

Supplementary Information for

An *Aspergillus nidulans* bZIP response pathway hardwired for defensive secondary metabolism operates through *aflR*

Wenbing Yin¹, Saori Amaike², Dana J. Wohlbach⁴, Audrey P. Gasch⁴, Yi-Ming Chiang⁵, Clay C. Wang⁵, JinWoo Bok¹, Marko Rohlf⁶, Nancy P. Keller^{1,3}

¹ Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, WI, United States

² Department of Plant Pathology, University of Wisconsin-Madison, WI, United States

³ Department of Bacteriology, University of Wisconsin-Madison, WI, United States

⁴ Department of Genetics, University of Wisconsin-Madison, WI, United States

⁵ Department of Pharmacology and Pharmaceutical Sciences, University of Southern California, School of Pharmacy, CA, United States

⁶ J.F Blumenbach Institute of Zoology and Anthropology, Georg August University Göttingen, Germany

*Corresponding author:

Phone: (608) 262-9795; fax: (608) 262-8418

E-mail: npkeller@wisc.edu

Running title: bZIP regulation of secondary metabolism in *Aspergillus nidulans*

Table S1. PCR primer sets utilized in this study

Name of the primer	Oligonucleotide sequence (5'-3')	Uses
<i>A. fumigatus pyrG</i> For	TGCCTCAAACAATGCTCTTC	<i>A. fumigatus pyrG</i> marker
<i>A. fumigatus pyrG</i> Rev	CAAGGTATCGTCGGGAGGT	<i>A. fumigatus pyrG</i> marker
gpdA(p)::pyrG For	GTGACGACAATACCTCCCGACACCT GGCATCCGGATGTCTGAAGGCTTG	<i>A. nidulans gpdA</i> amplicon
gpdA_rev	CATGGTGATGTCTGCTCAAG	<i>A. nidulans gpdA</i> amplicon
OErsmA_5F_for	CGTCGGATCTGTCTGACTTTC	<i>OE::rsmA</i> 5' flank
OErsmA_5F_rev	CGAAGAGGGTGAAGAGCATTGTTTG AGGCAGTCTCGAGTGCAAAGTCTGC	<i>OE::rsmA</i> 5' flank
OErsmA_3F_for	AACAGCTACCCCGCTTGAGCAGACA TCACCCCTGAGACACTGCGATGAA TC	<i>OE::rsmA</i> 3' flank
OErsmA_3F_rev	CAAACCTGGGTCCGAGGAGATC	<i>OE::rsmA</i> 3' flank
OErsmA_NEST_for	AAACCAGGTCAACGTCATTGATTTC	<i>OE::rsmA</i> cassette amplicon
OErsmA_NEST_rev	ATCACACTGCGCACTAGTCTTCAAC	<i>OE::rsmA</i> cassette amplicon
HisMBPRsmA_for	CCAAGGCCTTAGCAGGTGCATGTGG ACGTCCAATCAATATCCCTACTATG GGGGC	<i>rsmA</i> cDNA amplicon for Quickchange
HisMBPRsmA_rev	AAGCTTGCCTGCAGGCCATGGCTAG CCCGTCACAACAACCCATCCGGGTC GGTCGTCAGACTGTCTGATGAAGCC	<i>rsmA</i> cDNA amplicon for Quickchange
pKLD116_for	CTTTCGGGCTTTGTTAGCAG	sequencing
pKLD116_rev	GCGGATCCTCTTCCGGGACTGGAAG CAGG	sequencing
aflRtrpC_for	ATTGAATCCGTGGCTGGTATCTGTG CCAG	<i>aflR</i> complementation plasmid
aflRtrpC_Rev	GGGCTCGATATCTACCGACAAACAG GAGAAATAACAATTCAAC	<i>aflR</i> complementation plasmid
aflR (p)mu1_for	GTTGAATTGTTATTTCTCCTGTTTGT CGGTAGATATCGAGCCC	RsmA site mutation
aflR (p)mu1_rev	GCTGTACAGACTTTTAAGTTATGGC GACTCAGCCAGCTGG	RsmA site mutation
aflR (p)mu2_for	CCAGCTGGCTGAGTCGCCATAACTT AAAAGTCTGTACAGC	Yap1 site mutation
aflR (p)mu2_rev	GTTGCATCTCGTGCTCCAG	Yap1 site mutation
aflRtrpC screening_for	CGACGTACCATCCAAGAACC	<i>trpC</i> mutants screening
aflRtrpC screening_rev	CGGAGTATTGACATTGCATTGG	<i>trpC</i> mutants screening
<i>A.f</i> pyroAgpd_for	CATTGTGCAACGCCCTTTGCAGAGC TATCGGTCAGGGTGTGTATTCAAGT TCG	<i>A. fumigatus pyroA</i> amplicon
<i>A.f</i> pyroAgpd_rev	CAAACGCCGAACCTTGAATACACACC CTGACCGATAGCTCTGCAAAGGGC	<i>A. fumigatus pyroA</i> amplicon
gpdpyroA_for		<i>A. nidulans gpdA</i> amplicon

gpdpyroA_rev	GGTGATGTCTGCTCAAGCGG	<i>A. nidulans</i> <i>gpdA</i> amplicon
OEaflR5F_for	CACCTTGGGTTGTGCAGGATC	<i>OE::aflR</i> 5' flank
OEaflR5F_rev	GTGCTTCCCCAATGCAATGTCAATA CTCCGGATATTTGCATATGATACAG GCCCC	<i>OE::aflR</i> 5' flank
OEaflR3F_for	AACAGCTACCCCGCTTGAGCAGACA TCACCATGGAGCCCCCAGCGATCAG C	<i>OE::aflR</i> 3' flank
OEaflR3F_rev	GCGGATCCTCAGGCGTGGCGGAGGA TGC	<i>OE::aflR</i> 3' flank
RsmA For	CAGAATTCCTTCCCGCTCTACG	<i>rsmA</i> probe
RsmA Rev	GCACCACACTCTCCTCAATTGC	<i>rsmA</i> probe
aflR_for	AGAGTTGCATCTCGTGCTCCAG	<i>aflR</i> probe
aflR_rev	CTTGAAGACAATAAGTGAGACGAG	<i>aflR</i> probe
StcU_for	ATGTCCTCCTCCGATAATTACC	<i>stcU</i> probe
StcU_rev	CTTTCCACTGATCCATTCGGC	<i>stcU</i> probe
aflJ Nor_for	ATGACCGGTGCTAACAAAGTAAAG	<i>aflJ</i> probe
aflJ Nor_rev	CACCTTGGGTTGTGCAGGATC	<i>aflJ</i> probe
VeA+for	TGTGTTATCCCATCAAGAGG	Screening progeny for <i>veA</i>
VeA+ rev	CTTGGCGCTGTAGACGATAA	Screening progeny for <i>veA</i>

Table S2. Transcription factor binding sites in secondary metabolism gene clusters.

Upstream regions (1000 bp) were queried for presence of AflR or Yap-like binding sites; motif presence is indicated by blue and purple boxes, respectively.

Gene	Description	AflR	Yap
Sterigmatocystin cluster			
AN7804	<i>stcW</i> FAD-containing monooxygenase		
AN7805	<i>stcV</i> Putative norsolorinic acid reductase		
AN7806	<i>stcU</i> Versicolorin reductase		
AN7807	<i>stcT</i> Putative translation elongation factor 1 gamma		
AN7808	<i>stcS</i> Sterigmatocystin biosynthesis P450 monooxygenase		
AN7809	Uncharacterized		
AN7810	<i>stcQ</i> Putative aflatoxin biosynthesis protein		
AN7812	<i>stcN</i> Putative versicolorin B synthase		
AN7814	<i>stcK</i> Fatty acid synthase beta		
AN7815	<i>stcJ</i> Fatty acid synthase alpha		
AN7816	<i>stcI</i> Putative sterigmatocystin biosynthesis lipase/esterase		
AN7817	Uncharacterized		
AN7818	<i>stcF</i> Sterigmatocystin biosynthesis P450 monooxygenase		
AN7819	<i>aflJ</i> Putative co-regulator in sterigmatocystin biosynthesis		
AN7820	<i>aflR</i> C6 zinc finger transcription factor		
AN7821	<i>stcE</i> Norsolorinic acid reductase		
AN7824	<i>stcB</i> Sterigmatocystin biosynthesis P450 monooxygenase		
AN7825	<i>stcA</i> Polyketide synthase		
Monodictyphenone cluster			
AN0146	<i>mdpC</i> Putative versicolorin ketoreductase		
AN0147	<i>mdpD</i> Putative flavin-containing monooxygenase		
AN0148	<i>mdpE</i> C6 zinc finger transcription factor		
AN0149	<i>mdpF</i> Putative zinc-dependent hydrolase		
AN0150	<i>mdpG</i> Polyketide synthase		
AN0153	Putative MYB DNA binding protein		
AN0154	Uncharacterized		
AN10021	<i>mdpA</i> Putative regulatory protein		
AN10022	<i>mdpH</i> Homologous to DUF 1772 superfamily		
AN10023	<i>mdpL</i> Uncharacterized		
AN10035	<i>mdpI</i> Putative AMP-binding CoA ligase		
AN10038	<i>mdpJ</i> Putative glutathione S transferase		
AN10039	Putative histidine acid phosphatase		
AN10044	<i>mdpK</i> Putative oxidoreductase		
Prenyl xanthone cluster			
AN6784	<i>xptA</i> Putative dimethyl-allyl-tryptophan synthase aromatic prenyltransferase		
AN6785	Uncharacterized		
AN6786	Putative beta-1,4-endoglucanase		
AN6787	Putative cytochrome P450		
AN6788	Uncharacterized		
AN6789	Uncharacterized		
AN6790	Uncharacterized		
AN6791	Putative polyketide synthase		
AN6792	<i>gfdB</i> Putative NAD+ dependent glycerol 3-phosphate dehydrogenase		

Table S2. (continued)













AN6793	Uncharacterized	
AN6794	Uncharacterized	
Asperthecin cluster		
AN5998	Uncharacterized	
AN5999	Predicted role in arginine or pyrimidine metabolism	
AN6000	<i>aptA</i> Polyketide synthase	
AN6001	<i>aptB</i> Putative hydrolase	
AN6002	<i>aptC</i> Putative monooxygenase	
AN6003	Uncharacterized	
AN6004	Uncharacterized	
Penicillin cluster		
AN2621	<i>acvA</i> Delta-(L-alpha-aminoadipyl)-L-cysteiny-D-valine synthetase	
AN2622	<i>ipnA</i> Isopenicillin-N synthase	
AN2623	<i>aata</i> Isopenicillin-N N-acyltransferase	
Terrequinone A cluster		
AN8513	<i>tdiA</i> Single-module nonribosomal peptide like synthetase	
AN8514	<i>tdiB</i> Asterriquinone prenyltransferase	
AN8516	<i>tdiD</i> Putative aminotransferase	
AN8518	<i>tdiC</i> Putative NADPH-dependent quinone reductase	
AN8520	<i>tdiE</i> Protein required for terrequinone A biosynthesis	
Asperfuranone cluster		
AN1027	Uncharacterized	
AN1028	Uncharacterized	
AN1029	<i>afmA</i> Homology to citrinin biosynthesis transcriptional activator	
AN1030	Uncharacterized	
AN1031	<i>afmB</i> Putative efflux pump	
AN1032	<i>afmC</i> Putative oxidoreductase	
AN1033	<i>afmD</i> Putative salicylate hydroxylase	
AN1034	<i>afmE</i> Putative polyketide synthase	
AN1035	<i>afmF</i> Putative FAD/FMN-dependent oxygenase	
AN1036	<i>afmG</i> Polyketide synthase	
AN1037	<i>odeA</i> Oleate delta-12 desaturase	
AN11287	Uncharacterized	
AN11288	Uncharacterized	
Pantothenate cluster		
AN1777	Uncharacterized	
AN1778	<i>panB</i> Putative ketopantoate hydroxymethyl transferase	
AN1779	<i>nimO</i> Required for DNA synthesis and mitotic checkpoint control	
AN1780	Uncharacterized	
AN1781	Uncharacterized	
AN1782	Uncharacterized	
AN1783	Uncharacterized	
AN1784	Putative polyketide synthase	
AN1785	Uncharacterized	
AN1786	Uncharacterized	
AN1787	Uncharacterized	
AN1788	Uncharacterized	
AN1789	Uncharacterized	

Table S2. (continued)

Other SM cluster




AN7080	Uncharacterized	
AN7081	Uncharacterized	
AN7083	Uncharacterized	
AN7084	Putative polyketide synthase-like enzyme	
AN7085	Uncharacterized	
AN7086	Uncharacterized	
AN7087	Uncharacterized	
AN7088	Uncharacterized	
AN7089	Uncharacterized	
AN10887	Uncharacterized	
AN10886	Uncharacterized	
AN10885	Uncharacterized	

Fig. S1 LC-MS analysis of secondary metabolite production by wild type (WT) and *OE::rsmA* respectively on malt extract agar

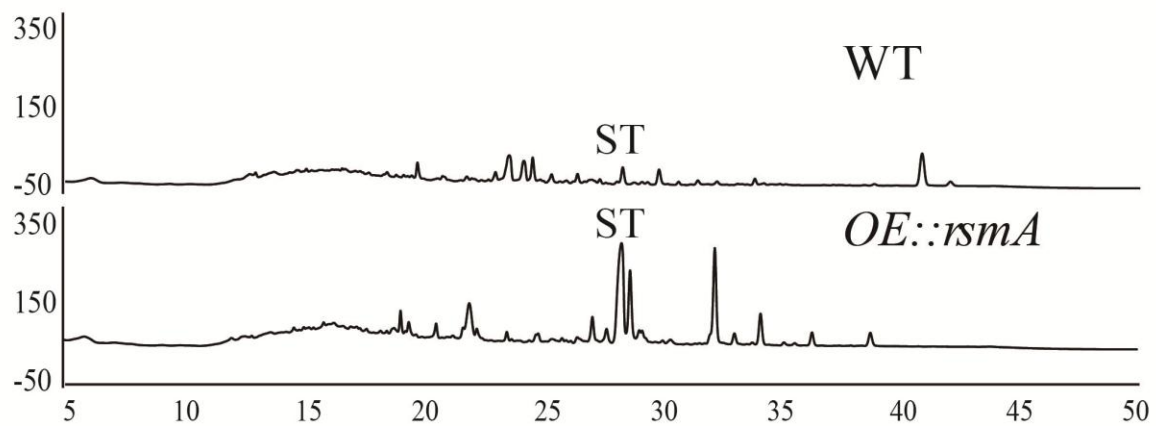


Fig. S2 MEME analysis of RsmA binding motif from RsmA regulated genes in *OE::rsmA* microarray

A total of 329 genes that were significantly up-regulated (FDR = 0.05) by more than 2-fold in *OE::rsmA* as compared to WT were used as target genes. The examination was carried out in 1000 bp upstream of target genes by searching for 6-12 nt motifs in either strand. Two conserved motifs (GCN4: TGACTCA and Yap 1: TTACTAA) were found.

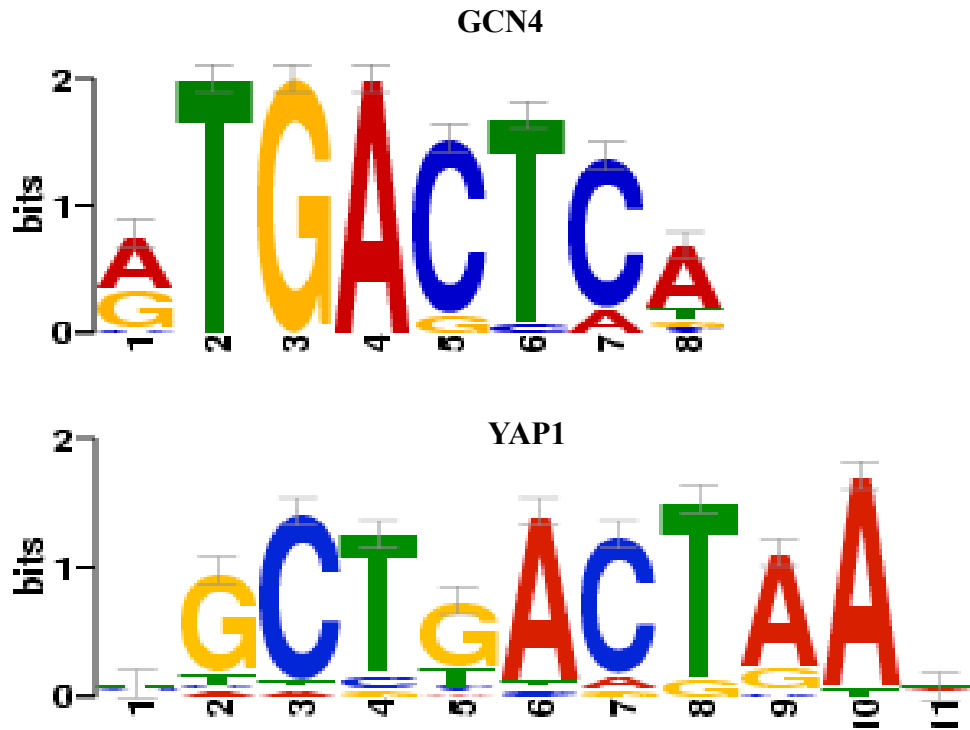


Fig. S3 Phenotypes of *aflR* at *trpC* locus mutants

These mutants contain WT (RDIT9.32), *aflR* with WT promoter at *trpC* locus (TWY18.14), *aflR* and promoter with R* mutation at *trpC* locus (TWY19.15), *aflR* and promoter with Y1* mutation at *trpC* locus (TWY20.4) and *aflR* and promoter with R* and Y1* mutations at *trpC* locus (TWY21.20) which were grown under standard conditions.

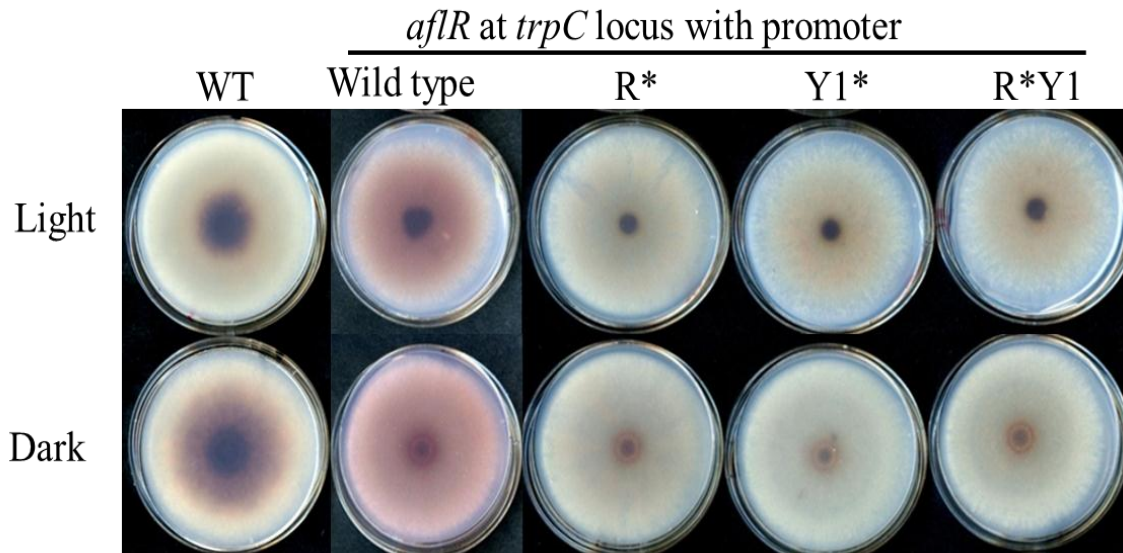


Fig. S4 AflR overexpression replicates an RsmA overexpression phenotype and deletion of *aflR* in RsmA overexpression returns a WT phenotype

Panel A. Shown are bottom side of plates of AflR overexpression (*OE::aflR*, RWY20.3) and RsmA overexpression, *aflR* deletion (*OE::rsmA*, Δ *aflR*, RSA15.2) double mutant strains grown on solid GMM media.

Panel B. TLC analysis of chloroform extracts of secondary metabolite production by strains shown in panel A (ST=sterigmatocystin standard).

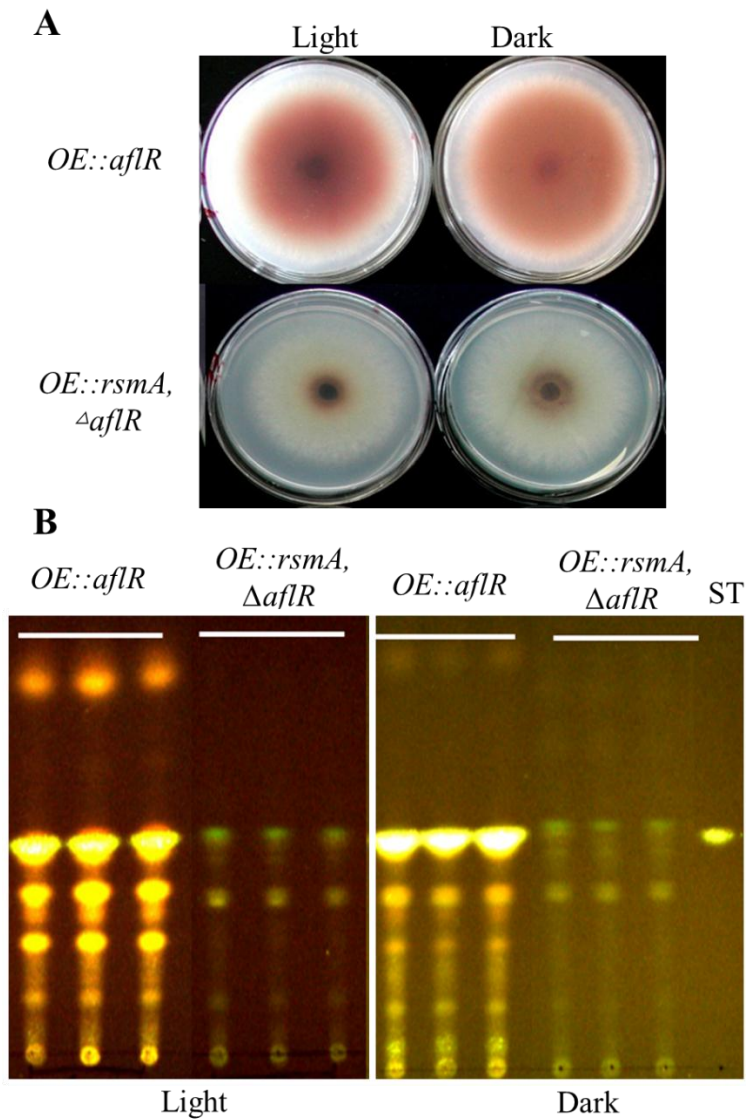


Fig. S5 Expression and purification of recombinant RsmA

Panel A. A Coomassie blue-stained SDS-PAGE gel is shown. Lanes: M, molecular weight markers; 1, total soluble proteins from uninduced bacterial cultures; 2, 3, 4 and 5, total soluble proteins from 1.5 h, 4.5 h, 3 h and 16 h induced bacterial cultures at 25 C, respectively; 6, 7 and 8, total soluble proteins from 1.5 h, 4.5 h and 16 h induced bacterial cultures at 37 C, respectively. The band corresponding to recombinant RsmA is indicated by the arrow.

Panel B. Purification of recombinant RsmA protein. Lanes: M, molecular weight markers; 1, purified recombinant RsmA protein.

