Escherichia coli transcription factor NusG binds to 70S ribosomes

Shivalika Saxena[1], Kamila K. Myka[1], Robert Washburn[1], Nina Constantino[2], Donald L. Court[2], and Max E. Gottesman[1*]

SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY TABLE

Table S1: Intermediate strains used for construction

Strain	Genotype	Construction / Reference
7006	N99 *nusE*71:Tn*10 galK*$^-$	P1.K2016 x N99
10289	W3110 *luc*:N *cI*857 *cro*(ΔRBS) *nutR* tRI *cII*::*lacZ*:kan *nusE*100:Tn*10*	P1.RR229 x NC438
11491	TUC680 *nusE*71:Tn*10*	P1.7006 x TUC680
12422	MDS42 *rpoB**35	P1.N4849 x RSW712
K2016	*nusE*71 *zhb*-3082::Tn*10*	(Schauer *et al.*, 1996)
N99	*galK*2 *strA*	(Griffo *et al.*, 1989)
N4849	MG1655 *rpoB**35	(Trautinger *et al.*, 2005)
NB885	W3110 Δ*(argF-lac)U169 gal::IS2-490* pglΔ8 rpoC*:[His6-kan] *nusG* F165A	M. Bubunenko, Court lab strain
NC438	W3110 *luc*:N *cI*857 *cro*(ΔRBS) *nutR* tR1 *cII*::*lacZ*:kan	N. Constantino, Court lab strain
RR229	N99 λpR-*cro-nutR*-tR1-*gal nusE*100:Tn*10*	R. Robledo, Gottesman lab strain
RSW712	MDS42 *rpoB**35 *argE*:Tn*10*	(Washburn and Gottesman, 2011)
SS2132	TUC680 *nusE*-M88/D97A:Tn*10*	ssDNA recombineering into 11491 using pSIM6
TUC680	W3110 *gal::IS2-490* pglΔ8* [*lacZ<>cIII-N*$^+$ p_L] λ*cI*857 [p_R- (*cro-bioA*)Δ]	Court lab strain
XL2-Blue	*endA1 supE44 thi-1 hsdR17 recA1 gyrA96 relA1 lac* [*F´ proAB lacI*^{q}*ZΔM15* Tn*10* Amy Cam^R]	Invitrogen

SUPPLEMENTARY FIGURES

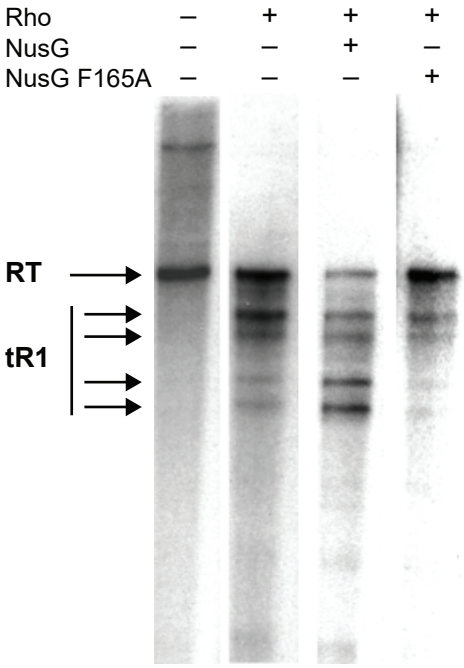

Figure S1: A representative gel of *in vitro* transcription with NusG and NusG F165A.

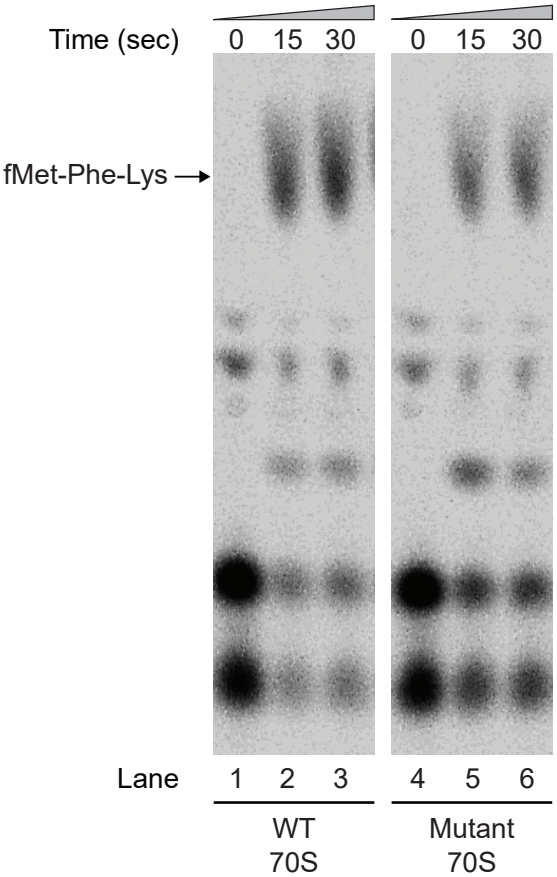

Figure S2: A representative electrophoretic (eTLC) plate showing formation of fMet-Phe-Lys tripeptide over time with wild-type and mutant ribosomes. Lanes 1, 2 and 3: WT ribosomes, Lanes 4, 5 and 6: mutant ribosomes. The 0, 15 and 30 sec time points represent the interval before the reaction was quenched.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Bacterial strains and plasmid construction

Generation of strain 11601 carrying the *nusE M88A* and *nusE D97A* mutations. Strain TUC680 was P1-transduced with a lysate from strain 7006 (*nusE*71:Tn*10*) to obtain strain 11491 (Table S1). TUC680 contains a defective λ prophage in which transcription from the λ*pL* promoter is repressed by *c*I857 repressor at 32°C. At 42°C, RNAP transcribing from λ*pL* is antiterminated by the N-Nus protein complex. This allows transcription to pass through the *gal* leader *IS2* element resulting in *gal* operon expression and a Gal^+ phenotype. The *nusE*71 mutation blocks N-Nus antitermination; 11491 is thus Gal^-. To generate the strain containing the *nusE* M88/D97A mutations, a 72 bp oligonucleotide (5'-TGA TTA ACC CAG GCT GAT CTG CAC CGC TAC ACC GGC AGC CAG ATC CAG ACG CGC CAG **CGC** ATC AAC GGT TTT-3') was recombineered into 11491 transformed with pSIM6 (Datta *et al.*, 2008). This oligo encodes the antisense sequence of *nusE* gene that corrects the *nusE71* (A86D) sequence and introduces the *nusE* M88/D97A mutations. The (antisense) codons changed are underlined and the wild type alanine (antisense) codon A86 is in bold. The change from *nusE*71 to *nusE* M88/97A restores the Nus^+ and Gal^+ phenotypes. Gal^+ recombinants were selected on minimal M63 galactose agar plates supplemented with biotin. The *nusE* M88/D97 mutation was then transduced into MDS42, selecting for tetracycline resistant colonies to yield 11601. All primers were obtained from IDT.

Construction of strains for the antibiotic sensitivity testing. Strains KM848, KM850, KM852 and KM854 were constructed as follows. A kanamycin cassette was amplified from a Keio library strain (Baba *et al.*, 2006), digested with XbaI and ligated with pKD3 (Datsenko and Wanner, 2000) also digested with XbaI. The resulting plasmid contained a <kan> cassette instead of a <cat> cassette, in the same orientation. The plasmid was cut with NdeI and ligated with a fragment of $nusG^+$ or *nusG* F165A obtained by PCR from MDS42 and 10780, respectively, using primers *nusG*_NdeI_F (5'-GTA TCA CAT ATG GAA GTG GTT GAA ATC CGT-3') and *nusG*_NdeI_R (5'-TGT TTG CAT ATG GGG TTA GGC TTT TTC AAC-3'). The resulting two plasmids served as templates for the PCR with primers *nusG*_rcmb_F (5'-ATT ATG AAC CGC CTG CAG CA-3') and *nusG*_rcmb_R (5'-CGC CTT GTG CAA CGA TTA AAT CGC CGC TTT TTT GAT CGC TTG TAG GCT GGA GCT GCT TCG-3'). The two PCR products were used for recombineering using pKD46 (Datsenko and Wanner, 2000) into MDS42 and 12422. P1vir lysates were prepared from the four strains (MDS42 $nusG^+$/F165A::<kan>; 12422 $nusG^+$/F165A::<kan>) and used for transduction into MDS42 and 12422, resulting in strains KM848, KM850, KM852 and KM854. The <kan> cassette in all four strains is inserted between the third and fourth nucleotide after the *nusG* stop codon. All PCRs were performed using NEB Q5 High-Fidelity Polymerase.

Construction of the pTrc-NusG-F165A plasmid. The ampicillin resistant plasmid pTrc-NusG (pRM431) was a gift from R. Landick (Mooney *et al.*, 2009). Its mutant derivative pTrc-NusG-F165A (pCu2121) was constructed by site-directed mutagenesis using primers 5'-AGT GTC TGT

TTC TAT Cgc CGG TCG TGC GAC CCC G-3' and 5'-AGT GTC TGT TTC TAT Ccg CGG TCG TGC GAC CCC G-3' to amplify *nusE*. PCR was performed using Pfu TURBO DNA Polymerase from Stratagene. XL-2 blue cells from Invitrogen were used to select ampicillin-resistant transformants of pTrc-NusG-F165A. p-Trc-NusG-F165A transformants were lethal when overexpressed by addition of IPTG on LB-Amp agar and were sensitive to 4 μg ml^{-1} of chloramphenicol on LB-Amp-Cm plates at 37°C.

In vitro transcription

Transcription templates were generated using GoTaq DNA polymerase (Promega, Madison, WI) and primers 5'-GTT AAA TCT ATC ACC GCA AGG GAT-3' and 5'-CGT AGA GCC TCG TTG CGT-3'. These oligos amplify a 500 bp fragment of phage λ including the λ *pR* promoter and the Rho-dependent terminator tR1. Open complexes were formed by preincubating 0.1 pmol template with 2 pmol RNAP (New England Biolabs, Ipswich, MA) and 40 units rRNasin Plus (Promega, Madison, WI) in 50 μl transcription buffer (20 mM Tris-acetate (pH 7.9), 60 mM potassium acetate, 4 mM magnesium acetate, 1 mm DTT, 0.25 mg ml^{-1} bovine serum albumin, and 5% (v/v) glycerol) for 5 min at 37°C. Where indicated, 2 pmol Rho and/or 2 pmol NusG or 2 pmol NusG F165A were added. Transcription was initiated by addition of 200 μM GTP, CTP and ATP and 50 μM UTP plus 1 μCi of [α-^{32}P] ATP. After 10 min. the reactions were terminated by addition of 50 μl stop mix (375 mM sodium acetate (pH 5.2), 62.5 mM EDTA) and precipitated with 3 volumes 95% (v/v) ethanol. Extracted RNA was then resolved on a denaturing polyacrylamide gel (7 M urea) and analyzed by autoradiography.

In vitro translation and eTLC

The individual components required for this assay were purified and obtained from R. Gonzalez (Columbia University). For more detail, see supplementary material for Ning *et al.* (2014) and Fei *et al.* (2010). The pre-initiation complex consisted of 3 μM ribosomes (WT or mutant), 110 μM IF1, 99 μM IF2, 206 μM IF3, 50 mM GTP, 5x initiation polymix in a final volume of 1.54 μl. Tubes were kept at 37°C for 10 min and then shifted to RT. The above complex was mixed with 110 μM of mRNA (Met, Phe, and Lys) and 28.5 μM of S^{35} labeled fMet-tRNA in a final volume of 2.25 μl to enable 70S complex formation. The reaction was kept at 37°C for 20 min and then transferred on ice. The final reaction was set up with 0.5 μl of 70S complex, 1 μl of solution 5 and 1 μl of solution 4 (Fei *et al.*, 2010). The reaction was run at 37°C for 0, 15, 30, 60 and 300 sec and then quenched with the addition of 1 μl KOH. Samples were stored at -80°C until the eTLC separation. 2-5 μl of sample was mixed with dye and spotted on a TLC plate (aluminum cellulose plate, 1055520001, EMD Millipore) and run at 1200 V for 30 min. Signals were read using a phosphorimager screen.

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