

Supplementary Materials

Supplement procedures

Bacterial strains, plasmids, and media.

The *yqgF*⁺ strains and the *yqgF*^{ts} mutant used in this study were derivatives of MG1655. Isolation of the *yqgF*^{ts} mutant was described previously [Iwamoto, 2012]. Strains were grown routinely in LB and Antibiotic Medium 3 (Becton Dickinson, USA) or on Antibiotic Medium 3 agar plates.

Antibiotics were added at the following concentrations: ampicillin (Ap), 25 µg/ml; kanamycin (Km), 50 µg/ml; and tetracycline (Tc) 0.5 µg/ml.

Fractionation of ribosomes.

To prepare ribosomes for proteomic analysis, the *yqgF*^{ts} mutant and the isogenic *yqgF*⁺ strain were grown to log phase at 30°C for 2 h in Antibiotic Medium 3 and then shifted to 42°C and incubated for 1 h. To prepare the standard ¹⁵N-labeled ribosome for the proteomic analysis, the *yqgF*⁺ strain was grown to stationary phase in M9-glucose media containing ¹⁵NH₄Cl and then subcultured in fresh medium at a dilution of 1:100 at 30°C. After these culture steps were repeated three times, the cells were again subcultured in fresh medium (at a dilution of 3:200) at 40°C for 4.5 h and then shifted to 42°C and incubated for 4.5 h. To examine the ribosome profile, the $\Delta(rrnB$ leader) mutant and control strain were grown at 37°C for 2 h in Antibiotic Medium 3 and then shifted to 30°C and incubated for 2 h.

Next, the cells were harvested, washed, suspended in Buffer A (20 mM Tris-HCl [pH 7.5], 10 mM MgCl₂, 100 mM NH₄Cl, 6 mM β -mercaptoethanol), and lysed by freezing and thawing. The cell lysates were centrifuged at 30,000 · g for 10 min at 4°C and 500 µl of the supernatant from each sample was layered on top of a 5–40 % or 5–20 % sucrose gradient (12 ml) before centrifugation at 150,000 · g for 2.5 h at 4°C. Samples (200 µl each) were collected from the bottoms of the tubes.

LC-MS/MS analysis of peptides.

Purified fractions of standard ¹⁵N-labeled 70S ribosomes were mixed with the same amount of non-labeled *yqgF*⁺ or *yqgF*^{ts} 70S ribosomes, as determined by A₂₆₀ measurements. Samples were prepared

as described previously [Taoka et al., 2004]. The resulting peptide samples were analyzed on a nanoscale LC-MS/MS system [Taoka et al., 2009] with quadrupole-orbitrap hybrid mass spectrometer (Q Exactive, Thermo Fisher Scientific Inc., MA, USA). Peptides were detected in MS mode to select a set of precursor ions for analysis by data-dependent collision-induced dissociation mass spectrometry (MS/MS). Every second, the ten ions with the largest signal intensities were subjected to MS/MS analysis. The MS/MS signals were converted by Proteome Discoverer (Thermo Fisher Scientific Inc.) and a search performed against the UniProt database (version SwissProt_2013_05) using MASCOT version 2.3.2 (Matrix Science Ltd., London, UK) and the following parameters: fixed modification: carbamidomethyl (Cys); variable modifications: oxidation (Met), N-acetylation, pyroglutamine; maximum missed cleavages: 2; peptide mass tolerance: 15 ppm; MS/MS tolerance: 0.8 Da. The criteria for identification was based on the vendor's definitions. More stringent criteria were set for protein assignment: > 2 peptides with $p < 0.05$ were considered to indicate a 'hit'. Using the hit peptides, quantitation of proteins was performed with Proteome Discoverer. LC-MS/MS analysis was performed three times per sample, and the ratios $yqgF^{ts}$ standard and $yqgF^+$ standard of each r-protein was averaged over the triplicates. Next, the averaged ratios of the $yqgF^{ts}$ samples were divided by the averaged ratios of the $yqgF^+$ samples. This analysis was performed three times using independent cultures and the ratio ($yqgF^{ts}$ standard) / ($yqgF^+$ standard) was averaged over the independent replicates. In the cases of S12, L31, L35 and L36, ratios based on detection of light and heavy peptides were significantly deviated; therefore, all values of $yqgF^{ts}$ standard or $yqgF^+$ standard derived from detected peptides in same sample were averaged.

Taoka M, Yamauchi Y, Shinkawa T, Kaji H, Motohashi W, Nakayama H, Takahashi N, Isobe T: Only a small subset of the horizontally transferred chromosomal genes in *Escherichia coli* are translated into proteins. *Mol Cell Proteomics* 2004;3:780–787

Taoka M, Yamauchi Y, Nobe Y, Masaki S, Nakayama H, Ishikawa H, Takahashi N, Isobe T: An analytical platform for mass spectrometry-based identification and chemical analysis of RNA in ribonucleoprotein complexes. *Nucleic Acids Res* 2009;37:e140.

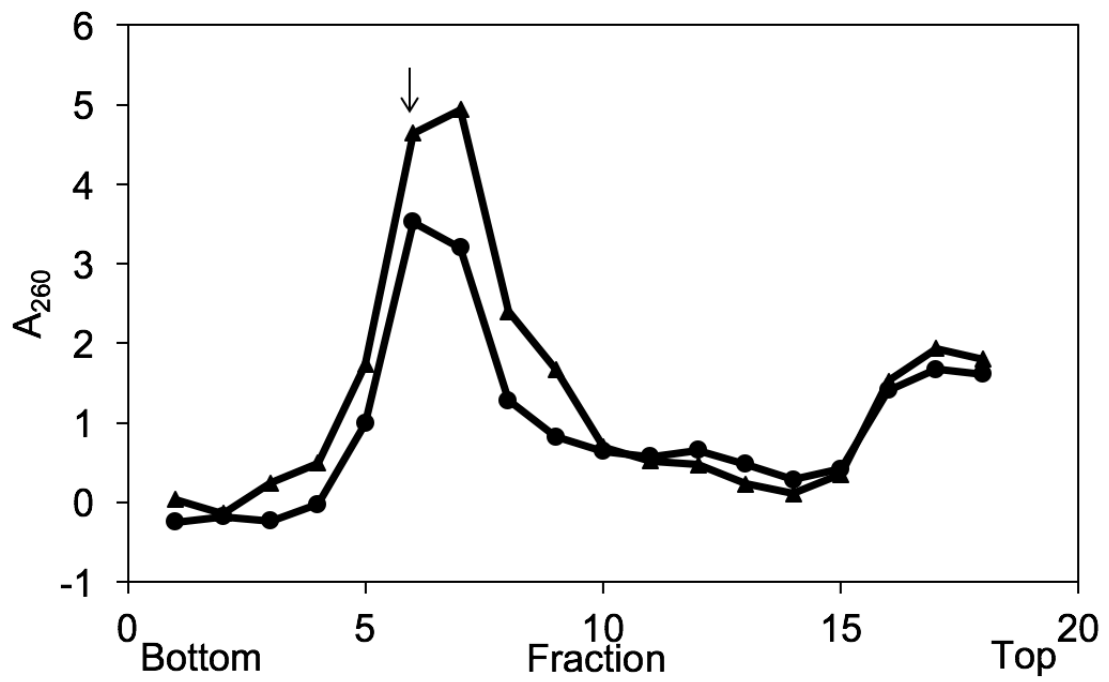


Fig. S1. Profile of ribosome for r-protein analysis.

Circle and triangle indicate the profile of $yqgF^+$ and $yqgF^{ts}$ ribosomes respectively. Arrow head shows the fraction used for followed analysis.

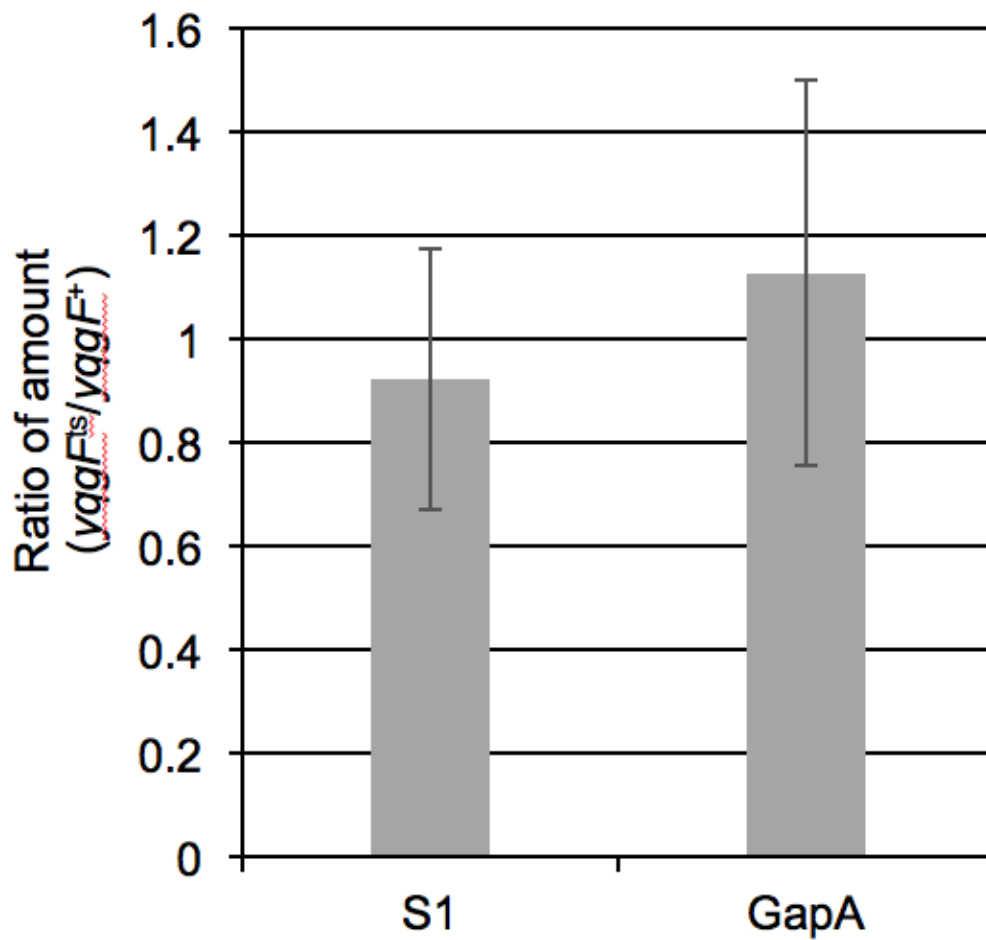


Fig. S2. Relative amount of ribosomal protein S1 in cell extract.

The relative amount was calculated by averaging $yqgF^{ts}/yqgF^{+}$ ratios of the peptides uniquely hit to ribosomal protein S1. Ten percent of high and low ratio were eliminated as outliers (53 ratios were used). A ratio of GapA was similarly analyzed and used for internal control.

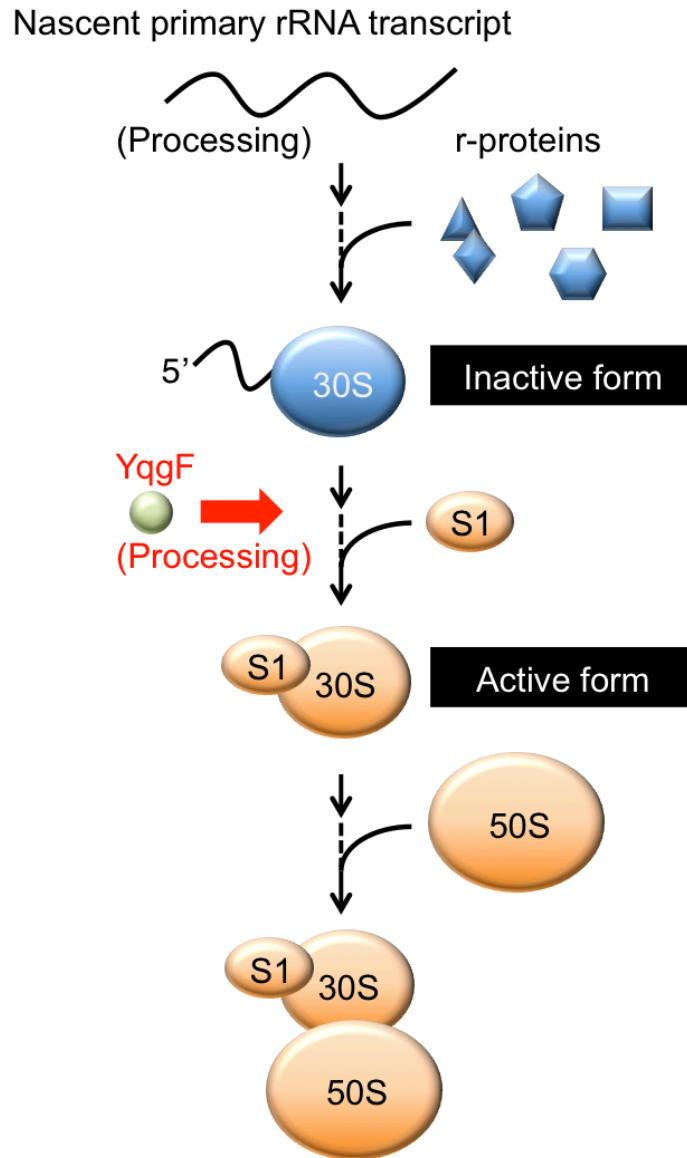


Fig. S3. Model of YqgF function.

rRNAs are assembled into ribosomes along with r-proteins after processing of the primary transcript. The 5'-leader region of pre-16S rRNA is required for ribosome assembly and blocks the assembly of S1 into the 30S subunit to avoid activation of the immature 30S subunit. When ribosome assembly is completed, YqgF processes the 5'-leader of pre-16S rRNA and then S1 binds to the 30S subunit. In this manner, 30S subunit is converted from the inactive to the active form.

Table. S1. LC-MS/MS analysis (1)

r-protein	Averaged ratio of r-protein to standard						Ratio of r-protein ($yqgF^{ts}/yqgF^+$)			
	$yqgF^+$ ^a			$yqgF^{ts}$ ^a			Sample1 ^c	Sample2 ^c	Sample3 ^c	Average ^d
	Sample1 ^b	Sample2 ^b	Sample3 ^b	Sample1 ^b	Sample2 ^b	Sample3 ^b				
S1	0.827	1.703	1.271	0.268	0.418	0.349	0.324	0.245	0.275	0.281 ± 0.040
S2	0.995	1.106	1.122	1.094	1.157	1.086	1.099	1.046	0.968	1.038 ± 0.066
S3	0.980	1.065	1.047	0.986	1.068	0.957	1.006	1.004	0.914	0.974 ± 0.053
S4	0.983	1.069	1.066	1.018	1.077	0.985	1.036	1.007	0.923	0.989 ± 0.058
S5	0.963	1.047	1.085	1.002	1.098	0.966	1.040	1.049	0.890	0.993 ± 0.089
S6	0.994	1.062	1.107	1.027	1.064	0.974	1.033	1.002	0.880	0.972 ± 0.081
S7	0.955	1.093	1.125	1.033	1.137	1.047	1.082	1.040	0.931	1.017 ± 0.078
S8	0.958	1.022	1.111	1.022	1.050	0.997	1.067	1.027	0.897	0.997 ± 0.089
S9	0.990	1.061	1.082	1.012	1.082	0.999	1.023	1.019	0.924	0.989 ± 0.056
S10	0.982	1.053	1.070	1.067	1.060	0.965	1.087	1.007	0.902	0.999 ± 0.093
S11	1.050	1.048	1.078	1.045	1.084	0.910	0.995	1.034	0.844	0.958 ± 0.100
S12	0.987	1.056	1.001	ND	1.065	0.979	ND	1.009	0.977	0.993 ± ND
S13	1.006	1.058	1.056	1.070	1.057	0.952	1.064	0.999	0.902	0.988 ± 0.082
S14	0.979	1.037	1.032	0.937	1.009	0.977	0.957	0.972	0.947	0.959 ± 0.013
S15	0.995	1.065	1.080	1.020	1.063	0.943	1.025	0.998	0.873	0.965 ± 0.081
S16	0.982	1.068	1.062	1.049	1.052	0.932	1.069	0.985	0.878	0.977 ± 0.096
S17	0.934	1.066	1.094	1.034	0.997	0.985	1.108	0.936	0.900	0.981 ± 0.111
S18	0.960	1.090	1.091	1.229	1.195	1.084	1.281	1.096	0.994	1.123 ± 0.145
S19	1.050	1.039	0.997	0.959	1.108	0.976	0.914	1.066	0.978	0.986 ± 0.076
S20	0.887	1.051	1.035	1.030	1.096	0.966	1.161	1.042	0.934	1.046 ± 0.114
S21	1.071	1.354	1.271	0.998	0.842	0.882	0.932	0.622	0.694	0.749 ± 0.162
L1	0.999	1.126	1.109	1.094	1.136	1.055	1.096	1.009	0.952	1.019 ± 0.073
L2	0.914	1.045	1.034	0.942	1.078	0.964	1.031	1.032	0.932	0.998 ± 0.057
L3	1.018	1.034	1.015	1.019	1.060	1.008	1.001	1.025	0.993	1.006 ± 0.017
L4	0.947	1.063	1.094	1.002	1.088	0.983	1.058	1.023	0.899	0.993 ± 0.084
L5	0.973	1.089	1.090	1.019	1.101	0.984	1.048	1.011	0.903	0.987 ± 0.075
L6	0.982	1.092	1.114	1.074	1.084	0.968	1.094	0.992	0.869	0.985 ± 0.113
L7/L12	0.948	1.174	1.125	1.099	1.102	1.026	1.160	0.939	0.911	1.003 ± 0.136
L9	0.997	1.108	1.105	1.060	1.119	1.030	1.064	1.009	0.932	1.002 ± 0.066
L10	0.934	1.142	1.104	1.082	1.149	1.020	1.159	1.006	0.924	1.030 ± 0.119
L11	1.034	1.099	1.112	1.157	1.119	1.009	1.119	1.018	0.907	1.015 ± 0.106
L13	0.986	1.018	1.021	1.036	1.075	0.989	1.051	1.056	0.969	1.025 ± 0.049
L14	0.988	1.108	1.095	1.057	1.088	0.997	1.070	0.981	0.911	0.987 ± 0.080
L15	0.962	1.069	1.077	1.001	1.082	0.984	1.040	1.012	0.914	0.989 ± 0.066
L16	0.981	1.050	1.036	0.965	1.057	0.994	0.984	1.007	0.960	0.984 ± 0.024
L17	0.998	1.081	1.119	1.007	1.089	1.028	1.010	1.007	0.918	0.978 ± 0.052
L18	0.971	1.068	1.050	0.996	1.066	1.002	1.026	0.998	0.954	0.993 ± 0.036
L19	0.950	1.055	1.097	1.041	1.082	0.976	1.096	1.026	0.889	1.004 ± 0.105
L20	0.962	1.111	1.074	1.071	1.080	0.998	1.113	0.972	0.929	1.005 ± 0.096
L21	0.985	1.179	1.120	0.959	1.070	0.988	0.973	0.907	0.882	0.921 ± 0.047
L22	1.003	1.071	1.111	1.003	1.085	1.023	1.000	1.013	0.920	0.978 ± 0.050
L23	0.912	1.429	1.063	0.982	1.080	1.058	1.076	0.756	0.995	0.942 ± 0.167
L24	0.949	1.074	1.066	1.029	1.066	0.958	1.085	0.993	0.899	0.992 ± 0.093
L25	0.966	1.102	1.112	1.040	1.085	1.007	1.077	0.985	0.906	0.989 ± 0.086
L27	0.974	1.045	1.048	1.041	1.109	0.954	1.069	1.062	0.910	1.013 ± 0.090
L28	1.004	1.067	1.042	1.022	1.132	0.990	1.018	1.061	0.950	1.010 ± 0.056
L29	0.941	0.968	1.068	1.017	1.029	0.876	1.081	1.062	0.820	0.988 ± 0.145
L30	0.978	1.016	1.026	1.077	0.987	0.903	1.101	0.972	0.881	0.984 ± 0.111
L31	ND	ND	ND	1.050	ND	ND	ND	ND	ND	ND
L32	0.916	1.071	1.023	1.082	1.161	0.966	1.181	1.084	0.945	1.070 ± 0.119
L33	0.927	1.119	1.035	0.988	0.985	1.152	1.066	0.880	1.113	1.020 ± 0.123
L34	0.970	1.080	0.987	1.040	1.126	0.967	1.072	1.042	0.980	1.032 ± 0.047
L35	ND	ND	ND	1.050	ND	ND	ND	ND	ND	ND
L36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

a. The strain which ribosome was purified from

b. The ribosomes which were purified from the cells independently cultured from different colonies. ND shows that we could not correctly or at all measure the ratio of protein to standard in three times of mass spectrometry analysis

c. The ribosomes which were purified from the cells independently cultured from different colonies. ND shows that we could not calculate the ratio of protein ($yqgF^{ts}/yqgF^+$) in order to the ratio of protein to standard was ND

d. An average and standard deviation of the ratio of r-proteins in three samples. ND shows that we could not calculate standard deviation or average in order to the ratio of protein ($yqgF^{ts}/yqgF^+$) was ND in any or all samples respectively.

Table. S2. LC-MS/MS analysis (2)

r-protein	Averaged ratio of r-protein to standard						Ratio of r-protein ($yqgF^{ts}/yqgF^+$)			
	$yqgF^+$ ^a			$yqgF^{ts}$ ^a			Sample1 ^c	Sample2 ^c	Sample3 ^c	Average ^d
	Sample1 ^b	Sample2 ^b	Sample3 ^b	Sample1 ^b	Sample2 ^b	Sample3 ^b				
S1	0.839	2.015	1.557	0.270	0.422	0.514	0.322	0.210	0.330	0.287 ± 0.067
S12	1.044	0.942	1.009	1.740	1.105	0.952	1.668	1.173	0.944	1.261 ± 0.370
L31	1.899	1.563	1.737	2.063	2.006	3.241	1.086	1.284	1.866	1.412 ± 0.406
L35	ND	0.774	1.725	ND	1.159	1.845	ND	1.007	0.923	0.989 ± ND
L36	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

a. A strain which ribosome was purified from

b. The ribosomes which were purified from the cells independently cultured from different colonies. ND shows that we could not at all measure the ratio of protein to standard in three times of mass spectrometry analysis

c. The ribosomes which were purified from the cells independently cultured different colonies. ND shows that we could not calculate the ratio of protein ($yqgF_{ts}/yqgF^+$) in order to the ratio of protein to standard was ND

d. An average and standard deviation of ratio of r-proteins in three samples. ND shows that we could not calculate standard deviation or average in order to the ratio of protein ($yqgF_{ts}/yqgF^+$) was ND in any or all samples respectively.