Repressor activity of the RpoS/σ^S-dependent RNA polymerase requires DNA binding

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SUPPLEMENTARY DATA

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Table S1: Bacterial strains and plasmids used in this study

Strain or Plasmid	Characteristics	Source or reference
JM109	*E. coli* K-12, *recA*1 *supE*44 *endA*1 *hsdR*17 *gyrA*96 *relA*1 *thi* Δ(*lac-proAB*) F'(*traD*36 *proAB*$^+$ *lacI*^{q} *lacZ*ΔM15)	(40)
ATCC14028	*Salmonella enterica* serovar Typhimurium, wild-type strain	American Type Culture Collection
VF7928	ATCC14028 Δ*rpoS*::Cm	(8)
VF8158	VF7928 with the Cm cassette eliminated	(8)
VFC326	ATCC14028 Δ*rpoS*::*tet*RA	(12)
VFC331	ATCC14028 Δ*rpoS* (scarless in frame deletion of *rpoS*)	(12)
VFC391	ATCC14028 $rpoS_{db}$	This study
VF9579	ATCC14028 $rpoS_{420}$::Cm	This study
VF8293	ATCC14028 Δ*rssB*::Cm	This study
VFC117	ATCC14028 Δ*ompD*::Cm	This study
VFC781	ATCC14028 Δ*ynfM*::Cm	This study
VF8132	ATCC14028 *katE-lacZ*	(16)
VF8721	ATCC14028 *katN-lacZ*	(16)
VF9583	ATCC14028 *sdhA-lacZY*-Cm	(16)
VFC165	ATCC14028 *ompD-lacZY*-Km	This study
VFC360	ATCC14028 *ompD-lacZY*-Cm	This study
VFC789	ATCC14028 *ynfM-lacZY*-Km	This study
VFD33	ATCC14028 *ynfM-lacZY*-Cm	This study
VF7969	ATCC14028 ΔSTM2922::Km	(16)
VF7975	ATCC14028 ΔSTM2922::Km Δ*rpoS*::Cm	(16)
VF9356	VF7975 with the Cm cassette eliminated	(16)
VF9682	ATCC14028 ΔSTM2922::Km $rpoS_{R141S}$	This study
VF9676	ATCC14028 ΔSTM2922::Km $rpoS_{A157T}$	This study
VF9849	ATCC14028 ΔSTM2922::Km $rpoS_{db}$	This study
VF8082	VF7969 *katE-lacZ*	(16)
VFD86	VF9356 *katE-lacZ*	This study
VF9879	VF9849 *katE-lacZ*	This study
VF8088	VF7969 *katN-lacZ*	(16)
VF8089	VF7975 *katN-lacZ*	(16)
VFD85	VF9356 *katN-lacZ*	This study
VF9721	VF9682 *katN-lacZ*	This study
VF9717	VF9676 *katN-lacZ*	This study
VF9885	VF9849 *katN-lacZ*	This study
VF9613	VF9356 *sdhA-lacZY*-Cm	This study
VF9891	VF9849 *sdhA-lacZY*-Cm	This study
VFC384	VF7969 *ompD-lacZY*-Cm	This study
VFC385	VF9356 *ompD-lacZY*-Cm	This study
VFC388	VF9849 *ompD-lacZY*-Cm	This study
VFD35	VF7969 *ynfM-lacZY*-Cm	This study
VFD36	VF9356 *ynfM-lacZY*-Cm	This study
VFD37	VF9849 *ynfM-lacZY*-Cm	This study
VFC802	ATCC14028 *ynfM-lacZY*-Km	This study
VFC803	VFC331 *ynfM-lacZY*-Km	This study
VFC804	VFC391 *ynfM-lacZY*-Km	This study
VFC259	VF7969 Δ*ompD*::Cm	This study
VFC260	VF9356 Δ*ompD*::Cm	This study

VFC261	VF9849 Δ*ompD*::Cm	This study
VFC314	ATCC14028 *sdh*-mut1	This study
VFC315	VF8158 *sdh*-mut1	This study
VFD984	ATCC14028 *sdh*-mut2	This study
VFD987	VF8158 *sdh*-mut2	This study
MA10200	LT2 ΔGifsy-1 Δ*isrE*::*aadA* Δ*ryhB*::*cat*	N. Figueroa-Bossi[a]
VFB867	ATCC14028 Δ*isrE*::*aadA* Δ*ryhB*::*cat*	This study
VFB868	VF8158 Δ*isrE*::*aadA* Δ*ryhB*::*cat*	This study
Plasmids		
pACYC184	cloning vector, Cm^R, Tet^R	(68)
pSTK4	*rpoS* cloned into pACYC184, Cm^R	(69)
pQE30	vector for expression of His-tagged proteins, Cb^R	Qiagen
pQE30*rpoS*	Expresses His_6-σ^S	(70)
pQE30$rpoS_{R141S}$	Expresses His_6-σ^S_{R141S}	This study
pQE30$rpoS_{A157T}$	Expresses His_6-σ^S_{A157T}	This study
pQE30$rpoS_{db}$	Expresses His_6-σ^S_{db}	This study
pUCC52-2922K	*rpoS* and downstream sequences in pUC19, Cb^R	(16)
pVF9551	pUCC52-2922K with mutation $rpoS_{A157T}$	This study
pVF9647	pUCC52-2922K with mutation $rpoS_{R141S}$	This study
pVF9793	pUCC52-2922K with mutation $rpoS_{db}$	This study

[a] Albontin, Bossi, Figueroa-Bossi, manuscript in preparation (RyhB and IsrE are also named RyhB1 and RyhB2, respectively (12, 63))

Table S2. Oligonucleotides used in this study.

Name	Sequence (5' – 3')	Purpose
ompD-P1	GAGGAAACACGCTAAGAAAATTATAAGGATTATTAAAA TGAAAGTGTAGGCTGGAGCTGCTTC	Construction of Δ*ompD*::Cm
ompD-P2	CATCAAGAGAAAAAGCCAGCCCTGAAAGGACTGGCTTT GTATTCAGCATATGAATATCCTCCTTAG	Construction of Δ*ompD*::Cm
rssB-P1	GCCACTATTGATTAAAGCCAGTCAGGGGAGAGAACATG ACGCAGTGTAGGCTGGAGCTGCTTC	Construction of Δ*rssB*::Cm
rssB-P2	CGGTAAAGCAATTTCCGCTCACTCTTCCGTTTGGTCATTC CGCCATATGAATATCCTCCTTAG	Construction of Δ*rssB*::Cm
ynfM-P1	GTCTCCTGTTGACGGAGATGTAAAGCAAGGATTTAACGT GGTGTAGGCTGGAGCTGCTTC	Construction of Δ*ynfM*::Cm
ynfM-P2	GCGATAGCGCACGTTGCTATCGCCGGATTATTTCTTTTCA CATATGAATATCCTCCTTAG	Construction of Δ*ynfM*::Cm
*sdh*del1-TetFw	AAACTATATGTAGGTTAATTGTAATGATTTTGTGAACGTC TTAAGACCCACTTTCACATT	Construction of *sdh*-mut1/mut2
*sdh*del1-TetRv	TGGGTGGCTCGGGATTGCAGGGTATTCCGGAGACCTGGC GGCCTAAGCACTTGTCTCCTG	Construction of *sdh*-mut1/mut2
*sdh*mut2-Fw	GACAAACTATATGTAGGTTAATTGTAATGATTTTGTGAA CGTCCTATACTTAAGCCAGGT	Construction of *sdh*-mut2
*sdh*mut2-Rv	CGCTGGGTGGCTCGGGATTGCAGGGTATTCCGGAGACCT GGCTTAAGTATAGGACGTTCA	Construction of *sdh*-mut2
*sdh*mut1-Fw	GACAAACTATATGTAGGTTAATTGTAATGATTTTGTGAA CGTCCACTACGGCCGCCAGGT	Construction of *sdh*-mut1
*sdh*mut1-Rv	CGCTGGGTGGCTCGGGATTGCAGGGTATTCCGGAGACCT GGCGGCCGTAGTGGACGTTCA	Construction of *sdh*-mut1
$rpoS_{420}$-P1	GCTTATCCGTGCAGTCGAGAAGTTTGACCCGGAACGCGG GTTCGTGTAGGCTGGAGCTGCTTC	Construction of $rpoS_{420}$::Cm
$rpoS_{420}$-P2b	GATGCACATATTGAACTCATGGCGACTTCGCCGGTAAAA GGAGCATATGAATATCCTCCTTAG	Construction of $rpoS_{420}$::Cm
HK1	AGGCTCGGATCCATGAGTCAGAATACGCTGAAAGTTCAT	Cloning of *rpoS* in pQE30
HK2	TTCCGAAAGCTTTTACTCGCGGAACAGCGCTTCGATATT	Cloning of *rpoS* in pQE30
G469Af	CGCCAGACAATCGAACGGACGATCATGAACCAAACC	Site-directed mutagenesis of *rpoS*
G469Ar	GGTTTGGTTCATGATCGTCCGTTCGATTGTCTGGCG	Site-directed mutagenesis of *rpoS*
C421Af	CCGGAACGCGGGTTCAGCTTCTCAACATACGCAACC	Site-directed mutagenesis of *rpoS*
C421Ar	GGTTGCGTATGTTGAGAAGCTGAACCCGCGTTCCGG	Site-directed mutagenesis of *rpoS*
CL-*sdhA*-Fw	CTACCGTTCTCCCGTCTT	Quantitative real-time PCR
CL-*sdhA*-Rv	GAAAATCGTGGTGTGGTTT	Quantitative real-time PCR
CL-*sdhB*-Fw	GAATAATGGGCAAAATCCAC	Quantitative real-time PCR
CL-*sdhB*-Rv	TAAACTTATCCGGGTTCCA	Quantitative real-time PCR
CL-*sdhC*-Fw	CACCAGCCTCTCTTCTCC	Quantitative real-time PCR
CL-*sdhC*-Rv	TTCCAGATAGCCAAAATCC	Quantitative real-time PCR
CL-*sdhD*-Fw	TTCTTCTCATCGGCCTTC	Quantitative real-time PCR
CL-*sdhD*-Rv	CCCACACCACAACAATC	Quantitative real-time PCR
CL-*rpoZ*-Fw	GACGCTGTAGAGAAAATTGG	Quantitative real-time PCR
CL-*rpoZ*-Rv	CGTCGAGGATCTGGTTGT	Quantitative real-time PCR

Supplementary References

68. Chang,,A.C. and Cohen,S.N. (1978) Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *J. Bacteriol.*, **134**, 1141-1156.

69. Kowarz,,L., Coynault,C., Robbe-Saule,V. and Norel,,F. (1994) The *Salmonella typhimurium katF (rpoS)* gene: cloning, nucleotide sequence, and regulation of *spvR* and *spvABCD* virulence plasmid genes. *J. Bacteriol.*, **176**, 6852-6860.

70. Monteil,V., Kolb,A., D'Alayer,J., Beguin,P. and Norel,F. (2010) Identification of conserved amino acid residues of the *Salmonella* sigmaS chaperone Crl involved in Crl-sigmaS interactions. *J. Bacteriol.*, **192**, 1075-1087.

71. Thompson,J.D., Higgins,D.G. and Gibson,T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673-4680.

SUPPLEMENTARY FIGURES

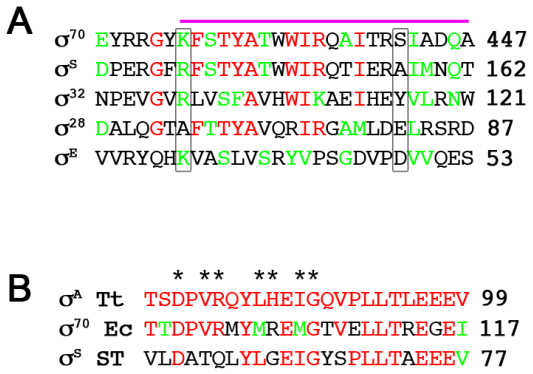

Figure S1. Amino acid sequence comparison between DNA-binding regions of σ^S and other sigma factors. (A) Sequence alignment of *S*. Typhimurium σ^S, σ^{70}, σ^{32} σ^{28} and σ^E in the region containing the amino-acid substitutions in σ^S_{db} (R141 and A157 are shown in boxes). The bar in magenta corresponds to a DNA-binding α helix in housekeeping sigma factors (57). (B) Sequences of sigma factor region 1.2 (Figure 2A) in σ^S and housekeeping sigma factors (σ^{70} and σ^A). σ^A is from *Thermus thermophilus* (Tt), σ^{70} from *E. coli* (Ec) and σ^S from *Salmonella* Typhimurium (ST). * denotes interactions between σ region 1.2 of housekeeping RNAP and non template-strand ssDNA of discriminator element (GGG) (57). The alignments were generated with ClustalW (71).

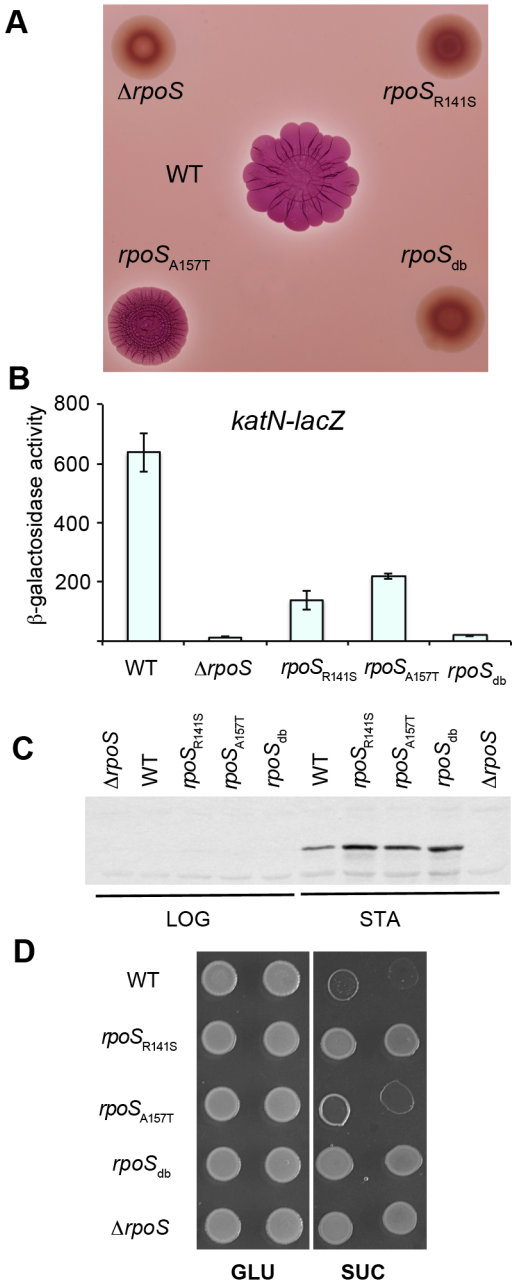

Figure S2. Physiological characterization of *Salmonella rpoS* mutants. Expression of the rdar morphotype (A) and of a *katN-lacZ* transcriptional fusion (B) in the *Salmonella* wild-type strain VF7969 and its *rpoS* mutant derivatives VF9356, VF9682, VF9676 and VF9849 (Table S1). β-galactosidase activity was measured in cultures grown for 18h in LB at 37°C. All results are shown as the mean and standard deviation of at least three independent experiments. (C) Cellular σ^S levels immunodetected with anti-σ^S antibodies, in exponential (LOG, OD_{600} of 0.4) and stationary (STA, OD_{600} of 4) phase cultures of *Salmonella* strains expressing either wild-type σ^S (VF7969) or its variants (VF9356, VF9682, VF9676, and VF9849, Table S1). Ten micrograms of total protein were loaded into each slot. Similar results were obtained in repeat experiments. (D) Ability of the wild-type strain and Δ*rpoS* mutants to grow at the expense of glucose (GLU) and succinate (SUC) as a sole carbon source (5 µl of cultures diluted to OD600 of 1.0 and 0.05 were spotted).

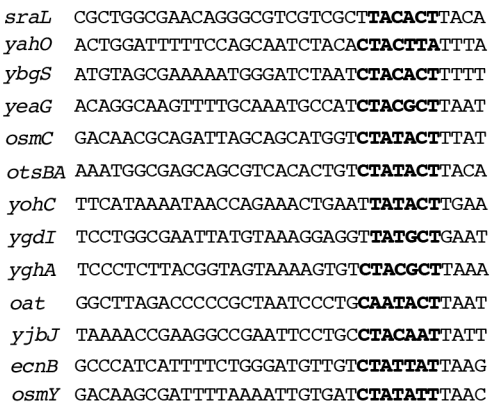

Figure S3. Promoter sequences of σ^S-regulated genes in Table 1 used to construct the logograph on Figure 7A. Promoter sequences are from (24, 62, 63). The -10 region is shown in bold face.

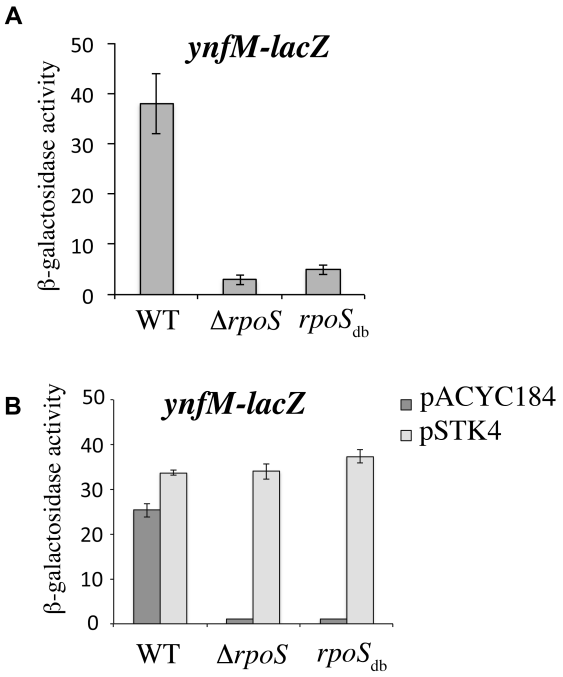

Figure S4. **Expression of a *ynfM-lacZ* transcriptional fusion.** (A) Expression of a transcriptional *ynfM-lacZ* fusion in the wild-type (WT) strain VF7969, and its *rpoS* derivatives VF9356 and VF9849, grown for 18h in LB at 37°C. (B) Plasmid pSTK4 carrying the *rpoS* gene and the empty vector pACYC184 were used in complementation experiments for *ynfM-lacZ* expression in strains ATCC14028, VFC331 and VFC391 (Table S1). Results shown are the mean and standard deviation of at least three independent experiments.

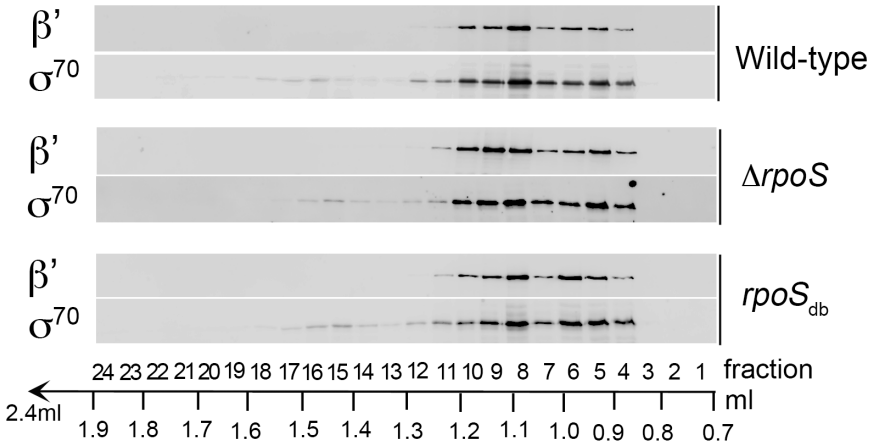

Figure S5. Distribution of free and holoenzyme forms of σ^{70} in the wild-type strain and the Δ*rpoS* and $rpoS_{db}$ mutants. Whole cell lysates from wild-type strain VF7969, the Δ*rpoS* mutant (VF9356) and the $rpoS_{db}$ mutant (VF9849) were fractionated by size exclusion chromatography and the relative concentration of RNAP subunits was subsequently analyzed in the fractions by immunoblot using monoclonal antibodies against the β' subunit of RNA polymerase and a monoclonal anti-σ^{70} antibody (Neoclone CP004). The elution profiles were similar for the wild-type, Δ*rpoS* and $rpoS_{db}$ strains. Two populations of σ^{70} were found. The major one co-eluted with the β' subunit of core RNAP and was interpreted to represent holoenzyme-associated σ^{70} (in fractions 4-11), while the other, in fractions 14-17, represented free (unbound) σ^{70}. The percentage of total σ^{70} in fractions corresponding to free σ^{70} was very low for the three strains suggesting that most σ^{70} molecules were associated with RNAP in stationary phase. Two independent experiments were performed with similar results (σ^{70} in fractions 4-11 represented 94 to 98 % of total σ^{70} for the three strains, calculated using the IMAGEJ software).