Residue substitutions near the redox center of *Bacillus subtilis* Spx affect RNA polymerase interaction, redox control and Spx-DNA contact at a conserved cis-acting element.

Ann A. Lin, Don Walthers, and Peter Zuber

SUPPLEMENTAL MATERIAL

Figure S1. Effect of R92 substitutions in Spx on the transcription of *trxB-lacZ*. Spx R92 residue was substituted with alanine(R92A), glutamine(R92Q), or lysine(R92K), and the resulting mutant alleles were introduced in the *amyE* locus and under IPTG control in *spx* null mutant strain bearing *trxB-lacZ*. Strains were grown in DS medium until the OD_{600} reached 0.4. Then, each culture was divided into two flasks, and 1 mM IPTG was added to one flask to induce SpxDD expression. Samples were taken at time intervals and β-galactosidase activities were measured. Symbols: circles, SpxDD; diamonds, Spx(R92A)DD; squares, Spx(R92Q)DD; triangles, Spx(R92K)DD. Open symbols with broken lines represent cells cultured without IPTG, and closed symbols with solid lines represent cell culture with IPTG.

Figure S2. Effect of Spx(G52R, R91A) mutant on *trxB-lacZ* transcription and RNAP binding. The IPTG-inducible allele encoding Spx(G52R, R91A)DD was introduced into the *amyE* locus of the Spx null mutant

strain bearing *trxB-lacZ*. (A) The β-galactosidase assay was performed as described in Fig. 3 and the highest activities of Spx mutants were selected. The ratio of mutant activity to that of the control SpxDD was calculated. (B) The production of Spx protein in the strains was evaluated by Western blot analysis using anti-Spx antibody. (C, D) Purified Spx(G52R, R91A)ΔCHA protein was used for in vitro RNAP interaction assay. The SAd-RNAP or Holo-RNAP was incubated with SpxΔCHA or Spx(G52R, R91A)ΔCHA and followed by the anti-HA affinity chromatography. The interaction between Spx proteins and SAd-RNAP (C)/Holo-RNAP (D) was analyzed by SDS-PAG gel. Abbreviation: I, input and E, eluate.

Figure S3. Spx/RNAP/*trxB* crosslinking result at position -11, -21, -46, -49, -52 on *trxB* promoter. Crosslinking reactions were performed as described in Expermental procedures. The nucleotide positions indicated here are crosslinking sites on the *trxB* promoter. Bands of crosslinked products on the gel corresponding to ββ′, σ^A, α and Spx are indicated. The band representing presumed uncleaved radiolabeled DNA substrate is also shown.

Table S1. *B. subtilis* strains and plasmids

Strain or plasmid	Relevant genotype or characteristics	Source or reference
***B. subtilis* strains**		
JH642	*trpC2 pheA1* (parental strain)	James Hoch
LAB545	*trpC2 pheA1* SPβ *c2del2*::Tn917:: pMMN92(*srfA-lacZ*)	(43)
ORB5853	*trpC2 pheA1 sigA*(L366A) *neo rpoC*-His_{10} *cat*	(42)
ORB6129	*trpC2 pheA1* SPβ*c2del2*::Tn917:: pMMN92(*srfA-lacZ*) *amyE*::pSN56(P*spankhy-spx*^{DD})	(32)
ORB7262	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*)	(32)
ORB7276	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pSN56(P*spankhy-spx*^{DD})	(32)
ORB7282	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pMMN683(P*spankhy-spxR60E*^{DD})	(32)
ORB7316	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pYZ14 (P*spankhy-spxC10A*^{DD})	(32)
ORB7821	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL37(P*spankhy-spxR92A*^{DD})	This study
ORB7822	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL38(P*spankhy-spxR92Q*^{DD})	This study
ORB7904	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL56(P*spankhy-spxC10A,R92A*^{DD})	This study
ORB7906	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL58(P*spankhy-spxR60E,R92A*^{DD})	This study
ORB7945	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL64(P*spankhy-spxK62E,R92A*^{DD}]	This study
ORB7946	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pMMN684(P*spankhy-spxK62E*^{DD})	This study
ORB7979	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL65(P*spankhy-spxC10A,R60E*^{DD})	This study
ORB7981	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL67(P*spankhy-spxC10A,K62E*^{DD})	This study
ORB8268	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL88(P*spankhy-spxR91A*^{DD})	This study
ORB8269	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL93(P*spankhy-spxF64A*^{DD})	This study
ORB8270	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL94(P*spankhy-spxF64Y*^{DD})	This study
ORB8271	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*)	This study

	amyE::pAL95(P*spankhy-spxQ65A*^{DD})	
ORB8272	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL96(P*spankhy-spxN68A*^{DD})	This study
ORB8273	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL97(P*spankhy-spxN70A*^{DD})	This study
ORB8274	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL98 (P*spankhy-spxY5A*^{DD})	This study
ORB8275	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL99 (P*spankhy-spxY5F*^{DD})	This study
ORB8309	*trpC2 pheA1* SPβ*c2del2*::Tn917:: pMMN92(*srfA-lacZ*) *amyE*::pAL88(P*spankhy-spxR91A*^{DD})	This study
ORB8310	*trpC2 pheA1* SPβ*c2del2*::Tn917:: pMMN92(*srfA-lacZ*) *amyE*::pAL37(P*spankhy-spxR92A*^{DD})	This study
ORB8323	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL106(P*spankhy-spxR91A,R92A*^{DD})	This study
ORB8565	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL127(P*spankhy-spxR92K*^{DD}]	This study
ORB8566	*trpC2 pheA1* Δ*spx*::*neo thrC*::pDYR9(*trxB-lacZ*) *amyE*::pAL128(P*spankhy-spxG52R,R91A*^{DD}]	This study
Plasmids		
pAL37	pDR111 with *spxR92A*^{DD}	This study
pAL38	pDR111 with *spxR92Q*^{DD}	This study
pAL45	pDR111 with *spx*Δ*CHA*	(19)
pAL46	pTYB4 with *spx*Δ*CHA*	(19)
pAL54	pTYB4 with *spx*^{R92A}	This study
pAL56	pDR111 with *spxC10A,R92A*^{DD}	This study
pAL58	pDR111 with *spxR60E,R92A*^{DD}	This study
pAL64	pDR111 with *spxK62E,R92A*^{DD}	This study
pAL65	pDR111 with *spxC10A,R60E*^{DD}	This study
pAL67	pDR111 with *spxC10A,K62E*^{DD}	This study
pAL73	pTYB4 with *spx*^{C10A}Δ*CHA*	(19)
pAL74	pTYB4 with *spx*^{G52R}Δ*CHA*	(19)
pAL75	pTYB4 with *spx*^{R60E}Δ*CHA*	(19)
pAL82	pTYB4 with *spx*^{R92A}Δ*CHA*	This study
pAL88	pDR111 with *spxR91A*^{DD}	This study
pAL93	pDR111 with *spxF64A*^{DD}	This study
pAL94	pDR111 with *spxF64Y*^{DD}	This study
pAL95	pDR111 with *spxQ65A*^{DD}	This study
pAL96	pDR111 with *spxN68A*^{DD}	This study
pAL97	pDR111 with *spxN70A*^{DD}	This study
pAL98	pDR111 with *spxY5A*^{DD}	This study
pAL99	pDR111 with *spxY5F*^{DD}	This study
pAL106	pDR111 with *spxR91A,R92A*^{DD}	This study
pAL121	pTYB4 with *spx*^{R91A}	This study
pAL122	pTYB4 with *spx*^{R91A}Δ*CHA*	This study
pAL127	pDR111 with *spxR92K*^{DD}	This study
pAL128	pDR111 with *spxG52R,R91A*^{DD}	This study
pAL129	pTYB4 with *spx*^{G52R,R91A}Δ*CHA*	This study
pDG793	*thrC* integration vector with promoter-less *lacZ* reporter	(49)
pDR111	*amyE* integration vector with P*spankhy* promoter	(38)
pDW4	TOPO vector with *trxB* promoter	This study
pDW10	pRLG770 with *trxB* promoter	This study
pDYR9	pDG793 with *trxB*(-115~+47)-*lacZ*	(22)
pMMN470	pTYB4 with *spx*	(37)
pMMN683	pDR111 with *spxR60E*^{DD}	(32)
pMMN684	pDR111 with *spxK62E*^{DD}	(32)
pRLG770	transcription vector	(44)
pSN56	pDR111 with *spx*^{DD}	(13)

pSN64	pTYB4 with *sigA*	(21)
pTYB4	*E. coli* expression vector for IMPACT™ system	New England BioRads
pUC18	cloning vector	
pUC19	cloning vector	
pYZ14	pDR111 with *spxC10A*^{DD}	(50)

Table S2. oligonucleotides used in this study

oligonucleotide	sequence	purpose
oAL25	GATGATCGGAGCGCGAAGC	*spx* R92A reverse
oAL24	GCTTCGCGCTCCGATCATC	*spx* R92A forward
oAL27	GATGATCGGTTGGCGAAGC	*spx* R92Q reverse
oAL26	GCTTCGCCAACCGATCATC	*spx* R92Q forward
oAL62	ATCGGACGGGCAAGCAAACC	*spx* R91A reverse
oAL61	GGTTTGCTTGCCCGTCCGAT	*spx* R91A forward
oAL65	GTTCAAAAGTAGCTCAAAAACTGA	*spx* F64A forward
oAL66	TCAGTTTTTGAGCTACTTTTGAAC	*spx* F64A reverse
oAL68	TCAGTTTTTGATATACTTTTGAAC	*spx* F64Y reverse
oAL67	GTTCAAAAGTATATCAAAAACTGA	*spx* F64Y forward
oAL70	ACATTCAGTTTAGCGAATACTTTTG	*spx* Q65A reverse
oAL69	CAAAAGTATTCGCTAAACTGAATGT	*spx* Q65A forward
oAL72	ACGTTCACAGCCAGTTTTTG	*spx* N68A reverse
oAL71	CAAAAACTGGCTGTGAACGT	*spx* N68A forward
oAL74	GATTCAACAGCCACATTCAG	*spx* N70A reverse
oAL73	CTGAATGTGGCTGTTGAATC	*spx* N70A forward
oAL76	GCTTGGTGATGTTGCTAGTGTA	*spx* Y5A reverse
oAL75	TACACTAGCAACATCACCAAGC	*spx* Y5A forward
oAL78	GCTTGGTGATGTAAATAGTGTA	*spx* Y5F reverse
oAL77	TACACTATTTACATCACCAAGC	*spx* Y5F forward
oAL88	GATCGGAGCGGCAAGCAAACC	*spx* R91A,R92A reverse
oAL87	GGTTTGCTTGCCGCTCCGATC	*spx* R91A,R92A forward
oLA106	ATGATGATCGGTTTGCGAAGCA	*spx* R92K reverse
oAL105	TGCTTCGCAAACCGATCATCAT	*spx* R92K forward
oDW7	GGAATTCATCTGTTGACTCTATGAGCAATC	*trxB* (-200~+20) forward
oDW8	CAAGCTTCCCCATCAAACGTATTCCTTAC	*trxB* (-200~+20) reverse
oAL35	TCCCCCGGGAGCATAATCAGGAACATC	*HA* reverse (pTYB4)
oAL38	CTGAGGACCGAACAGATG	*spx* G52R forward
oAL39	CATCTGTTCGGTCCTCAG	*spx* G52R reverse
oDYR06-032	TAAACCATCTTTAAGCTTTTGGGCTCTTTC	*trxB* (-115~+47) reverse
oDYR07-052	GCGAATTCGGCCTTCTATAAACAGAAGGC	*trxB* (-115~+47) forward
oMMN01-135	AGAGGAGTGAAGATCCATGGTTACACTATAC	*spx* forward (pTYB4)
oMMN01-137	TAACTCCCGGGGTTTGCCAAACGCTGTGCTT	*spx* reverse (pTYB4)
oMMN01-173	CGAGGAAGCTTAGATGTTCATCCTACTA	*spx* forward (pDR111)
oMMN01-174	TACCAGCAGGTCGACAAATAAAAGAAGG	*spx* reverse (pdR111)
oSN03-66	CACATCACCAAGCGCGACTTCATGCAGAA	*spx* C10A forward
oSN03-67	TTCTGCATGAAGTCGCGCTTGGTGATGTG	*spx* C10A reverse
oDYR06-02	[Biotin]-CTGAACTAGAGGCCAAGGCTCTTTCTTTATTAACATATAC	*trxB* template (forward) for crosslinking
oDYR06-03	ATCCCCATCAAACGTATTCCTTAC	*trxB* template (reverse) for crosslinking
oDYR06-05	ATCCCCATCAAACGTATTCCTTACCCATATCC*T	*trxB* -11 crosslinking probe
oDYR07-35	ATCCCCATCAAACGTATTCCTTACCCATATCCTATAAAATCT*C	*trxB* -21 crosslinking probe
oAL47	ATCCCCTCAAACGTATTCCTTACCCATATCCTATAAAATCTCATGGTGA TACGCTA*T	*trxB* -36 crosslinking probe
oAL49	ATCCCCTCAAACGTATTCCTTACCCATATCCTATAAAATCTCATGGTGA TACGCTATTTT*T	*trxB* -40 crosslinking probe
oAL51	ATCCCCTCAAACGTATTCCTTACCCATATCCTATAAAATCTCATGGTGA TACGCTATTTTTTTG*C	*trxB* -44 crosslinking probe
oAL52	ATCCCCTCAAACGTATTCCTTACCCATATCCTATAAAATCTCATGGTGA TACGCTATTTTTTTGCTCAA*C	*trxB* -49 crosslinking probe
oDYR06-08	ATCCCCTCAAACGTATTCCTTACCCATATCCTATAAAATCTCATGGTGA TACGCTATTTTTTTGCT*C	*trxB* -46 crosslinking probe
oDYR06-09	ATCCCCTCAAACGTATTCCTTACCCATATCCTATAAAATCTCATGGTGA TACGCTATTTTTTTGCTCAACAC*G	*trxB* -52 crosslinking probe