E. coli 6S RNA complexed to RNA polymerase maintains product RNA synthesis at low cellular ATP levels by initiation with non-canonical initiator nucleotides

Supplemental Information

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SUPPLEMENTARY MATERIALS AND METHODS

Engineering the MIII ssrS E. coli construct

pCAGO electroporation into NEB® 10-β Electrocompetent E. coli

The 6S RNA^{M}-encoding *E.coli* strain*, MIII ssrS*, was generated via CAGO (Zhao et al., 2017) using NEB® 10-β Electrocompetent *E. coli.* 100 μl cells were thawed on ice for 10 min then electroporated at 2000 V, 200 Ω, and 25 μF in 1 mm cuvettes with 1 pg/ng pCAGO encoding Ampicillin resistance (GenScript). Positive transformants from LB-agar plates containing 100 μg/ml Ampicillin were identified by agarose gel electrophoresis of colony PCR products.

MIII ssrS cassette construction

100 ng/μl total *E. coli* nucleic acid extract was used as input for PCRs of the left and right homology arms flanking the middle region encoding a chloramphenicol marker, N20PAM region, and RNA Mango III. DNA primers 5′-TCA TTT CGT TGT AAC CAG GCA TTT CG-3′ and 5′-CCA GGT CTC AGT CCC CGA GCA TGC TCA CCA ACC G-3′ were used to generate the left arm, whereas 5′-CCA GG TCT CAA AAC TGC GAC GAC ACA TTC ACC-3′ and 5′-CAC GTC ACG CAC ATC CAA TTT TGG-3′ were used for the right arm. The middle region encoding the chloramphenicol marker, N20PAM region, and 40 bp left homology were then amplified from the pEditing_Cassette plasmid (GenScript) using primers 5′-CCA GGT CTC AGG ACT TCA TGT GCA GCT CCA TC-3′ and 5′-CCA GGT CTC ATT CGC CGA GCA TGC TCA CCA ACC GCG GAG CGC CAC ATT CTT GTG CAA CGT CAT CTC GTT CTC-3′. Finally, the section encoding RNA Mango III to replace *ssrS* “TCC GT” and a part of the right homology arm was generated by annealing and extending DNA primers 5′-CCA GGT CTC A<u>CG AAG GAA GGA TTG GTA</u>

TGT GGT ATA TTC GCC GA-3′ and 5′-CCA GGT CTC AGT TTT AAG GCT TCT CGG CGA ATA TAC CAC ATA CCA ATC C-3′ (MIII-encoding regions are underlined). The amplicons were subsequently gel-purified using NEB spin columns and ligated together by Golden Gate Assembly (NEB) at a 1:1:1:1 mole ratio (58 ng Left homology arm; 100 ng marker-N20PAM; 4.66 ng Mango III-right homology arm; 39.67 ng (right homology arm)). Assembly conditions were prepared in 1X NEB T4 DNA Ligase buffer, 0.20 mg/ml BSA (NEB), 10% BsaI-HFv2, and 1.33 X 10^5 cohesive end units/ml NEB T4 Ligase, then placed in the thermocycler for the Golden Gate Assembly program as described in Zhao et al. 2017. The assembled cassette was then gel-purified as described earlier and amplified using the outer primers at 0.5 μM.

Chromosomal integration of the MIII ssrS cassette

Colonies containing pCAGO were inoculated and grown overnight at 30℃ in 5 ml LB-Amp (100 μg/ml Ampicillin) shaking at 400 rpm. 50 μl of each culture was then aliquoted into 50 ml of LB-Amp and exposed to the same conditions. 0.1 mM IPTG was added to each 50 ml culture at the exponential phase (OD_{600}=~0.2-0.8). Cells were made electrocompetent after at least 60 min of IPTG induction by first centrifugation at 7430 RCF for 10 minutes at 4℃, washed as in (Zhao et al. 2017), then electroporated at 1600 V; 200 Ω; 25 μF in 1 mm cuvettes with 433 ng cassette. Shocked cells then received 250-300 μl SOC media and were shaken at 30℃ for 2 hours, then plated on LB-agar-Amp (100 μg/ml)-Chloramphenicol plates (30 μg/ml) plates with 1% glucose. The plates were incubated overnight at 30℃ and colonies encoding the integrated cassette were identified by agarose gel electrophoresis of colony PCR products using 5′-CCA GGT CTC AGG ACT TCA TGT GCA GCT CCA TC-3′ and 5′-CAC GTC ACG CAC ATC CAA TTT TGG-3′ as primers at 0.5 μM to monitor for MIII insertion. Two colonies were screened and observed with an insertion frequency of 100%.

Red intrachromosomal recombination of MIII ssrS cassette

Colonies bearing the integrated construct were inoculated in 5 ml LB-Amp, relative to a colony-free LB-Amp control. After 70 min of growth by shaking at 400 rpm, at 30℃, each LB-Amp culture was spiked with IPTG to 0.1 mM. Each culture was then spiked with arabinose to 2 g/l after 140 min of growth and grown overnight. LB-Amp plates were then respectively streaked with 5 μl aliquot from each overnight culture. Twenty colonies suspected to be fully edited were identified by agarose gel electrophoresis of colony PCR products, relative to one un-edited colony, and one clone-free reaction. *E. coli* plates verified by sequencing to have successful intrachromosomal recombination were kept.

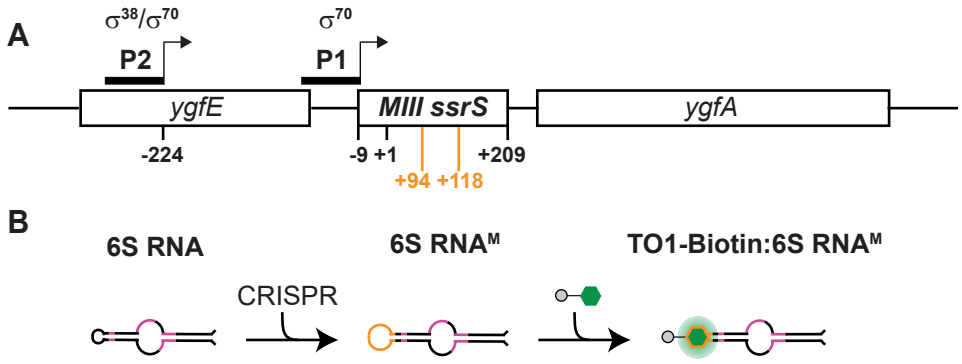
Plasmid curing and validation of MIII ssrS E. coli

Colonies with the integrated cassette were then each inoculated into 2 ml LB media, relative to a colony-free LB media control, and shaken at 250 rpm overnight at 42℃ to cure out pCAGO. 5 μl of each inoculation was then plated on to LB, LB-Amp (100 μg/ml), and LB-Chloramphenicol agar plates (30 μg/ml). To verify pCAGO-curing, colonies from LB plates were then utilized as input for colony PCR with primers 5′-ATG TCT GAA TTA GTT GTT TTC AAA GC-3′ and 5′-TCA GAT CCT TCC GTA TTT AGC-3′ at 0.5 μM final and screened for pCAGO-curing by agarose gel electrophoresis. Overnight-shaken cultures and monoclonal plates of edited, pCAGO-free colonies were kept, and each of the cultures were prepared into glycerol stocks. Furthermore, six colonies (one unedited; five derived from the prepared overnight inoculations) were screened by Colony PCR with primers 5′-GCA AGA TCT GAA AGA ACG CAC-3′ and 5′-CTT TGC GGA TGG TAA TTC AGG-3′ (0.5 μM final) on a locus presumably bearing the *MIII ssrS* gene. The PCR reactions were purified using the QIAquick PCR purification kit (Qiagen) and sent for Sanger sequencing (Eurofins) for sequencing.

SUPPLEMENTARY FIGURE

Supplementary Figure 1. Engineering *MIII ssrS E. coli* encoding functional 6S RNA^{M}. *(A)* Map of the *MIII ssrS* gene. The first and last positions enclosing the MIII-encoding region are shown in orange. The *ygfA* cistron is still proximal. ***(B)*** From left to right: wildtype *E. coli* encoding *ssrS* are modified by CRISPR (Zhao et al. 2017) into *MIII ssrS* encoding 6S RNA^{M} ("M" referring to RNA Mango III, shown in orange). RNA Mango III of 6S RNA can bind to thiazole orange of TO1-Biotin (TO1 shown in green, and Biotin shown in light gray).

Figure S1


A
σ38/σ70
P2
σ70
P1
ygfE
MIII ssrS
ygfA
-224
-9 +1
+209
+94 +118

B
6S RNA
6S RNAM
TO1-Biotin:6S RNAM
CRISPR