol/chloroform/isopropanol alcohol extraction and ethanol precipitation. Pellets were washed with 70% EtOH and resuspended in 10 μL of TE. Approximately 1–2 μL was used for PCR. Quantitative PCR (qPCR) was used to determine the amounts of genomic DNA immunoprecipitated in the ChIP experiments. qPCR was performed by using the primer pairs redDpF/redDpR and adpApF/adpApR (Table S2) on Rotor-Gene Q and detected by the SYBR Green detection system with the following program: 95 °C for 15 min, 1 cycle; 95 °C for 10 s and 65 °C for 30 s, 40 cycles. The relative levels of P*adpA* and P*redD* coprecipitated with ScbR2 were calculated by comparison with their respective IgG controls.

Construction of AdpA Overexpression Strains. To construct the plasmid pEadpA, *adpA* was amplified with primers adpAF and adpAR (Table S2). The pIMEP fragments were amplified with primer pairs IMEPF/IMEPR. Then, the pIMEP and *adpA* fragments were enzymatic assembled (1). To construct the plasmid pRadpA, *adpA* was amplified with primers adpAF2 and adpAR2. The pGusT-ermE-E* fragments were amplified with primer pairs GusTF and GusTR. The two fragments were enzymatic assembled (1). The plasmids pEadpA and pRadpA were transformed into *S. coelicolor* M145 by conjugation, and the transformants were used to observe the effects of *adpA* expression on Red production in liquid SMM or on plate; M145 was included as control.

Preparations of Jadomycin Pathway Intermediates and Other Angucyclines. Jadomycin B (JdB)–Nle (norleucine), JdB–Hse (homoserine), and JdA were isolated from *Streptomyces venezuelae*

ISP5230 cultures as described (5). We isolated 2,3-dehydro-UWM6 (DHU) from VS668 culture as described (6). Gaudimycin A and C were provided by M. Metsä-Ketelä (University of Turku, Turku, Finland) (7). Landomycin D and A were provided by Biao Y. (Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences) (8).

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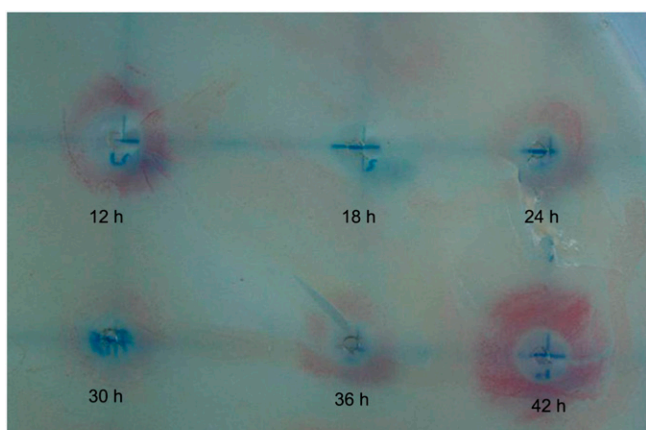

Fig. S1. Plate assay of the responses of *S. coelicolor* (grown on SMM agar) to extracts of *S. venezuelae* ISP5230 grown after different times in glucose–Mops medium.

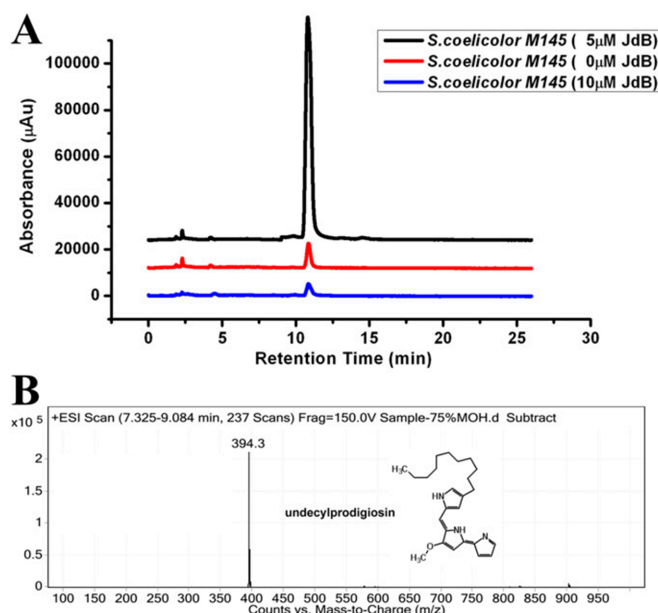

Fig. S2. Identification of Red by MS. (*A*) HPLC analysis of Red produced by *S. coelicolor* M145 in varying concentrations of JdB. (*B*) MS analysis of Red. The compound generates $[M+H]^+$ ion at *m/z* of 394.3, matching the calculated molecular weight of Red.

Fig. S3. Gel mobility shift assays of the dissociation of ScbR2 from different DNA probes induced by JdB. Shown is the dissociation of ScbR2 from P*adpA* (*A*), P*redD* (*B*), P*scbA* (*C*), and P*kasO* (*D*). The assays were performed in 0.3 μM ScbR2, with 0.12 nM P*adpA* (*A*), P*redD* (*B*), P*scbA* (*C*), and P*kasO* (*D*) probes. The concentrations of JdB are indicated.

Fig. S4. Red production levels of *S. coelicolor* M145 in response to different concentrations of JdB. The columns and bars indicate means ± SDs of Red production levels. The red line above the columns shows the trend of the production levels fitted by Gaussian function.

Fig. S5. Growth curves of *S. coelicolor* M145 in SMM containing different concentrations of JdB.

Fig. S6. Phenotypes and *adpA*p activities of *S. coelicolor* M145, M145::scbR2, and ΔScbR2. (*A*) The phenotypes of these strains on SMM. (*B*) The activities of *adpA*p measured as GusA units (GU). Values are means and SDs from three replicates.

Fig. S7. The activities of *redD*p and *adpA*p at different concentrations of JdB in ΔscbR2. The activities of *redD*p and *adpA*p were measured as GusA units. Values are means and SDs from three replicates.

Fig. S8. Gel mobility shift assays of the DNA-binding activity of ScbR2 in response to DhU. The assays were performed in 0.3 μM ScbR2 with 0.13 nM P*scbA*.

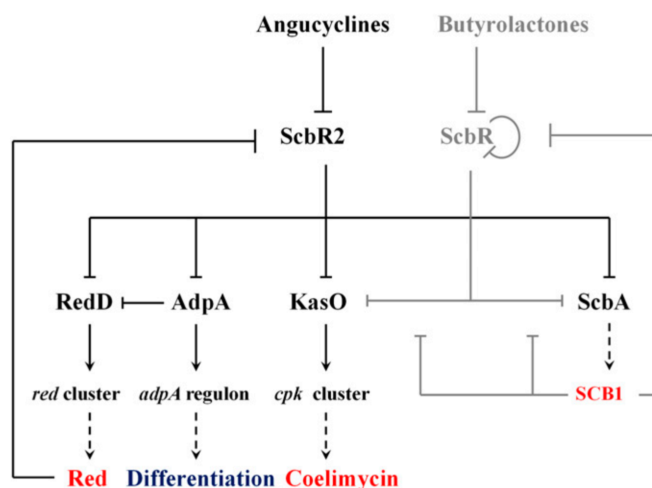

Fig. S9. The signal response network in *S. coelicolor* M145 controlled by ScbR2 and ScbR. The regulatory relationships governed by ScbR and ScbR2 are illustrated by arrows. Arrows, activation; blunt-end arrows, repression; dashed-line arrows, production of small molecules or phenotypes.

Table S1. Strains and plasmids used in this study

Names	Relevant genotypes and purposes	Sources
Strains		
S. coelicolor M145	Prototrophic derivative of *S. coelicolor* A3 (**2**)	Ref. 1
S. venezuelae ISP5230	Source of JdB	Ref. 2
M145::EadpA	AdpA overexpression strain with an integrated pEadpA in the genome	This work
M145::RadpA	AdpA controlled-expression strain with an integrated pRadpA in the genome	This work
ΔScbR2	Same as scbR2DM, the *scbR2* disruption mutant	Ref. 3
ΔScbR2::scbR2	Same as scbR2COM, the *scbR2* complementation strain of ΔScbR2	Ref. 3
M145::scbR2	Same as scbR2OE, *scbR2* overexpression strain	Ref. 3
DH5α	Host for reporter system	Novagen
BL21 (DE3)	Host for protein expression plasmids with T7-derived promoter	Novagen
Plasmids		
pET23b-ScbR2	For his_6-ScbR2 expression (Amp^R)	Ref. 3
pScbR2	For ScbR2 expression in the reporter system (Cm^R)	Ref. 3
pOkasOlux	pCS26-Pac containing the *kasO* promoter-controlled *luxCDABE* reporter genes (Kan^R, Hyg^R)	Ref. 3
pLC803	*Escherichia coli–Streptomyces* shuttle plasmid, with φBT1-*int, ori*T, and *attP* (Ap^R)	Xiaoming Ding
pGusT-ermE-E*	Derived from pSET152, carrying a *gusA* reporter gene controlled by the riboswitch promoter ermE-E* (Ap^R)	Ref. 4
pLCgus	Derived from pLC803 (φBT1-*int,ori*T, and *attP*), carrying a promoterless *gusA* reporter gene	This work
pLCgus-adpAp	pLCgus with *adpA* promoter inserted in front of *gusA* (Hyg^R)	This work
pLCgus-redDp	pLCgus with *redD* promoter inserted in front of *gusA* (Hyg^R)	This work
pIMEP	Derived from pSET152, with *ermE*p* and a ribosomal binding site sequence inserted upstream of the multiple cloning site of pSET152 (Ap^R)	Ref. 3
pEadpA	pIMEP with *adpA* inserted downstream of *ermE*p* for *adpA* overexpression (Ap^R)	This work
pRadpA	pGusT-ermE-E* in which *gusA* was replaced by *adpA* to put *adpA* under the control of riboswitch controlled promoter ermE-E* (Ap^R)	This work

Ap^R, apramycin resistance; Cm^R, chloramphenicol resistance; Hyg^R, hygromycin resistance; Kan^R, kanamycin resistance; *ori*T, origin of transfer from plasmid RK2; Tc^R, tetracycline resistance.

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Table S2. Primers used in this study

Primers	Sequence (5′–3′)	Applications
PscbAF	CTGCACCCTGGTCCGGTGGACA	EMSA
PscbAR	TGAGGATCTCCGTGATCGTGGCA	
HrdBF	TGATGAGCAGGCTGCGCCTTCCTCGCGC	EMSA
HrdBR	GCCCCTCAGCCTTTCCCCGCTCAATGAG	
PkasOF	GTGTATGTCACGGACGAGGAG	EMSA
PkasOR	GTTGGCCTGCAACAGCAGGTA	
RedDpF	CCCGCAGCCCATGATGACAATGTGCAC	EMSA
RedDpR	GGTGCCGCCCGATCTTTGGCTGGCTC	
PAdpAF	CCGGCAGCGGGCGCGCGGACG	EMSA
PAdpAR	TTGCGGCGTCGCCCGGACAGC	
PredZF	ATGACGCGGTCGCAGCAC	EMSA
PredZR	GGCCGCACCTTCTTCGTG	
PredVF	TTGTGGGAGGAGGGACTCAGC	EMSA
PredVR	GGACGGCCGCTTCACCTG	
PredQF	GCTTGTCGTAGGTGGTGCTC	EMSA
PredQR	AGGTGTCCATCGTCCGCTC	
redDpQF	GTTCCGTTGAACCGGAGTGC	ChIP-qPCR
redDpqR	GTCATCCACCGAACGATCGGAT	
adpApqF	TGCAACGCTTCGTGATCGAC	ChIP-qPCR
adpApqR	GGTCAAGATCGAATCTACTG	
gusAF	GGCTGCAGCTCACGGTAACTGATGCCAGTGCCAAGCTTGGGCTGC	pLCgus
gusAR	GTGCCCTGAATGAACTGCAGGACGAGGCAGTAGTCCTGTCGGGTTTCGCCAC	
LCF	CTGCCTCGTCCTGCAGTTCATTC	pLCgus
LCR	GCATCAGTTACCGTGAGCTGC	
adpApF	GGCCGCAGACCGGTCATCTTGTTGCCTCCTTAGCAGACTGCTAAGCCCCCCTCGGTG	pLCgus-adpAp
adpApR	TTCTCGACCTCGTCATCGTCAAGTGTTAATAGGGTCTTCGTCTGCTGCTGCGGCGTTCC	
redDpF	ACGGGCCGCAGACCGGTCATCTTGTTGCCTCCTTAGCAGCCACCGAACGATCGGATTCG	pLCgus-redDp
redDpR	TCTCGACCTCGTCATCGTCAAGTGTTAATAGGGTCTTCGTCGATCACGGCGACCGTGTG	
pLCgusF	GAAGACCCTATTAACACTTGACG	pLCgus-adpAp&pLcgus-redDp
pLCgusR	CTGCTAAGGAGGCAACAAGATG	
IMEPF	TACCGAATTCATCTGAATCGATGCGTAATCATG	pEadpA
IMEPR	ATCTCTAGATCCGTTCCGCTAG	
adpAF1	TGCCGGTTGGTAGGATCTAGCGGAACGGATCTAGAGATATGAGCCACGACTCCACCG	pEadpA
adpAR1	TATGACATGATTACGCATCGATTCAGATGAATTCGGTAGTTCGAGGGAGCCGTCTGCTC	
GusTF	TGATAAGCATTACCCTGTTATCTC	pRadpA
GusTR	GCGGTGGAGTCGTGGCTCATCTTGTTGCCTCCTTAGCAGG	
adpAF2	CCTGCTAAGGAGGCAACAAGATGAGCCACGACTCCACCGC	pRadpA
adpAR2	TCCTTCTTGTTGTCTAGAGATAACAGGGTAATGCTTATCACGGCGCGCTGCGCTGGC	