

Supplemental Material

Title:

The δ subunit of RNA polymerase is required for rapid changes in gene expression and competitive fitness of the cell.

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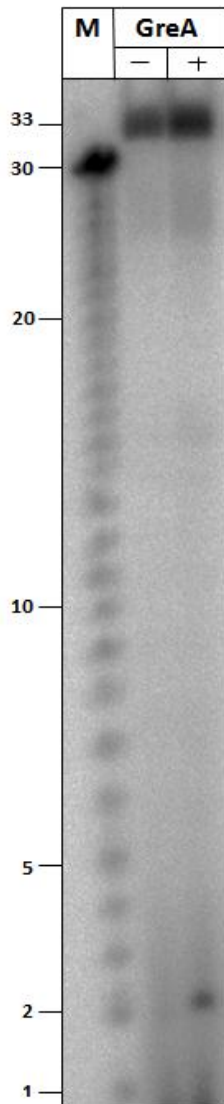
LIST OF PRIMERS

Table S1. *List of primers*

Primer number	Sequence (5' → 3')	Expression vector
194	CGGGTACCAAACAATATTCACAGGAAGAG	pSG1729
210	CGGGATCCTCAAAGCGTCTCGGAGAAGAAG	pGEX-5X-3
211	CGGAATTCGTATCGTTCTTATACAAACC	pGEX-5X-3
212	CCGCTCGAGGTATCAGATTTTCATCTTCAAAC	pGEX-5X-3
213	AAGGAAAAAAGCGGCCGCTTTGTGATGTGCGGAATGAC	pGEX-5X-3
235	CGGAATTCTCAGTGGTGGTGGTGGTGGTGGTTTAATTCCTCTTCTTC	pSG1729
483	CGGAATCCCCGCCATGTCCAAACTGATG	pDG3661
484	CGAAGCTTATAAGAAAAATGAGGGGTGA	pDG3661
GREA1	CGGGATCCCATCGAAGGTCGTATGGCACAAAGAGAAAGTTTTTC	pGEX-1
GREA2	CGGAATTCTTATTATGAAATTTTCACAATTTTCACGAGC	pGEX-1
931	GCTCTAGAGGAGGTGGATGATATGCACCACCACCACCACATT GAAGGCAGAATGTTTCAAATTGGCGATAAC	pSG1729
963	TGGGTACCATGTTTCAAATTGGCGATAAC	pSG1729

GRE A ACTIVITY

Figure S1. *B. subtilis* GreA protein is active *in vitro*.



B. subtilis GreA promotes formation of active elongation complexes *in vitro*. In the presence of GreA (+) more elongation complexes (~ 2x) were formed than in its absence (-). Active complexes were visualized by the addition of ^{32}P αUTP that was incorporated by RNAP to the 3' end of the RNA, resulting in the radiolabeled 33 nt RNA shown in the gel. A dinucleotide was also observed in the presence of GreA, likely generated by the cleavage of the 3' end of RNA in some backtracked complexes, another indication of GreA activity. M, ladder generated from 5' end labeled 30 nt RNA. A representative gel is shown. The experiment was repeated three times with identical results.

The elongation complexes were assembled as described (1), using the same sequences of respective nucleic acids with the exception of the first three nucleotides incorporated into the RNA that in this case were Us. Briefly, 2-fold molar excess of RNA was mixed with template DNA (12.5 pmol) in water and the oligonucleotides were annealed as in (1). Subsequently, 5 pmol of RNAP was incubated with a 2-fold molar excess of the annealed hybrid for 15 min at room temperature while gently shaking. 40 pmol of the non-template DNA was then added and the mixture was incubated for 10 minutes at 37°C. 50 pmol of GreA/buffer was added to the elongation complexes and incubated for another 10 minutes. 1 μl of 10x diluted [$\alpha^{32}\text{P}$] UTP (10 mCi/ml) was added per reaction, followed by an incubation at 30°C for 15 min. Reactions were stopped with equal volumes of formamide stop solution (95% formamide, 20 mM EDTA, pH 8.0). Samples were loaded onto 14% polyacrylamide gels and electrophoresed. The dried gels were scanned with Molecular Imager_FX (BIO-RAD).

STARVATION FOR AMINO ACIDS

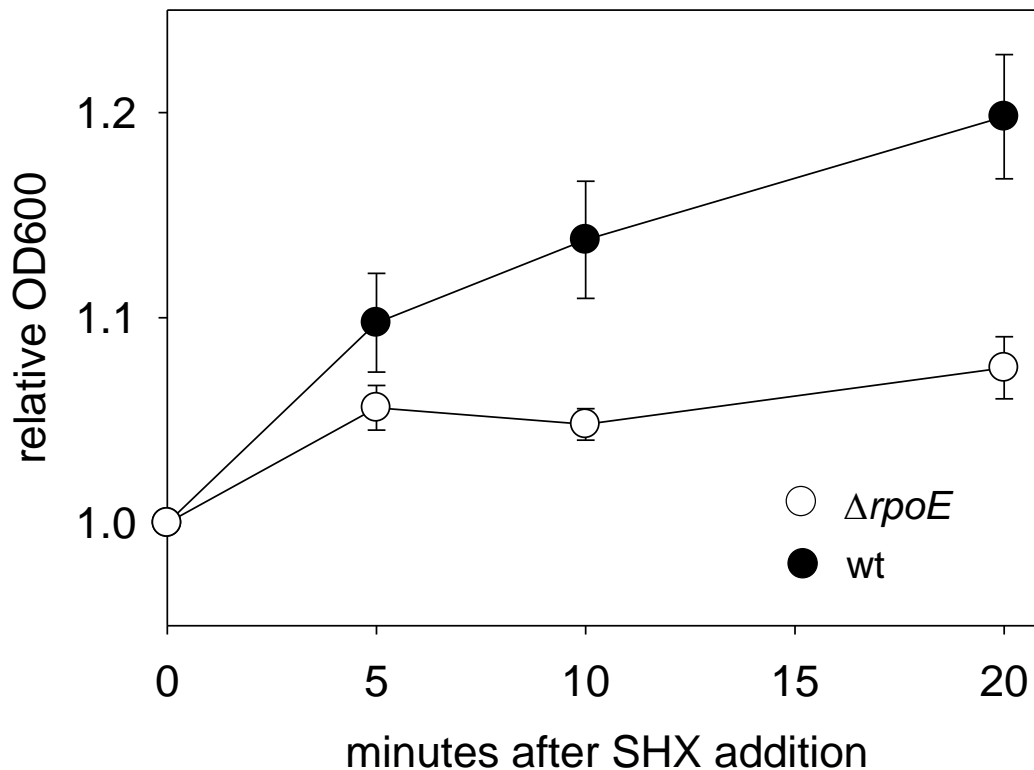


Figure S2. Starvation for amino acids

Cells (wt: RLG7555; $\Delta rpoE$: LK643) were grown in MOPS, 0.4% glucose, and 25 $\mu\text{g/ml}$ of all 20 amino acids at 37°C. Starvation for amino acids was induced at OD600~0.3 by the addition of 3 mM (final concentration) serine hydroxamate (Sigma-Aldrich) at time zero. Growth of the bacterial strains was measured at 600 nm at indicated time points (OD600 at time zero was set as 1 to aid in visualizing the differential growth of the strains). The data are averages \pm SD from five independent experiments.

List of References

1. Komisarova, N., Kireeva, M. L., Becker, J., Sidorenkov, I., and Kashlev, M. (2003) Engineering of elongation complexes of bacterial and yeast RNA polymerases. *Methods Enzymol*, **371**: 233- 251.