Supporting Information

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SI Text

Mutagenesis of Era by Single-Strand (ss) DNA Recombineering. The TUC540 strain was made from HME45 with λ Red functions by counterselection using recombineering (1, 2). The TUC565 strain was created by using standard P1 transduction (3), in which a P1 lysate of strain SDF189 with the *era* gene linked to a *yfhB*::ΔTn*10* marker (4) was transduced into the TUC540 strain. Next, a plasmid pSIM5 with λ Red functions (2) was introduced into TUC565 to generate the Era(E209Q) mutant (TUC572) by ssDNA recombineering (5, 6). TUC565 has two copies of wild-type *era* gene; one is in the *rnc* operon and the other in the λ *pL* operon. A P1 lysate of TUC572 was used to transduce the W3110 strain to tetracycline-resistant TUC573 carrying the Era647 change. Bacteria were grown in LB broth with antibiotics as needed: 10 μg/mL of chloramphenicol or 6.25 μg/mL of tetracycline.

ssDNA recombineering (5–7) was performed with the following three different 70-mer ssDNAs containing changes in the wobble positions of the five codons surrounding the E209 codon for the three Era mutants: 5′AACGTTCGATCTCCACGGTCACGGAGTATTGGTAG*CG*C*G*GC*A*CCGAGGAAACGCATCAGTTTTTCGCGGAT, XMZ299 (E209A); 5′AACGTTCGATCTCCACGGTCACGGAGTATTGGTAG*CTTG*GC*A*CCGAGGAAACGCATCAGTTTTTCGCGGAT, XMZ301 (E209K); and 5′AACGTTCGATCTCCACGGTCACGGAGTATTGGTAG*CTGG*GC*A*CCGAGGAAACGCATCAGTTTTTCGCGGAT, XMZ303 (E209Q), in which the underline represents changed nucleotides with respect to the wild type and the italic font indicates the change to the mutant amino acids. DNAs were purchased (Integrated DNA Technologies) as salt-free without further purification. Colonies on LB were screened at 42 °C using PCR with primers specific to the mutations created (7). A total of 10% colonies carried the E209Q change. The mutations were confirmed by sequencing. Colony phenotypes were tested on L agar plates at 25, 32, 37, and 42 °C.

Isothermal Titration Calorimetry (ITC) Experiment. The Era and RNA samples were prepared by buffer exchange in 20 mM Tris (pH 7.5), 200 mM NaCl, 2.5 mM $MgCl_2$, 0.1 mM EDTA, and 0.24 mM nonhydrolyzable GTP-analogue GDPNP (GNP). Then, the solutions were concentrated using Amicon Ultra-15 centrifugal filters (Millipore), 10 kDa for the protein and 3 kDa for the RNAs. The final concentrations were determined by NanoDrop 2000 (Thermo Scientific) and adjusted to the working concentration with the same buffer.

The RNA-Era interactions were studied using an isothermal titration microcalorimeter, iTC_{200} (GE Healthcare/MicroCal), at 25 °C. A typical experiment included injection of 18 aliquots (2.1 μL each) of approximately 0.2 mM ligand (RNA) solution into a protein solution of approximately 0.022 mM in the ITC cell (volume ~200 μL). The set of titrations was preceded by a single 0.5 μL injection to eliminate the effect of diffusion at the ligand/protein interface at the titration syringe tip during the thermal equilibration of the calorimeter prior to injections. The experiments were run at the High feedback mode/gain setting. The stirring speed was 1,000 RPM. The duration of injection in seconds was usually twice the value of injection volume. An additional set of injections was run in a separate experiment with buffer in the cell instead of the protein solution. These blank experiment data were subtracted from the main ligand-into-protein experiment data.

The binding isotherms were integrated to give the enthalpy change ΔH plotted as a function of the molar ratio of RNA and Era. When necessary, prior to the integration procedures the baseline was manually adjusted to minimize the background noise. The initial titration point was always discarded.

The ΔH/molar ratio plot had a sigmoidal shape, representing the fractional saturation of the binding sites by the ligand (8, 9). The Origin 7.0-based software provided by GE/MicroCal was used for data analysis and the one set of sites model was used as the basic option. The association constant K_a, which is the reciprocal of the dissociation constant K_d ($K_a = 1/K_d$), was determined from the slope of the central linear part of the fractional saturation curve. The Gibbs free energy change ΔG and the entropy change ΔS were calculated based on equations:

$$\Delta G = -RT \ln K_a = \Delta H - T\Delta S, \qquad \textbf{[S1]}$$

where ΔH was derived from original ΔH/molar ratio plots (8).

Crystallization and Data Collection. The concentration of Era was 20 mg/mL in 25 mM Tris (pH 7.2) and 150 mM NaCl. The concentration of KsgA was 27 mg/mL in 25 mM Tris (pH 7.2), 150 mM NaCl, and 2 mM *tris*(2-carboxyethyl)phosphine (TCEP). The protein-RNA mixture [molar ratio Era:RNA(:KsgA) = 1.0:1.2(:1.0)] was heated at 70 °C for 5 min and slowly cooled down to room temperature. Then, 10 mM GNP and 10 mM $MgCl_2$ were added and the mixture was kept at room temperature for another 30 min. A Hydra II Plus One (Matrix Technologies Corporation) crystallization robot was employed. Crystals were grown at 19 ± 1 °C. To crystallize the Era-GNP-RNA301 complex, each drop contained 0.4 μL Era-GNP-RNA301 mixture and 0.2 μL reservoir solution containing 0.2 M calcium acetate and 40% (vol/vol) 2-methyl-2,4-pentanediol (MPD). The crystals appeared in 30 d and reached the final size of $0.15 \times 0.10 \times 0.05$ mm^3 after six more days. To crystallize the Era-GNP-RNA301-KsgA complex, each drop contained 0.3 μL Era-GNP-RNA301-KsgA mixture and 0.3 μL reservoir solution containing 0.1 M sodium acetate (pH 5.0) and 20% (vol/vol) MPD. The crystals appeared in 30 d and reached the final size of $0.10 \times 0.05 \times 0.02$ mm^3 after seven more days. X-ray diffraction data were collected at the 22-bending magnet beamline of the Southeast Regional Collaborative Access Team at the Advanced Photon Source, Argonne National Laboratory. Data processing was carried out with the HKL2000 program (10). Crystal data and processing statistics are summarized in Table S2.

Structure Solution and Refinement. The structures were solved by molecular replacement using program PHASER (11) with the structure of Era-GNP-RNA100 [Protein Data Bank (PDB) ID code 3IEV] and of KsgA (PDB ID code 3FTF) as search models after ligand and solvent molecules were removed. The structures were refined using PHENIX (12) and REFMAC (13). Bulk solvent correction was employed. The $2F_o - F_c$ and $F_o - F_c$ electron density maps were calculated for the inspection and improvement of the structures during refinement. Solvent molecules and ions were added at the later stages of the refinement and verified with annealed omit maps. The Era-GNP-RNA301 structure contains one Era polypeptide chain (amino acids 1–301) with a His residue (amino acid 0, from cloning) at the N terminus, one GNP molecule, nucleotides 1506–1539 of RNA301, two MPD molecules, one Mg^{2+} ion, five acetate (ACT) ions, one Ca^{2+} ion, and

91 water oxygen atoms. The Era-GNP-RNA301-KsgA structure contains one Era (amino acids 1–301) with a His residue (amino acid 0) at the N terminus, nucleotides 1506–1540 of RNA301, one KsgA molecule (amino acids 13–247, UniProt release no. O67680), one GNP, two MPD, one Mg^{2+}, two ACT, and 83 water oxygen atoms. The missing residues of both protein and RNA are disordered. Graphic work was carrying out using COOT (14). The structures were verified with annealed omit maps and assessed using PROCHECK (15) and WHAT IF (16). Illustrations were prepared with PyMOL (DeLano Scientific LLC).

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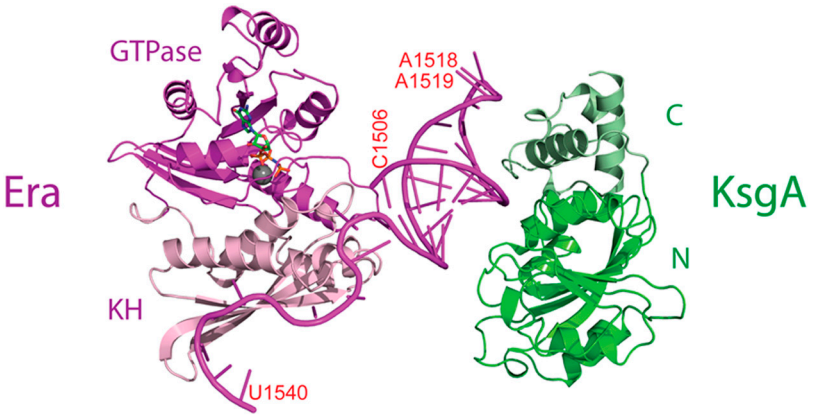

Fig. S1. The monomeric Era-GNP-RNA301-KsgA structure. Era and RNA are shown in magenta [darker, GTPase domain and RNA; lighter, K homology (KH) domain] and KsgA is shown in green (darker, N-terminal domain; lighter, C-terminal domain). The protein is represented by spirals (helices), arrows (strands), and tubes (loops), and the RNA by tube-and-sticks. The Mg^{2+} is indicated with a gray sphere and the GNP is shown as a stick model in atomic color scheme (C in green, N in blue, O in red, and P in orange).

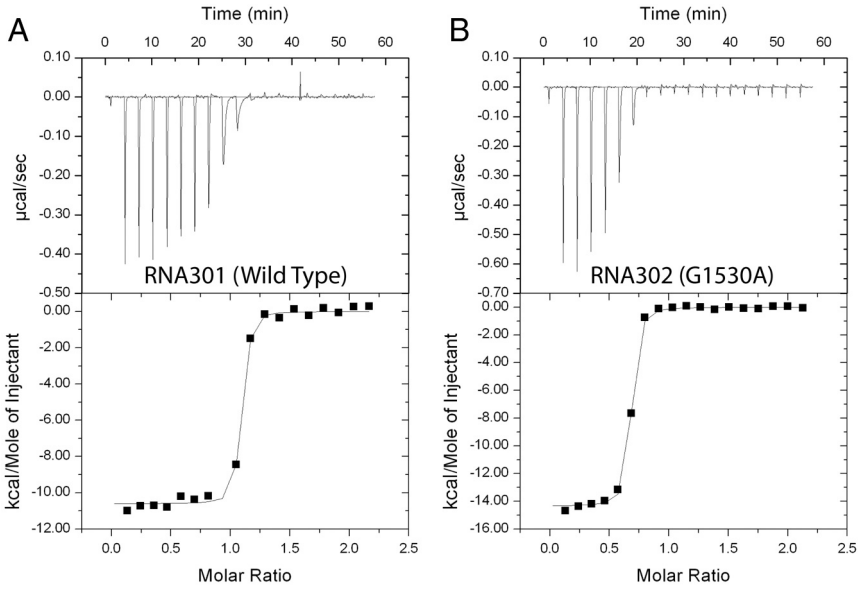

Fig. S2. ITC experiment with (*A*) RNA301 or (*B*) RNA302 binding to Era. See Table 1 for RNA sequences. The exothermic binding isotherm (*Top*) was integrated to give the enthalpy change plotted as a function of the molar ratio of RNA/Era (*Bottom*). The integrated enthalpy change data were used to calculate the dissociation constant K_d. Details for the ITC experiments and data analysis are provided in *SI Text*.

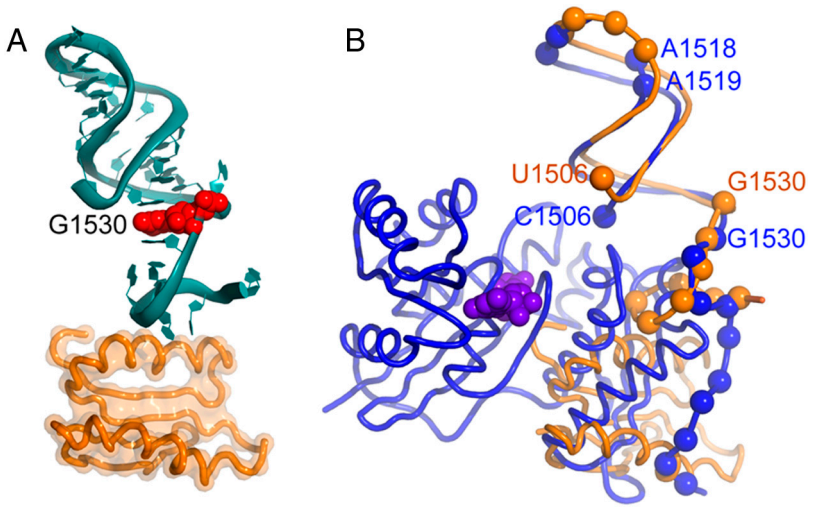

Fig. S3. Comparative analysis of Era-GNP-RNA301 (this work) and initiation factor 3 (IF3)-30S (PDB ID code 1I96). (*A*) In the IF3-30S structure, the C-terminal domain of IF3 (orange) contacts RNA300 (deep teal), but IF3 is approximately 20 Å away from G1530 (red). (*B*) Superposition of Era-GNP-RNA301 (blue) and IF3-RNA300 (orange) on the basis of h45. The phosphorus atoms in nucleotides 1506, 1516–1519 (the tetraloop of h45), and 1530–1539 (the GAUCACCUCC sequence) are indicated with spheres. The conformation of $_{1530}$GAUCACCUCC$_{1539}$ is dramatically different in the two structures.

Table S1. Strains and plasmid used for E209A, E209K, and E209Q mutants of Era

Strains or plasmid	Relevant genotype	Source
Strains		
W3110	*Escherichia coli* K-12 prototroph	Laboratory stock
HME45	W3110 *gal490 pgl Δ8* λ *cI857 Δ* (*cro-bioA*)	Laboratory stock
SDF189	W3110 *yfhB*::Δ Tn*10 ΔlacU169* λ *cI857 N-lacZ* Δ(*cro-bioA*) (TetR)	This study
TUC540	HME45 Δ(*int-cIII*)<>*era+*	This study
TUC565	TUC540 *yfhB*::ΔTn*10* (TetR)	This study
TUC572	TUC565 P_{rnc} *era* E209Q	This study
TUC573	W3110 *era* E209Q	This study
Plasmid		
pSIM5	pSC101 t^s λ *cI857 Δrex*<>*cat* Δ(*kil-N*) *exo bet gam* (CmR)	(2)

Table S2. X-Ray data collection and refinement statistics

Crystal	Era-GNP-RNA301	Era-GNP-RNA301-KsgA
Data collection		
Space group	C2	C2
Unit cell parameters		
a (Å)	88.95	195.50
b (Å)	85.61	68.09
c (Å)	67.19	73.59
α and γ (°)	90	90
β (°)	111.20	100.63
Resolution (Å)	30.0-2.05 (2.12-2.05)*	30.0-2.80 (2.90-2.80)
Measured reflections	148380	109730
Unique reflections	27512 (2143)	23217 (2114)
Completeness (%)	93.7 (72.9)	98.4 (91.0)
Multiplicity	5.5 (3.0)	4.8 (3.2)
R_{merge} (%) †	7.8 (66.5)	9.7 (61.4)
$I/\sigma(I)$	18.4 (2.2)	13.8 (1.7)
Refinement		
NonH atoms/*B*-factors (Å^2)	3315/62.3	5208/75.8
Protein/*B*-factors (Å^2)	2584/57.7	4478/70.5
RNA/*B*-factors (Å^2)	570/83.6	591/121.4
Heterogen/*B*-factors (Å^2)	70/64.0	56/56.1
Water/*B*-factors (Å^2)	91/59.1	83/49.7
R_{factor} (%)‡	19.1 (45.7)	17.1 (26.6)
R_{free} (%) ‡	24.4 (45.6)	22.6 (30.9)
rmsd bonds (Å)§	0.005	0.007
rmsd angles (°)	1.038	1.240
Ramachandran statistics (%)		
Most favored ϕ/ψ	92.2	85.0
Additionally allowed ϕ/ψ	7.4	13.3
Generously allowed ϕ/ψ	0.4	1.7
Disallowed ϕ/ψ	0.0	0.0

*Values in parentheses are for the highest resolution shell.

†$R_{merge} = \Sigma|(I - \langle I \rangle)|/\sigma(I)$, where I is the observed intensity.

‡R_{factor} and $R_{free} = \Sigma||F_{obs}| - |F_{calc}||/\Sigma|F_{obs}|$ where R_{free} was calculated over 5% of the amplitude chosen at random and not used in the refinement.

§rmsd, root-mean-square deviation.