Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Joseph Osmundson and Seth A. Darst

Biochemical insights into the function of phage G1 gp67 in *Staphylococcus aureus*

Bacteriophage 2013; 3(1)
http://dx.doi.org/10.4161/bact.24767

www.landesbioscience.com/journals/bacteriophage/article/24767

Supplemental Materials and Methods:

Protein Purification:

σ, σ^{A}_{4}, and Sau RNAP were purified as described.[1]

Gp67 was cloned into pET29a using NdeI and XhoI, removing the native stop codon, to produce a C-terminally His-tagged protein. Gp67 was expressed in BL21(DE3) cells with 0.5mM IPTG overnight at 18°C and purified using standard Ni- affinity chromatography in 1x protein buffer (20mM Tris pH 8.0, 0.5M NaCl, 5% glycerol, 0.5mM β-ME). For crystallization, gp67 was further purified by ion exchange chromatography and gel filtration, buffer exchanged into 10mM Tris pH 8.0, 0.2M NaCl, 1mM DTT), and concentrated to 5-10mg/ml.

Limited proteolysis:

Gp67, or the reconstituted complex of gp67 and σA4 were incubated at 5μM in 10μl in 1x proteolysis buffer (100 mM Tris HCl, pH 8.0 and 20 mM CaCl2) on ice for 10 minutes. Reactions were brought to 30°C before the addition of trypsin at the following molar ratios (protein:protease): 1:0, 1000:1, 100:1, 50:1, 10:1, 5:1. Reactions were allowed to proceed for 30 minutes and stopped with the addition of 1μl of 100mM PMSF. 10μl SDS loading buffer was added and samples boiled for 5 minutes at 95°C before being run on a 4-12% SDS-PAGE gel (Invitrogen).

Transcription Assays:

Transcription assays were performed on linear DNA fragments as described.[1]

Open promoter complex lifetime assays:

Open promoter complexes were assayed as previously described.[2] Briefly gp67 (1μM), or empty protein buffer, was incubated with σA (100nM) for 10 minutes before the addition of Sau core RNAP (50nM). The complex was incubated on ice for 10 minutes. For the aag and rrnA promoter, 50nM of linear DNA was added to the reaction in 1x Sau transcription buffer, as described. Open promoter complexes were allowed to form for 20 minutes at 37°C before being challenged by the addition of 1μM ds FullCon promoter fragment. At time points after the addition of the FullCon[3] promoter fragment, reactions were initiated by the addition of 200μM NTPs with α-P^{32} labeled ATP. Reactions were stopped after 5 minutes by the addition of 2x stop buffer and electrophoresed on a 12% Urea-PAGE gel.

For the G1-gp67 promoter, we assayed open promoter complexes by filter binding. The linear promoter fragment was end-labeled with P32 using polynucleotide kinase (PNK, New England Biolabs) under standard conditions. Open complexes were formed by incubation of holo +/- gp67 at the above concentrations at 37°C and challenged by the addition of 1μM unlabeled ds FullCon[3] promoter fragment. At time points after the addition of challenging DNA, 10μl aliquots were pipetted onto prewashed filter papers

(MF-Membrane Filters, 0.45μM, Millipore), allowed to bind for 10 seconds, and washed with 1x wash buffer (10mM Tris pH 8.0, 0.2M NaCl). Filter papers were dried and quantified on a phosphoimaging screen. All samples were normalized to the signal at time = 0.

Supplemental References:

1. Osmundson J, Montero-Diez C, Westblade LF, Hochschild A, Darst SA. Promoter-specific transcription inhibition in Staphylococcus aureus by a phage protein. Cell 2012; 151:1005-16.
2. Paul BJ, Barker M, Ross W, Schneider D, Webb C, Foster J, et al. DksA: A critical component of the transcription intiiation machinery that potentiates the regulation of rRNA promoters by ppGpp and the initiating NTP. Cell 2004; 118:311-22.
3. Gaal T, Ross W, Estrem ST, Nguyen LH, Burgess RR, Gourse RL. Promoter recognition and discrimination by EsigmaS RNAP. Molecular Microbiology 2001; 42:939-54.