

Multiple transcriptional factors regulate transcription of the *rpoE* gene in *Escherichia coli* under different growth conditions and when the lipopolysaccharide biosynthesis is defective

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Running title: *RpoN-, RpoD- and RpoS-dependent control of the rpoE transcription*

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Supplemental information

Table S1. Bacterial strains and plasmids used in this study

Strains/Plasmids	Relevant characteristic	Reference or source
Strains		
BW25113	<i>lacI</i> ^q <i>rrnB</i> _{T14} Δ <i>lacZ</i> _{WJ16} <i>hsdR514</i> Δ <i>araBAD</i> _{AH33} Δ <i>rhaBAD</i> _{LD78}	(62)
MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>) U169	Our collection
BL21(DE3)	F ⁻ <i>ompT hsdSB(rB⁻ mB⁻) gal dcm</i> (DE3)	Invitrogen
GK1942	BW25113 (pKD46)	(9)
SR4245	MC4100 ϕ (<i>rpoEP1-P5-lacZ</i>)	This study
SR7917	BW25113 ϕ (<i>rpoEP1-P5-lacZ</i>)	This study
SR18647	BW25113 ϕ (<i>rpoEP1-lacZ</i>)	This study
SR18868	BW25113 ϕ (<i>rpoEP2/P3-lacZ</i>)	This study
SR18987	BW25113 ϕ (<i>rpoEP2*-lacZ</i>) -12 and -24 mut	This study
SR19089	BW25113 ϕ (<i>rpoEP3*-lacZ</i>) -7C -11G	This study
SR18874	BW25113 ϕ (<i>rpoEP4-lacZ</i>)	This study
SR18583	BW25113 ϕ (<i>rpoEP5-lacZ</i>)	This study
SR10074	SR4245 <i>waaC</i> ::Tn10	This study
SR10182	SR7917 <i>waaC</i> ::Tn10	This study
SR10396	SR7917 <i>waaF</i> ::Tn10	This study
SR10401	SR7917 <i>rfaH</i> ::Tn10	This study
SR17505	SR7917 <i>rssB</i> ::Tn10	This study
SR8035	W3110 <i>waaC</i> \langle <i>aph</i>	(6)
SR8233	W3110 <i>waaC</i> \langle <i>cat</i>	(6)
GK2876	SR7917 <i>rpoS</i> \langle <i>aph</i>	This study
GK2880	SR7917 <i>rpoN</i> \langle <i>aph</i>	This study
GK2905	SR7917 <i>rpoN</i> \langle <i>aph waaC</i> \langle <i>cat</i>	This study
GK2986	SR7917 <i>crp</i> \langle <i>aph</i>	This study
GK2864	SR7917 <i>waaC</i> \langle <i>cat</i>	This study
GK2868	SR7917 <i>waaC</i> \langle <i>aph</i>	This study
GK4691	SR7917 <i>waaP</i> \langle <i>aph</i>	This study
GK4732	SR7917 <i>waaF</i> \langle <i>aph</i>	This study

GK4755	SR7917 <i>waaG</i> ◊ <i>aph</i>	This study
GK4762	SR7917 <i>rfaH</i> ◊ <i>aph</i>	This study
SR10438	SR7917 <i>ntrC</i> ◊ <i>aph</i>	This study
SR15415	SR7917 <i>rcsB</i> ◊ <i>aph</i>	This study
SR15422	SR7917 <i>rcsB</i> ◊ <i>cat</i>	This study
SR15506	SR7917 <i>rssB</i> ◊ <i>aph</i>	This study
SR15890	SR7917 <i>arcA</i> ◊ <i>aph</i>	This study
SR15894	SR7917 <i>arcB</i> ◊ <i>aph</i>	This study
SR17500	SR7917 <i>cya</i> ◊ <i>aph</i>	This study
SR18577	SR7917 <i>ecfLM</i> ◊ <i>aph</i>	This study
SR19015	SR7917 <i>lapA-lapB</i> ◊ <i>aph</i>	This study
SR19027	SR7917 <i>rcsB</i> ◊ <i>cat lapA-lapB</i> ◊ <i>aph</i>	This study
JW5702	BW25113 <i>crp</i> ◊ <i>aph</i>	(33)
JW5437	BW25113 <i>rpoS</i> ◊ <i>aph</i>	(33)
GK1943	BW25113 <i>waaP</i> ◊ <i>aph</i>	This study
GK2048	BW25113 <i>waaF</i> ◊ <i>cat</i>	This study
SR7938	BW25113 <i>waaO</i> ◊ <i>aph</i>	This study
SR7946	BW25113 <i>waaF</i> ◊ <i>aph</i>	This study
SR8021	BW25113 <i>waaG</i> ◊ <i>aph</i>	This study
SR15695	BW25113 <i>rcsF</i> ◊ <i>aph</i>	This study
SR17187	BW25113 <i>lapA-lapB</i> ◊ <i>aph</i>	(9)
SR18718	BW25113 <i>qseF</i> ◊ <i>aph</i>	This study
SR9237	BW25113 <i>waaO</i> ::3xFLAG◊ <i>frit</i>	(9)
SR16215	BW25113 <i>waaC</i> ::3xFLAG◊ <i>frit</i>	(9)
SR17110	BW25113 <i>rfaB</i> ::3xFLAG◊ <i>frit</i>	This study
GK4798	SR18987 <i>lapA-lapB</i> ◊ <i>aph</i>	This study
GK4803	SR18987 <i>rcsB</i> ◊ <i>cat lapA-lapB</i> ◊ <i>aph</i>	This study
SR18989	SR18987 <i>crp</i> ◊ <i>aph</i>	This study
SR18990	SR18987 <i>waaC</i> ◊ <i>cat</i>	This study
SR18993	SR18987 <i>rcsB</i> ◊ <i>cat</i>	This study
SR18997	SR18987 <i>rcsB</i> ◊ <i>cat waaC</i> ◊ <i>aph</i>	This study
SR19001	SR18987 <i>waaF</i> ◊ <i>aph</i>	This study
SR19077	SR18987 <i>waaG</i> ◊ <i>aph</i>	This study
SR19079	SR18987 <i>waaP</i> ◊ <i>aph</i>	This study
SR19083	SR18987 <i>waaO</i> ◊ <i>cat</i>	This study
SR18991	SR18987 <i>ecfLM</i> ◊ <i>aph</i>	This study
SR18936	SR18874 <i>rpoS</i> ◊ <i>aph</i>	This study
SR19013	SR18874 <i>rssB</i> ◊ <i>aph</i>	This study
SR19110	SR19089 <i>qseF</i> ◊ <i>aph</i>	This study
SR19112	SR19089 <i>rpoN</i> ◊ <i>aph</i>	This study
GK4815	SR19089 <i>qseF</i> ◊ <i>aph waaC</i> ◊ <i>cat</i>	This study
SR19055	SR19089 <i>waaC</i> ◊ <i>cat</i>	This study
SR19060	SR19089 <i>rpoN</i> ◊ <i>aph waaC</i> ◊ <i>cat</i>	This study
GK4853	BW25113 + pMF19 + pRS551	This study
GK4860	BW25113 + pMF19 + pSR9446	This study

Plasmids

pET28b	expression vector	Novagen
pET22b	expression vector	Novagen
pMF19	expresses WbbL rhamnosyltransferase sp ^R	(52)
pCP20	ts replicon with inducible FLP recombinase	(62)
pKD3	<i>oriR6K_g, bla(Amp^R), kan, rgnB(Ter), cat</i>	(62)
pKD13	<i>oriR6K_g, bla(Amp^R), kan, rgnB(Ter)</i>	(62)
pKD46	<i>araBp-gam-bet-exo, bla(Amp^R), repA101(ts)</i>	(62)

pRS415	<i>lacZYA</i> transcriptional fusion vectors	(63)
pRS550	<i>lacZYA</i> transcriptional fusion vectors	(63)
pRS551	<i>lacZYA</i> transcriptional fusion vectors	(63)
pCA24N	IPTG-inducible expression vector cm^R	(34)
JW5808	<i>pcnB</i> ⁺ in pCA24N cm^R	(34)
pSR9443	<i>virA</i> ⁺ in pOK12 kan^R	This study
pSR9446	<i>virA</i> ⁺ in pRS551 kan^R amp^R	This study
pSR18213	<i>virA</i> [*] in pSR9446	This study
pSR18976	<i>ntnC</i> ⁺ in pET28b kan^R	This study
pGK4661	<i>rcsA</i> ⁺ in pET22b amp^R	This study
pGK4662	<i>rcsB</i> ⁺ in pET22b amp^R	This study
pSR16691	<i>rpoS</i> ⁺ in pET24b kan^R	This study
pSR16739	<i>crp</i> ⁺ in pET22b amp^R	This study
pSR13036	<i>qseF</i> ⁺ in pET28b kan^R	This study
pSR13274	<i>qseG</i> ⁺ in pCA24N cm^R	This study
pSR14321	<i>yhdV</i> ⁺ in pCA24N cm^R	This study
JW3169	<i>rpoN</i> ⁺ in pCA24N cm^R	(34)
JW3818	<i>rfaH</i> ⁺ in pCA24N cm^R	(34)
pSR18621	<i>rpoEP1-lacZ</i> in pRS551 kan^R amp^R	This study
pGK4838	<i>rpoEP3*-lacZ</i> (-7C -11G) in pRS551	This study
pSR18980	<i>rpoEP2*-lacZ</i> (-12 and -24 mut) in pRS550	This study
pSR18854	<i>rpoEP2-P3-lacZ</i> in pSR415 amp^R	This study
pSR18861	<i>rpoEP4-lacZ</i> in pRS415 amp^R	This study
pSR18569	<i>rpoEP5-lacZ</i> in pRS550 kan^R amp^R	This study

Table S2. Primers

For gene disruptions:

waaG_for	5'-AAG CTG TTG CCA GAA GAT GCC CCT TCA GCT GAC AGG AAT GCA CAA TTA TGA TTC CGG GGA TCC GTC GAC C-3'
waaG_rev	5'-GCC AAA GTG TGG CAA GCG GCT CTT TTA ATT CAA CCA TCT AAA CCA CCT GTT GTA GGC TGG AGC TGC TTC G -3'
waaP_for	5'-GTA TAG CTT GCC AGA AAA AGC CGC GGA TAT CAT TAC AGG TGG TTT AGA TGA TTC CGG GGA TCC GTC GAC C-3'
waaP_rev	5'-TTA GTT CCA GTA CAT ACT AAT AAA TAT TTT TAT AAT CCT TTG CGT TGT GTT GTA GGC TGG AGC TGC TTC G-3'
rfaH_for	5'-AAA GCT TTT GCT ATC CTT GCG CCC CGA TTA AAC GGA TAA GAG TCA TTA TGA TTC CGG GGA TCC GTC GAC C-3'
rfaH_rev	5'-AAT GTC AAA ACA CTG TTT GGG ATT GCG TTT TAG AGT TTG CGG AAC TCG GTT GTA GGC TGG AGC TGC TTC G-3'
waaF_for	5'-CAT GGC CTG GCT GAA TCG CGA CGC ATA AGA GCT CTG CAT GCA TAT GAA TAT CCT CCT TAG-3'
waaF_rev	5'-CGA TCA AAA CCC GCA TCC GTC AGG CTT CCT CTT GTA ACA AGT GTA GGC TGG AGC TGC TTC-3'
resAF	5'-TAT TCA GGT AAG GGG AAT TAT CGT TAC GCA TTG AGT GAG GGT ATG CCA TGA TTC CGG GGA TCC GTC GAC C-3'
resAR	5'-ACT GGT GGG AAA CCA CCA GTC AGA ATG TGT TAG CGC ATG TTG ACA AAA ATT GTA GGC TGG AGC TGC TTC G-3'
resBF	5'-CAT TGA CAG TTA TGT CAA GAG CTT GCT GTA GCA AGG TAG CCT ATT ACA TGA TTC CGG GGA TCC GTC GAC C-3'
resBR	5'-CTA ACG CGT CTT ATC TGG CCT ACA GGT GAT TAG TCT TTA TCT GCC GGA CTT GTA GGC TGG AGC TGC TTC G-3'
resF-F	5'-GCT CCT GAT TCA ATA TTG ACG TTT TGA TCA TAC ATT GAG GAA ATA CTA TGA TTC CGG GGA TCC GTC GAC C-3'
resF-R	5'-AAC GCC TAT TTG CTC GAA CTG GAA ACT GCT CAT TTC GCC GTA ATG TTA AGT GTA GGC TGG AGC TGC TTC G-3'
ecfL_for	5'-ATC TTT TAA AAC CAG CGT GGC GTT AAC CGA TTC ACC AGG AAT AAT GAA TGA TTC CGG GGA TCC GTC GAC C-3'
ecfM_rev	5'-GTG AGT GAT ATG TTT CGG ATA ACA GGA AGT TAT CCG AAG CGA TGA GAG TTT GTA GGC TGG AGC TGC TTC G-3'

For cloning and overexpression of specific genes:

NtrC_F	5'-AGG TGA CGC ATA TGC AAC GAG GGA TAG-3'
NtrC_R	5'-CTT TAC ACA CAA GCT GTC TCG AGC TCC ATC C-3'
crpP_For	5'-CAG AGG ATA ACC GCA TAT GGT GCT TGG CAA ACC-3'
crp_Rev	5'-CCA CTC CGA CGC TCG AGA CGA GTG CCG TAA ACG-3'
resA_For	5'-GTG AGG GTA TCA TAT GTC AAC GAT TA-3'
resA_Rev	5'-CAC CAG TCA GAA TCT CGA GGC GCA TGT TGA C-3'
rirA_Rev	5'-GGC TCA ACA GGA TCC TGA ATA CTG ATA AC-3'
rirA_FE	5'-GCG AAA GAA ATG ATA GAA TTC CAC GTC-3'
rirA_RH	5'-CTT TAT GAC CAA GCT TTT TCG AAA TGG C-3'

For promoter cloning:

P1-P5 F	5'-CTG GCG ACG AAT TCA ACT GCA-3'
P1-P5 R	5'-GTT CCG TAA CGG ATC CGT TTT TGT T-3'
P1 F	5'-GTA GCG CCA GTG GAA TTC CGG CTG-3'
P1 R	5'-CGC ACA ACA GGA TCC TAT GCT GAC-3'
P2/P3 F	5'-GGT TTG GTG AAT TCA GCA TCA TGT TGT GCG GAT A-3'

RpoN-, RpoD- and RpoS-dependent control of the rpoE transcription

P2/P3 R 5'-CCT AAT CCT CCA GGA TCC AGT AAC TTC-3'
P4 F 5'-GTT ACA GTT GCT GAA TTC GCT GAC GCA GG -3'
P4 R 5'-GAA CAT CGG ATC CTC TGT GTA G-3'
P5 F 5'-AGT TAA ATG GGC ATT TC-3'
P5 R 5'-GTT CCG TAA CGC ATC TG-3'
P3 mut F 5'-CTA AAC AGA ATT CGG TCA GCA TAG CAT CAT GTT GTG CGG ATA
AAC ACC TGC TGT TTC AAT ATT TGT TAC AG-3'
P2 mut F 5'-GGT CAG CAT AGC ATC ATG TTG TGC GAA TAA ACA CCT ATT ATT
TTA ATA TTT GTT ACA G-3'

For promoter mapping:

P1_Race1 5'-CTC CCC AAA CCA AAT TTC CAC GC-3'
P1_Race2 5'-GTA AAG TTC CGT AAC GCA TCT G-3'

For gel shifts:

P1FL 5'-GGT TTG GTG AAT TCA GCA TCA TGT TGT GCG GAT A-3'
P1FS 5'-AAA AGA GAA TTC ACT GGC TGG TGG A-3'
P2/P3 R 5'-CCT AAT CCT CCA GGA TCC AGT AAC TTC-3'
P1BH_R 5'-CGC ACA ACA GGA TCC TAT GCT GAC-3'
RpoN_WF 5'-GGT CAG CAT AGC ATC ATG TTG TGC GGA TAA ACA CCT GCT ATT
TTA ATA TTT GTT ACA G-3'
GFP1_FOR 5'-AGT GAA AGT CCG GCT GCG CCG CTA-3'
GFP2_FOR 5'-GAA TGT TCA GGG AGA GTA TTC-3'
GFP3_FOR 5'-CTA AAC ATG GTT TGG TCA GC-3'
GFN_REV 5'-CCT CCA CCA GCC AGT AAC TTC-3'