Supplementary Data for:

The actinobacterial transcription factor RbpA binds to the principal sigma subunit of RNA polymerase

Aline Tabib-Salazar, Bing Liu, Philip Doughty, Richard A. Lewis, Somadri Ghosh, Marie-Laure Parsy, Peter Simpson, Kathleen O'Dwyer, Steve J. Matthews & Mark S. Paget

Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant genotype/comments	Source/reference
Strains		
***S. coelicolor* A3(2)**		
M145	Plasmid free derivative of wild-type	
J1915	M145 Δ*glkA119*	(1)
S101	J1915 Δ*rbpA::hyg* (Hyg^R)	(2)
S129	J1981 Δ*rbpA::hyg* (Hyg^R)	This work
J1981	M145 *rpoC::his*	(3)
E. coli		
ET12567 (pUZ8002)	*dam*, *dcm, hsdM*. pUZ8002 is a non-transmissible derivative of RK2 (Cm^R, Km^R)	(4)
BL21λDE3 (pLysS)	E. coli B F^- *ompT hsdS*($r_B^-m_B^-$) *dcm gal* λ(DE3) *endA* Hte (Tet^R)	(5)
BTH101	*cya-99* derivatives of (Spc^R)	
Plasmids		
pMT3000	*E. coli* cloning vector; *ori* pUC18 (Amp^R)	(6)
pBluescript II SK^+	*E. coli* cloning vector; *ori* pUC18 (Amp^R)	(7)
pKT25	Two-hybrid vector; T25 fragment of *B. pertussis* CyaA for N-terminal fusions (Km^R)	(8)
pUT18	Two-hybrid vector; T18 fragment of *B. pertussis* CyaA for C-terminal fusions (Amp^R)	(8)
pSET152	Integrative cloning vector; *ori* pUC18, *oriT* RK2, *int* ϕC31, *attP* ϕC31 (Apr^R).	(9)
pSETΩ	pSET152 derivative (Spc^R)	(10)
pIJ6902	pSET152-based expression vector, *tipAp* (Apr^R $Thio^R$).	(11)
pET15b	*E. coli* expression vector (His_6-tagged; Amp^R)	Novagen
pET20b	*E. coli* expression vector (native; Amp^R)	Novagen
pSX190	pSET152 containing C-terminally 3xFLAG-tagged *rbpA*.	This study
pSX233	pET20b containing *S. coelicolor rbpA*	(2)
pSX500	pET20b containing *M. tuberculosis rbpA*	This study
pSX505	pET15b containing *S. coelicolor rbpA* residues 1-75	This study
pSX510	pET15b containing *S. coelicolor rbpA* (R89A/R90A)	This study
pSX512	pMT3000::*rbpA* (2) in which an *Nde*I site has been engineered at the start codon. Includes promoter and terminator regions of *rbpA*.	This study
pSX528	pIJ6902 containing *rbpA* as an *Nde*I-*Bam*HI fragment from pSX233.	This study
pSX530	pSETΩ containing at the *Bam*HI site *a Bgl*II-fragment isolated from pMT3000::*rbpA*.	This study

Table S2. Oligonucleotides used in this study

Construction/Oligo nucleotide name		Primer sequence (5' to 3') (restriction sites indicated in bold)	
(A) Plasmid constructs			
rbpA_H3_rev		CCCGCTAAAGCTTCGCACTCTTGCG	
(B) Bacterial Two Hybrid Analysis			
S. coelicolor sigma factor fusions to T25 in pKT25 and *rbpA* fusions to T18 in pUT18			Amino acid coordinates
T25-hrdB (σ_2-σ_4)	F-	GGTCTAGAGACCGCCGACCCGGTCAAGGAC	211-511
	R-	GGG**GAATTC**CTAGTCGAGGTAGTCGCGCAG	
T25-hrdB ($\sigma_{1.1}$-σ_2)	F-	GG**TCTAGA**GGTGTCGGCCAGCACATCCCGTAC	1-347
	R-	GGG**GAATTC**CTAGCGCGCCTGGTCGGCCATCGC	
T25-hrdB (σ_2)	F-	GGTCTAGAGACCGCCGACCCGGTCAAGGAC	211-347
	R-	GGG**GAATTC**CTAGCGCGCCTGGTCGGCCATCGC	
T25-hrdB (σ_3-σ_4)	F-	GG**TCTAGA**GAGCTTCACACTGCTGCAGGAGC	348-511
	R-	GGG**GAATTC**CTAGTCGAGGTAGTCGCGCAG	
T25-hrdB (σ_4)	F-	GG**TCTAGA**GAGCTTCACACTGCTGCAGGAGC	435-511
	R-	GGG**GAATTC**CTAGTCGAGGTAGTCGCGCAG	
T25-hrdA (σ_2-σ_4)	F-	GG**TCTAGA**GTCCTCCGACCTGTTCCGGCAG	96-396
	R-	GGG**GAATTC**TCAGTCCAGGTAGCCCCTCAG	
T25-hrdC (σ_2-σ_4)	F-	GG**TCTAGA**GGAACCCGACCTGCTCGGC	35-339
	R-	GGG**GAATTC**TCAGCTCGCCCAGTCCAGC	
T25-hrdD (σ_2-σ_4)	F-	GG**TCTAGA**GGACCGCGATCTGGTCGGCATG	32-332
	R-	GGG**GAATTC**TCAGGCCGCCGCCTCGAAGC	
T25-sigB	F-	GG**TCTAGA**GATGACGACGACGACCGCGCGAGCCAC	1-281
	R-	GGG**GAATTC**TCAGGTGGTGCTGAGCATGCCTTCC	
T25-sigR	F-	GG**TCTAGA**GGTGGGTCCGGTCACTGGGAC	1-227
	R-	GGG**GAATTC**TCATGACCCCGAGCCTTTCG	
T25-sigE	F-	GG**TCTAGA**GATGGGCGAGGTGCTCGAGTTCG	1-177
	R-	GGG**GAATTC**TCAGGCCGCGCAACGCTCC	
T25-whiG	F-	GG**TCTAGA**GATGCCCCAGCACACCTCCG	1-280
	R-	GGG**GAATTC**TCAGCGGCCGAAACCCGCAAG	
rbpA-T18	F-	GG**GGATCC**GATGAGTGAGCGAGCTCTTCG	1-124
	R-	GGG**GAATTC**CCCGCACTCTTGCGGCTGTC	
rbpA$^{1-72}$*-T18*	F-	GG**GAATTC**CCCAGCCGCCAGC	1-72
	R-	CTCAGGGCCGTCGCCGTCAAC	
rbpA$^{1-90}$*-T18*	F-	GG**GAATTC**CCCAGCCGCCAGC	1-90
	R-	GGTGCGTCGCTCCATCAGCATGTC	
rbpA$^{73-124}$ *–T18*	F-	GG**GGATCC**GGAGAAGAAGGCCAAGCCCG	73-124
	R-	GGG**GAATTC**CCCGCACTCTTGCGGCTGTC	
M. tuberculosis sigma factor fusions to T25 in pKT25 and *rbpA* fusions to T18 in pUT18			
T25-sigA	F-	GG**TCTAGA**GGTGGCAGCGACCAAAGCAAG	1-528
	R-	GGG**GAATTC**TCAGTCCAGGTAGTCGCG CAG	
T25-sigA (σ_2)	F-	GG**TCTAGA**GTCCGCCGACTCGGTTCGCGCC	224-364
	R-	GGG**GAATTC**TCAGCGGGCCTGGTCGGCCATGGCGC	
T25-sigB	F-	CC**TCTAGA**GGCCGATGCACCCACAAGGGCCA	1-323
	R-	CC**GAATTC**CTGGCTCAGGATGTCCAGCT	
rbpA-T18	F-	GG**GGATCC**GATGGCTGATCGTGTCCTGAG	1-111
	R-	GG**GAATTC**CCGCCGCGCCGACGTGACCGAATG	
(C) Protein overexpression			
S. coelicolor proteins			
hrdB(σ_2-σ_4)	F-	GG**CATATG**ACCGCCGACCCGGTCAAGG	211-511
	R-	GG**AGATCT**CTAGTCGAGGTAGTCGCGCAGC	
hrdB (σ_2)	F-	GG**CATATG**ACCGCCGACCCGGTCAAGG	211-347
	R-	GG**AGATCT**TCAGCGCGCCTGGTCGGCCATC	
hrdB (σ_3-σ_4)	F-	GG**CATATG**ACCATCCGTATCCCGGTGCAC	348-511
	R-	GG**AGATCT**CTAGTCGAGGTAGTCGCGCAGC	
hrdB (σ_4)	F-	GG**CATATG**AGCTTCACACTGCTGCAGGAGC	435-511
	R-	GG**AGATCT**CTAGTCGAGGTAGTCGCGCAGC	
M. tuberculosis proteins			
rbpA	F-	GG**CATATG**GCTGATCGTGTCCTGAGG	1-111

	R-	CC**GGATCC**CGGGTCAGCCGCGCCGACGTG	
sigA (σ_2)	F-	GG**CATATG**TCCGCCGACTCGGTTCGCGCC	224-364
	R-	CC**GGATCC**CCATCAGCGGGCCTGGTCGGCCATGGC	

(D) Primers for *in vitro* transcription templates (distance from reverse primer to transcription start site indicated in parentheses)

atpI (187 nt)	F	CGCAATACCAGACAAGTTGC
	R	GCCGCGGGCACGGCAGCCTG
relAp1 (215 nt)	F	GGTCTGAACCACGCGAACCG
	R	TTGGGGCGCGGCTGCTGCTC
sacAp (200 nt)	F	GGCGTAGTGATGGCCGCACG
	R	GTCGCCCACCTGCAGTGTGC
Tuf3p (181 nt)	F	CCGCGCGGGAGGCGCTGCGG
	R	AAGCCTCGTGGCGGTGGTGG
rplJp (46 nt)	F	GGGAATTCGCGCCCGGCCCGCTCCGGTCGCCG
	R	GGAAGCTTGTCCTCTTTCGAACACACGGCAACG

(E) Primers for ChIP-qPCR of *rplJ* promoter region

rplJp_1	F	GCCGAGGCCGAGATGCAGAT
	R	GCCCCCGATGTACATGGCCT
rplJp_2	F	GTGAAGGTCACCGCCCCTCC
	R	AAGCGTACGTGAACGGGGCA
rplJp_3	F	CAGTCCTCCTTCGGGTCCGC
	R	TGTCCGTCAGCTCGGCAACC
rplJp_4	F	AAGGGCGGTGTCCTTGACGG
	R	TTGAAGGCACCCGCCAGCTT

Table S3. NMR and Refinement statistics

NMR and Refinement statistics		
	M. tuberculosis RbpA	*S. coelicolor* RbpA
NMR Distance and Dihedral Constraints		
Distance constraints		
Total NOE	484	474
Intraresidue	236	206
Interresidue	248	268
Sequential (|*i-j*|)=1)	101	74
Medium range (|*i-j*|)<4)	9	14
Long range (|*i-j*|)>5)	131	158
Total Dihedral angle Restraints	82	82
φ	41	41
ψ	41	41
Structural Statistics		
Violations (mean and SD)		
Distance constraints (Å)	0.024 ± 0.007	0.026 ± 0.004
Dihedral angle constraints (°)	0.19 ± 0.045	0.19 ± 0.056
Maximum dihedral angle violation (°)	0.25	0.65
Maximum distance constraint violation (Å)	0.167	0.26
Deviations from idealized geometry		
Bond length (Å)	0.002 ± 0.000	0.003 ± 0.000
Bond angle (°)	0.46 ± 0.007	0.46 ± 0.006
Impropers (°)	0.24 ± 0.01	0.22 ± 0.01
Average Pairwise rmsd[α](Å)		
Heavy	0.884	0.942
Backbone	0.377	0.517

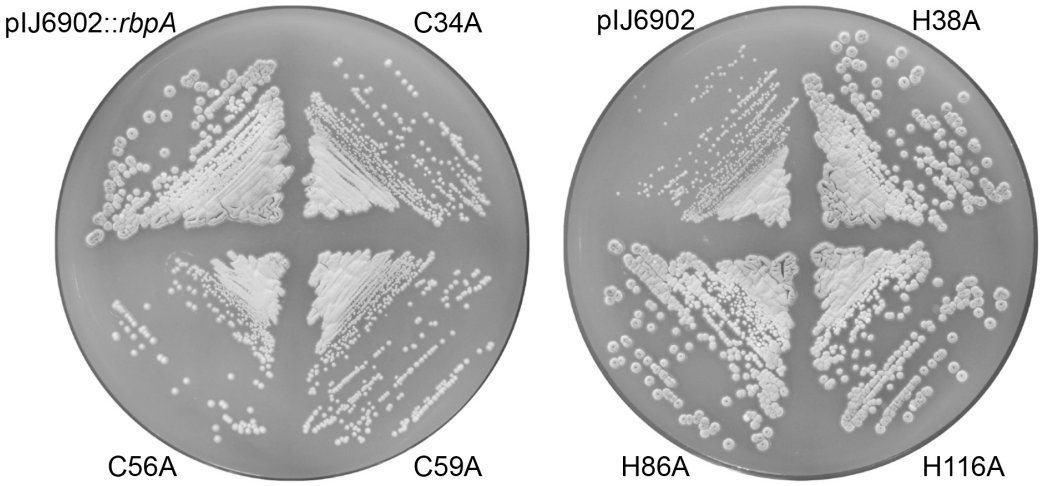

Figure S1. Mutational analysis of putative zinc ligands in *S. coelicolor* RbpA. Following site-directed mutagenesis *rbpA,* mutant genes were isolated as promoterless *Nde*I-*Bam*HI fragments and subcloned into pIJ6902, thereby placing them under control of the thiostrepton inducible promoter *tipAp*. The control plasmid pIJ6902::*rbpA* is equivalent to pSX528. Plasmids were introduced into *S. coelicolor* S101 (Δ*rbpA*::*hyg*) by conjugation and streaked to MS-agar plates plus 25 μg/ml thiostrepton. Plates were photographed after incubation for 4 days at 30^{o}C. All strains grew poorly and formed small colonies in the absence of thiostrepton.

Figure S2. Overlay of the RCD domain of *S. coelicolor* RbpA with homologous domains from the following structures: Mini-chromosome maintenance complex, green, RMSD of 2.6Å over 36 equivalent Cα atoms (12); ribosomal protein L27, orange, RMSD of 2.3Å over 38 equivalent Cα atoms(13) polypeptide chain release factor, yellow, RMSD of 2.5Å over 39 equivalent Cα atoms (14).

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