Supporting Information

Shoemaker and Green 10.1073/pnas.1113956108

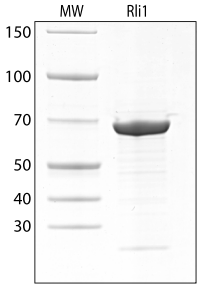

Fig. S1. Coomassie-stained SDS-PAGE of purified recombinant Rli1-His_6 from yeast. The expected molecular weight of Rli1 is 68 kDa. MW, molecular weight markers.

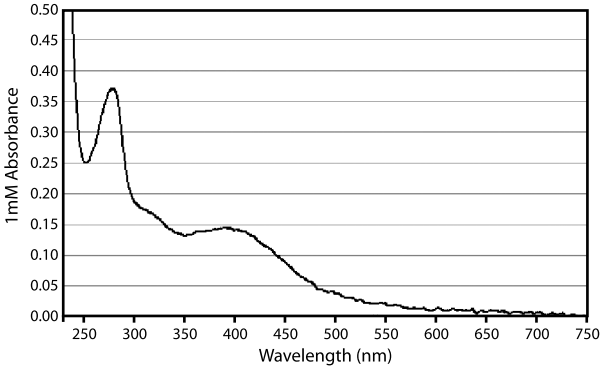

Fig. S2. UV-visible absorbance spectra of purified recombinant Rli1. Characteristic absorbance of 2[4Fe-4S] clusters is seen at A_{390}.

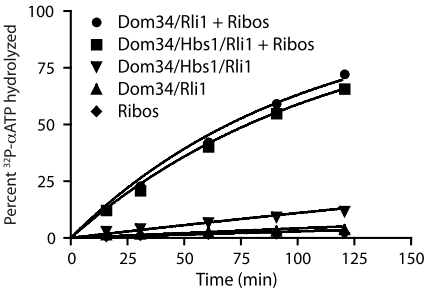

Fig. S3. ATP hydrolysis control reactions. Hydrolysis of ^{32}P-ATP to ^{32}P-ADP was measured by thin-layer chromatography at various time points in the presence of the factors indicated. Ribos, ribosomes.

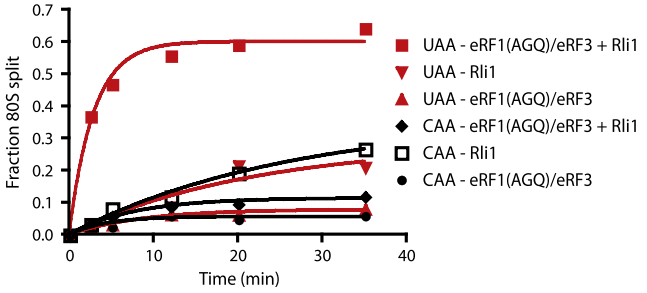

Fig. S4. Factor dependence of eRF1(AGQ)-mediated subunit dissociation on didpeptidyl-tRNA ribosome complexes containing an A-site stop codon (UAA) or nonstop codon (CAA). Subunit dissociation was measured by monitoring peptidyl-tRNA drop-off in a native gel system.

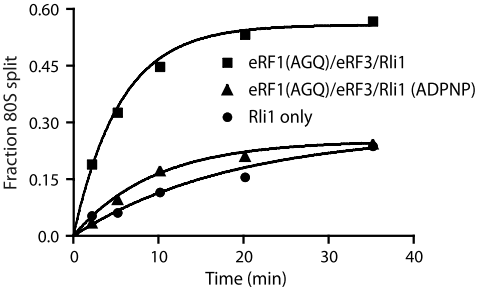

Fig. S5. ATP dependence of Rli1-mediated recycling in the presence of eRF1(AGQ):eRF3. Subunit dissociation was monitored as peptidyl-tRNA drop-off in a native gel system. Coordination between Rli1 and termination factors requires ATP hydrolysis.

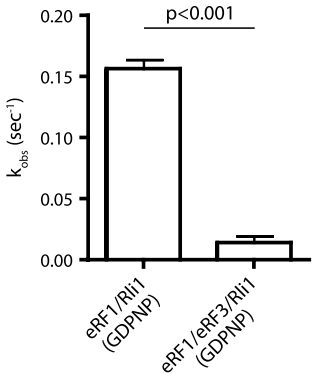

Fig. S6. Effect of GDPNP on eRF1:Rli1-mediated release in the presence and absence of eRF3 ($n \geq 3$, ±SEM). Reported rate constants were extrapolated by monitoring subunit dissociation via native gel electrophoresis. Statistics were determined using a Student's *t* test.

Table S1. DNA oligonucleotides used for cloning

Oligo	Sequence	Designation
oCS438	GAGCTCAAAAATGAGTGATAAAAACAGTCG	Rli1 F pYES
oCS439	GCATGCTTAGTGATGGTGATGGTGATGAATACCGGTGTTATCCA	Rli1 R pYES
oCS501	CCTCACTTAAGTTTTGTTGTTCTTGGCCATGGTGATGCGGGAAAATC	Hbs1 5′V176G
oCS502	GATTTTCCCGCATCACCATGGCCAAGAACAACAAAACTTAAGTGAGG	Hbs1 3′V176G
oCS517	ATATTGGATGCTCCTGGTGAGAAAATGTACGTTTCCGAG	eRF3 5′ H348E
oCS518	CTCGGAAACGTACATTTTCTCACCAGGAGCATCCAATAT	eRF3 3′ H348E
oCS545	CATATGAAGGTTATTAGTCTGAAAAAGG	Dom34 NdeI 5′
oCS546	CCCGGGCTCCTCACCATCGTCTT	Dom34 SmaI 3′

Table S2. DNA oligonucleotides used for in vitro transcription reactions

Oligo	Sequence	Designation
oCS230	TAATACGACTCACTATAGG	T7 clamp
oCS258	GAAGAA**CAT**AGAGAGAGAGAGAGATTCC TATAGTGAGTCGTATTA	mRNA +0
oCS552	GAGAGA**TTA**GAA**CAT**AGAGAGAGAGAGAGATTCCTATAGTGAGTCGTATTA	mRNA +9
oDE342	AGAGAGAGAGAGAGA**TTA**GAA**CAT** AGAGAGAGAGAGAGAGACCTATAGTGAGTCGTATTA	mRNA +18
oCS555	GAGAGAGAGAGAGAGAGAGA**TTA** GAA**CAT**AGAGAGAGAGAGAGATTCCTATAGTGAGTCGTATTA	mRNA +23
oCS557	AGAGAGAGAGAGAGAGAGAGAGAGAGA **TTA**GAA**CAT**AGAGAGAGAGAGAGA TT CC TATAGTGAGTCGTATTA	mRNA +30
oCS564	AGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA**TTA**GA A**CAT**AGAGAGAGAGAGAGATTCCTATAGTGAGTCGTATTA	mRNA +36
oCS556	GAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA GAGAGA**TTA**GAA**CAT**AGAGAGAGAGAGAGATTCC TATAGTGAGTCGTATTA	mRNA +47
oDE299	GTCATAGCTGTTTCCTGGAGAGAGAGAGAGAGAGAGAG AGAGAGAGAGAGAGAGAGA**TTA**GAA**CAT** AGAGAGAGAGAGAGAGACCTATAGTGAGTCGTATTA	mRNA +60

Start codons are **bold**. Stop codons are **bold** and underlined. T7 clamp binding sites are underlined.