Supporting Information

Armache et al. 10.1073/pnas.1010005107

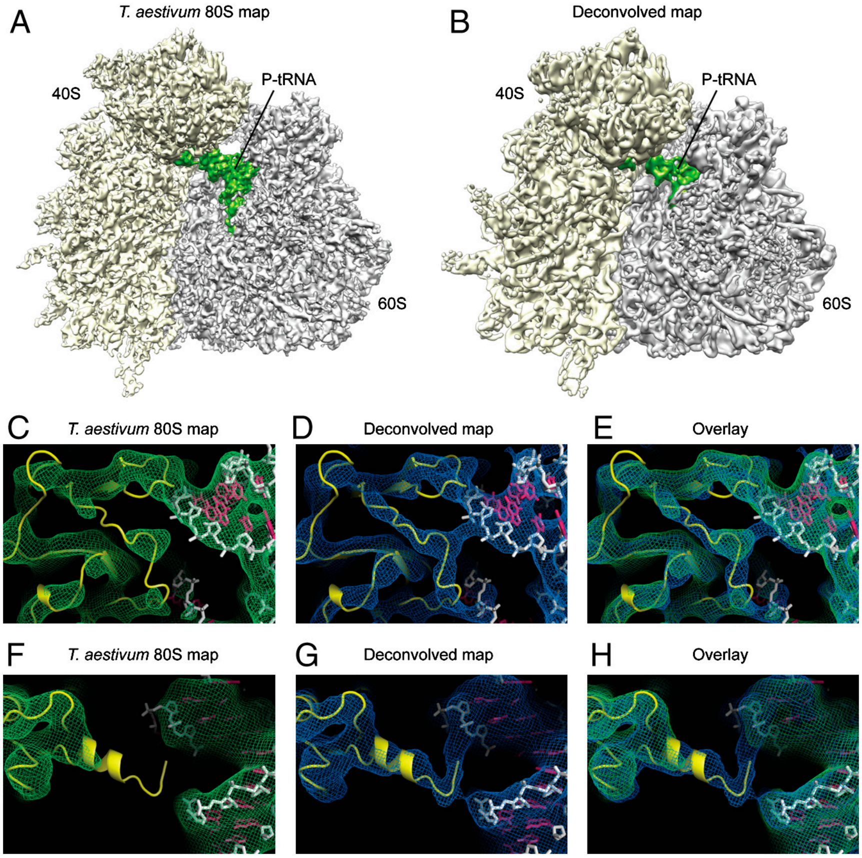

Fig. S1. Comparison of pre- and postdeconvolution sharpened maps. (*A*) pre- and (*B*) postdeconvolution cryo-EM maps of the *Triticum aestivum* 80S ribosome, with small and large subunits in yellow and gray, respectively, and P-tRNA colored green. Examples of (*C* and *F*) pre- and (*D* and *G*) postdeconvolution maps (mesh), with overlays shown in *E* and *H*, respectively. R proteins are shown as yellow ribbons and rRNA nucleotides with white backbone and red bases.

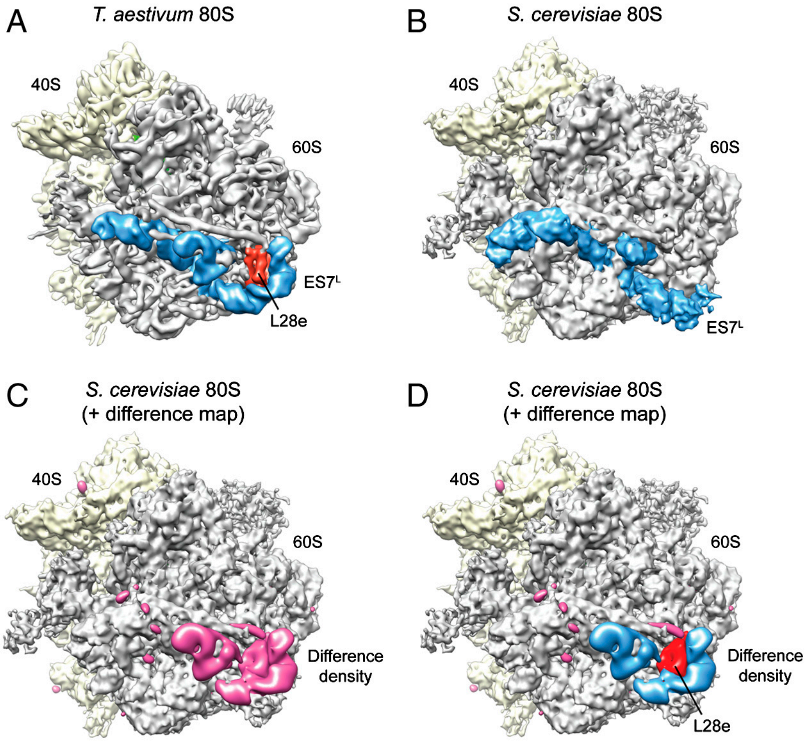

Fig. S2. Difference maps generated between *Triticum aestivum* and *Saccharomyces cerevisiae* identify position of r-protein L28e. (*A*) *T. aestivum* 80S reconstruction with ES7^L (blue) and L28e (red) positions highlighted. (*B*) *S. cerevisiae* 80S reconstruction with ES7^L (blue) highlighted. (*C*) *S. cerevisiae* 80S reconstruction superimposed with the difference density (magenta) calculated between the (*A*) *T. aestivum* map and (*B*) the *S. cerevisiae* map. (*D*) Same as *C* but with the regions of the difference density corresponding to ES7^L in *T. aestivum* colored blue, leaving a large region of extra density (red) that was assigned as r-protein L28e.

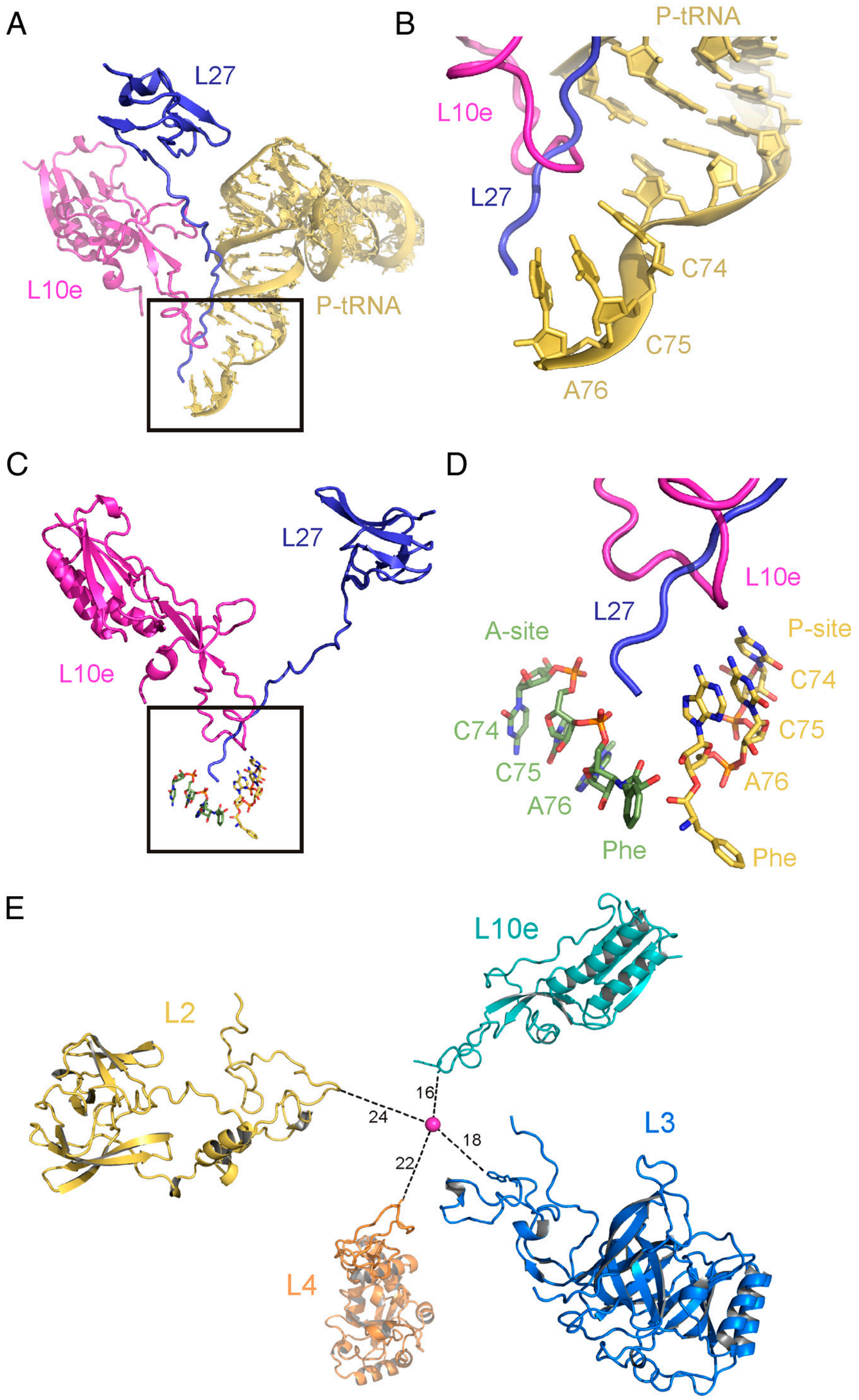

Fig. S3. Ribosomal proteins that approach the peptidyltransferase center of the ribosome. (*A* and *B*) Comparison of the relative positions of the N terminus of bacterial r-protein L27 (blue) and eukaryotic L10e (magenta) with a tRNA in the P site (yellow). (*C* and *D*) Comparison of the relative positions of the N terminus of bacterial r-protein L27 (blue) (1) and eukaryotic L10e (magenta) with the CCA-ends of tRNA mimics in A- (green) and P site (yellow) (2). (*E*) R-proteins L2p (yellow), L3p (blue), L4p (orange), and L10e (aqua) come within approximately 24, 22, 18, and 16 Å of the site of peptide bond formation, based on ref. 3.

1 Voorhees RM, et al. (2009) Insights into substrate stabilization from snapshots of the peptidyl transferase center of the intact 70S ribosome. *Nat Struct Mol Biol* 16:528–533.
2 Hansen JL, Schmeing TM, Moore PB, Steitz TA (2002) Structural insights into peptide bond formation. *Proc Natl Acad Sci USA* 99:11670–11675.
3 Nissen P, et al. (2000) The structural basis of ribosome activity in peptide bond synthesis. *Science* 289:920–930.

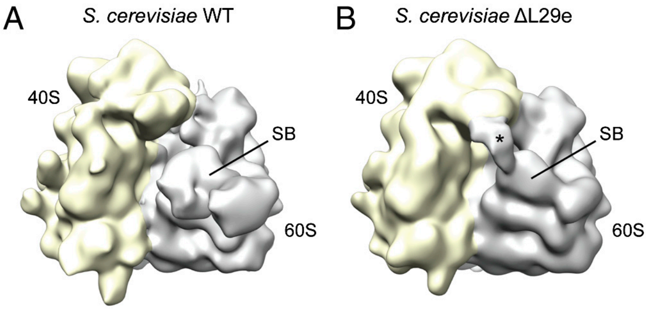

Fig. S4. Localization of ribosomal protein L29e. Reconstruction of (*A*) *Saccharomyces cerevisiae* WT 80S ribosome, compared to (*B*) reconstruction of *S. cerevisiae* 80S ribosomes isolated from a strain lacking the gene for L29e. In *B*, the rearranged position of the stalk base (SB) on the large subunit (gray) leads to a contact between the stalk (*) and the head of the small 40S subunit (yellow).

Table S1. Nomenclature for r proteins of the *Saccharomyces cerevisiae* and *Triticum aestivum*

	Family name	*S. cerevisiae name*	*Oryza sativa name*
1	S2p	rpS0	Sa
2	S3p	rpS3	S3
3	S3ae	rpS1	S3a
4	S4p	rpS9	S9
5	S4e	rpS4	S4
6	S5p	rpS2	S2
7	S6e	rpS6	S6
8	S7p	rpS5	S5
9	S7e	rpS7	S7
10	S8p	rpS22	S15a
11	S8e	rpS8	S8
12	S9p	rpS16	S16
13	S10p	rpS20	S20
14	S10e	rpS10	S10
15	S11p	rpS14	S14
16	S12p	rpS23	S23
17	S12e	rpS12	S12
18	S13p	rpS18	S18
19	S14p	rpS29	S29
20	S15p	rpS13	S13
21	S17p	rpS11	S11
22	S17e	rpS17	S17
23	S19p	rpS15	S15
24	S19e	rpS19	S19
25	S21e	rpS21	S21
26	S24e	rpS24	S24
27	S25e	rpS25	S25
28	S26e	rpS26	S26
29	S27e	rpS27	S27
30	S27ae	rpS31	S27a
31	S28e	rpS28	S28
32	S30e	rpS30	S30
33	RACK1	RACK1	RACK1

	Family name	*S. cerevisiae name*	*Oryza sativa name*

	Family name	*S. cerevisiae name*	*Triticum aestivum name*
1	L1p	rpL1	L1
2	L2p	rpL2	L2
3	L3p	rpL3	L3
4	L4e/L4p	rpL4	L4
5	L5p	rpL11	L11
6	L6p	rpL9	L9
7	L6e	rpL6	L6
8	L7ae	rpL8	L7a
9	L10p	rpP0	P0
10	L10e	rpL10	L10
11	L11p	rpL12	L12
12	L12p	rpP1/rpP2	P1/P2
13	L13p	rpL16	L13a
14	L13e	rpL13	L13
15	L14p	rpL23	L23
16	L14e	rpL14	L14
17	L15p	rpL28	L27a
18	L15e	rpL15	L15
19	L18p	rpL5	L5
20	L18e	rpL18	L18
21	L18ae	rpL20	L18a
22	L19e	rpL19	L19
23	L21e	rpL21	L21
24	L22p	rpL17	L17
25	L22e	rpL22	L22
26	L23p	rpL25	L23a
27	L24p	rpL26	L26
28	L24e	rpL24	L24
29	L27e	rpL27	L27
30	L28e	—	L28
31	L29p	rpL35	L35
32	L29e	rpL29	L29
33	L30p	rpL7	L7
34	L30e	rpL30	L30
35	L31e	rpL31	L31
36	L32e	rpL32	L32
37	L34e	rpL34	L34
38	L35ae	rpL33	L35a
39	L36e	rpL36	L36
40	L37e	rpL37	L37
41	L37ae	rpL43	L37a
42	L38e	rpL38	L38
43	L39e	rpL39	L39
44	L40e	rpL40	L40
45	L41e	rpL41	L41
46	L44e	rpL42	L44

Table S2. Summary of modeled wheat germ small subunit r proteins

Protein name	Protein family	Organism	Acc. no.	Size, aa	Modeled length, aa	Modeled range, aa	Percent modeled, %	Template	PDB ID
Sa	S2p	*Oryza sativa*	Q8H3I3	305	260	1–260	85	*Thermus thermophilus*	2J00_B
S2	S5p	*Oryza sativa*	Q84M35	274	263	1–263	96	*Escherichia coli*	
2QAL_E	S3	S3p	*Triticum aestivum*		Q8L804	227	208	12–219	92
Escherichia coli	2QAL_C								
S4*	**S4e**	***Oryza sativa***	**P49398**	**265**	**200**	**43–242**	**75**	***Thermoplasma acidophilum***	
3KBG_A	S5	S7p	*Oryza sativa*		Q93VC6	200	191	10–200	96
Pyrococcus horikoshii	1IQV_A								
S7	**S7e**	***Triticum aestivum***	**Q5I7K2**	**192**	**143**	**1–143**	**74**	***Polyalanine***	—
S9	S4p	*Oryza sativa*	Q2R1J8	195	195	1–195	100	*Thermus thermophilus*	2J00_D
S11	S17p	*Oryza sativa*	Q7XIK5	161	85	40–124	53	*Thermus thermophilus*	2J00_Q
S13	S15p	*Oryza sativa*	Q69UI2	151	121	31–151	80	*Escherichia coli*	
2QAL_O	S14	S11p	*Oryza sativa*		Q6H7T1	150	119	32–150	79
Thermus thermophilus	2J00_K								
S15	S19p	*Oryza sativa*	P31674	154	91	58–148	59	*Escherichia coli*	
2QAL_S	S16	S9p	*Oryza sativa*		Q0IQF7	149	126	24–149	85
Thermus thermophilus	2J00_I								
S17	**S17e**	***Oryza sativa***	**Q7XEQ3**	**141**	**141**	**1–141**	**100**	***Methanobacterium thermoautotrophicum***	
1RQ6_A	S18	S13p	*Triticum aestivum*		Q8L806	152	152	1–152	100
Escherichia coli	2QAL_M								
S19	**S19e**	***Oryza sativa***	**P40978**	**146**	**146**	**1–146**	**100**	***Pyrococcus abyssi***	
2V7F_A	S20	S10p	*Oryza sativa*	P35686	128	128	1–128	100	
Thermus thermophilus	2J00_J								
S21	**S21e**	***Oryza sativa***	**P35687**	**82**	**82**	**1–82**	**100**	**de novo**	—
S15a	S8p	*Oryza sativa*	Q84AP1	130	130	1–130	100	*Escherichia coli*	
2QAL_H	S23	S12p	*Oryza sativa*		Q8L4F2	142	142	1–142	100%
Thermus thermophilus	2J00_L								
S24	**S24e**	***Oryza sativa***	**Q6H541**	**138**	**98**	**5–102**	**71**	***Pyrococcus abyssi***	
2V94_A	**S25**	**S25e**	***Oryza sativa***		**Q53QG2**	**108**	**100**	**9–108**	**93**
Pyrococcus horikoshii	**1UB9_A**								
S26	**S26e**	***Oryza sativa***	**P49216**	**133**	**92**	**1–31; 73–133**	**69**	***Polyalanine***	—
S27	**S27e**	***Oryza sativa***	**Q6K5R5**	**86**	**50**	**33–82**	**58**	***Archeoglobus fulgidus***	
1QXF_A	**S28**	**S28e**	***Triticum aestivum***		**Q7X9K4**	**65**	**58**	**1–58**	**89**
Pyrococcus horikoshii	**1NY4_A**								
S29	S14p	*Triticum aestivum*	Q5I7K3	56	48	9–56	86	*Thermus thermophilus*	2J00_N
S30	**S30e**	***Oryza sativa***	**Q6K853**	**62**	**62**	**1–62**	**100**	**de novo**	—
RACK1	RACK1	*Triticum aestivum*	Q8LNY6	380	380	1–380	100	*Mus musculus*	2PBI_B

Acc., accession; PDB, Protein Data Bank.

*Bold rows indicate newly localized r proteins in this study, compared to the bacterial and archaeal X-ray structures.

Table S3. Summary of modeled wheat germ large subunit r proteins

Protein name	Protein family	Organism	Acc. no.	Size, aa	Modeled length	Modeled range	Percent modeled, %	Template	PDB ID
L1	L1p	*Triticum aestivum*	Q5I7L3	216	216	1–216	100	*Thermus thermophilus*	2HW8_A
L2	L2p	*Oryza sativa*	Q2QNF3	261	255	1–255	98	*Haloarcula marismortui*	1VQ8_A
L3	L3p	*Triticum aestivum*	Q7X744	389	389	1–389	100	*Haloarcula marismortui*	1VQ8_B
L4	L4p/L4e	*Oryza sativa*	Q6ZLB8	405	372	1–269;303–405	92	*Haloarcula marismortui*	1VQ8_C
L5	L18p	*Oryza sativa*	Q8L4L4	304	304	1–304	100	*Haloarcula marismortui*	1VQ8_N
L6*	**L6e**	***Triticum aestivum***	**Q5I7L4**	**219**	**219**	**1–219**	**100**	***Sulfolobus solfataricus***	**2JOY_A**
L7	L30p	*Triticum aestivum*	Q5I7K6	244	244	1–244	100	*Haloarcula marismortui*	1VQ8_W
L7a	L7ae	*Oryza sativa*	P35685	258	201	58–258	78	*Haloarcula marismortui*	1VQ8_F
L9	L6p	*Oryza sativa*	P49210	190	190	1–190	100	*Haloarcula marismortui*	1VQ8_E
L10	L10e	*Oryza sativa*	Q0ITS8	224	192	33–224	86	*Haloarcula marismortui*	3CC2_H
L11	L5p	*Triticum aestivum*	Q5I7L2	180	170	1–170	94	*H. marismortui/T. thermophilus*	1VQ8_D/2J01_G
L12	L11p	*Oryza sativa*	Q0JAI2	166	128	12–139	77	*Haloarcula marismortui*	2QA4_I
L13	**L13e**	***Oryza sativa***	**Q7XJB4**	**208**	**182**	**13–194**	**88**	***Polyalanine***	**—**
L14	**L14e**	***Oryza sativa***	**Q7XJ52**	**134**	**134**	**1–134**	**100**	***Sulfolobus solfataricus***	**2JOY_A**
L15	L15e	*Oryza sativa*	Q8H8S1	204	194	1–194	95	*Haloarcula marismortui*	3CC2_M
L13a	L13p	*Triticum aestivum*	Q5I7L1	206	206	1–206	100	*Haloarcula marismortui*	1VQ8_J
L17	L22p	*Oryza sativa*	Q6ZIA1	171	171	1–171	100	*Haloarcula marismortui*	1VQ8_R
L18	L18e	*Triticum aestivum*	Q5I7L0	188	163	1–163	87	*Haloarcula marismortui*	1VQ8_O
L18a	**L18ae**	***Oryza sativa***	**Q7XY20**	**178**	**167**	**1–167**	**94**	***Methanobacterium thermoautothropicum***	**2JXT_A**
L19	L19e	*Triticum aestivum*	Q943F3	209	189	1–189	90	*Haloarcula marismortui*	1VQ8_P
L21	L21e	*Triticum aestivum*	Q7XYC9	164	164	1–164	100	*Haloarcula marismortui*	1VQ8_Q
L22	**L22e**	***Oryza sativa***	**Q6YSX0**	**130**	**108**	**14–121**	**83**	***Artificial gene***	**2KL8_A**
L23	L14p	*Triticum aestivum*	Q5I7K4	140	140	1–140	100	*Haloarcula marismortui*	1VQ8_K
L24	L24e	*Oryza sativa*	Q5N754	162	75	1–75	46	*Haloarcula marismortui*	1VQ8_U
L23a	L23p	*Oryza sativa*	Q0JBZ7	152	122	31–152	80	*Haloarcula marismortui*	1VQ8_S
L26	L24p	*Oryza sativa*	Q2QXN5	150	130	1–130	87	*Haloarcula marismortui*	1VQ8_T
L27a	L15p	*Oryza sativa*	Q6EUQ7	144	144	1–144	100	*Haloarcula marismortui*	1VQ8_L
L27	**L27e**	***Oryza sativa***	**Q7XC31**	**136**	**99**	**1–99**	**73**	***Sulfolobus solfataricus***	**2JOY_A**
L28	**L28e**	***Oryza sativa***	**Q5TKP3**	**147**	**73**	**58–130**	**50**	**de novo**	**—**
L29	**L29e**	***Oryza sativa***	**Q9FP55**	**60**	**23**	**38–60**	**38**	***Oryctolagus cuniculus***	**1UTG_A**
L30	L30e	*Triticum aestivum*	Q5I7K9	112	112	1–112	100	*Saccharomyces cerevisiae*	1CN7_A
L31	L31e	*Triticum aestivum*	Q6ZGV5	123	120	1–120	98	*Haloarcula marismortui*	1VQ8_X
L32	L32e	*Oryza sativa*	Q3MST7	133	133	1–133	100	*Haloarcula marismortui*	1VQ8_Y
L34	**L34e**	***Triticum aestivum***	**Q5I7K8**	**119**	**119**	**1–119**	**100**	***Rhodobacter capsulatus***	**2PPT_A**
L35a	**L35ae**	***Oryza sativa***	**Q6I608**	**111**	**104**	**1–104**	**94**	***Pyrococcus furiosus***	**1SQR_A**
L35	L29p	*Triticum aestivum*	Q8L805	124	124	1–124	100	*Haloarcula marismortui*	1VQ8_V
L36	**L36e**	***Triticum aestivum***	**Q5I7L5**	**112**	**77**	**27–103**	**69**	***Archeoglobus fulgidus***	**2OEB_A**
L37	L37e	*Oryza sativa*	Q6Z8Y5	94	94	1–94	100	*Haloarcula marismortui*	1VQ8_1
L38	**L38e**	***Oryza sativa***	**Q8GVY2**	**69**	**69**	**1–69**	**100**	***Homo sapiens***	**1WH9_A**
L39	L39e	*Triticum aestivum*	Q5I7K7	51	51	1–51	100	*Haloarcula marismortui*	1VQ8_2
L40	**L40e**	***Oryza sativa***	**P35296**	**53**	**41**	**13–53**	**77**	***Sulfolobus solfataricus***	**2AYJ_A**
L41	**L41e**	***Oryza sativa***	**P62125**	**25**	**25**	**1–25**	**100**	**de novo**	**—**
L42	L44e	*Oryza sativa*	Q8H5N0	105	105	1–105	100	*Haloarcula marismortui*	1VQ8_3
L43	L37ae	*Oryza sativa*	Q5QM99	92	92	1–92	100	*Haloarcula marismortui*	3CC2_Z
P0	L10p	*Oryza sativa*	P41095	319	262	1–262	82	*Pyrococcus horikoshii and Methanocaldococcus janaschii*	3A1Y_G and 3JSY_A
P1	L12p	*Triticum aestivum*	Q5I7K5	110	58	6–63	53	*Pyrococcus horikoshii*	3A1Y_E
P2	L12p	*Triticum aestivum*	Q7X729	112	59	1–59	53	*Pyrococcus horikoshii*	3A1Y_F

Acc., accession; PDB, Protein Data Bank.

*Bold rows indicate newly localized r proteins in this study, compared to the bacterial and archaeal X-ray structures.

Table S4. Summary of modeled yeast small subunit r proteins

Protein name	Protein family	Organism	Acc. no.	Size, aa	Modeled length, aa	Modeled range, aa	Percent modeled, %	Template	PDB ID
rpS0	S2p	*Saccharomyces cerevisiae*	P32905	252	252	1–252	100	*Thermus thermophilus*	2J00_B
rpS2	S5p	*Saccharomyces cerevisiae*	P25443	254	254	1–254	100	*Escherichia coli*	2QAL_E
rpS3	S3p	*Saccharomyces cerevisiae*	P05750	240	204	12–215	85	*Escherichia coli*	2QAL_C
rpS4*	**S4e**	***Saccharomyces cerevisiae***	**P05753**	**261**	**200**	**43–242**	**77**	***Thermoplasma acidophilum***	**3KBG_A**
rpS5	S7p	*Saccharomyces cerevisiae*	P26783	225	199	27–225	88	*Pyrococcus horikoshii*	1IQV_A
rpS7	**S7e**	***Saccharomyces cerevisiae***	**P26786**	**190**	**143**	**1–143**	**75**	***Polyalanine***	**—**
rpS9	S4p	*Saccharomyces cerevisiae*	O13516	197	197	1–197	100	*Thermus thermophilus*	2J00_D
rpS11	S17p	*Saccharomyces cerevisiae*	P26781	156	85	39–123	54	*Thermus thermophilus*	2J00_Q
rpS13	S15p	*Saccharomyces cerevisiae*	P05756	151	121	31–151	80	*Escherichia coli*	2QAL_O
rpS14	S11p	*Saccharomyces cerevisiae*	P06367	137	119	19–137	87	*Thermus thermophilus*	2J00_K
rpS15	S19p	*Saccharomyces cerevisiae*	Q01855	142	88	49–136	62	*Escherichia coli*	2QAL_S
rpS16	S9p	*Saccharomyces cerevisiae*	P40213	143	126	18–143	88	*Thermus thermophilus*	2J00_I
rpS17	**S17e**	***Saccharomyces cerevisiae***	**P02407**	**136**	**136**	**1–136**	**100**	***Methanobacterium thermoautotrophicum***	**1RQ6_A**
rpS18	S13p	*Saccharomyces cerevisiae*	P35271	146	140	7–146	96	*Escherichia coli*	2QAL_M
rpS19	**S19e**	***Saccharomyces cerevisiae***	**P07280**	**144**	**144**	**1–144**	**100**	***Pyrococcus abyssi***	**2V7F_A**
rpS20	S10p	*Saccharomyces cerevisiae*	P38701	121	113	9–121	93	*Thermus thermophilus*	2J00_J
rpS21	**S21e**	***Saccharomyces cerevisiae***	**P0C0V8**	**87**	**87**	**1–87**	**100**	**de novo**	**—**
rpS22	S8p	*Saccharomyces cerevisiae*	P0C0W1	130	130	1–130	100	*Escherichia coli*	2QAL_H
rpS23	S12p	*Saccharomyces cerevisiae*	P32827	145	145	1–145	100	*Thermus thermophilus*	2J00_L
rpS24	**S24e**	***Saccharomyces cerevisiae***	**P26782**	**135**	**96**	**1–96**	**71**	***Pyrococcus abyssi***	**2V94_A**
rpS25	**S25e**	***Saccharomyces cerevisiae***	**Q3E792**	**108**	**85**	**24–108**	**78**	***Pyrococcus horikoshii***	**1UB9_A**
rpS26	**S26e**	***Saccharomyces cerevisiae***	**P39938**	**119**	**92**	**1–31;59–119**	**77**	**de novo**	**—**
rpS27	**S27e**	***Saccharomyces cerevisiae***	**P35997**	**82**	**50**	**31–80**	**61**	***Archeoglobus fulgidus***	**1QXF_A**
rpS28	**S28e**	***Saccharomyces cerevisiae***	**Q3E7X9**	**67**	**60**	**1–60**	**90**	***Pyrococcus horikoshii***	**1NY4_A**
rpS29	S14p	*Saccharomyces cerevisiae*	P41057	56	48	9–56	86	*Thermus thermophilus*	2J00_N
rpS30	**S30e**	***Saccharomyces cerevisiae***	**Q12087**	**63**	**63**	**1–63**	**100**	**de novo**	**—**
RACK1	RACK1	*Saccharomyces cerevisiae*	P38011	319	319	1–319	100	*Mus musculus*	2PBI_B

Acc., accession; PDB, Protein Data Bank.

*Bold rows indicate newly localized r proteins in this study, compared to the bacterial and archaeal X-ray structures.

Table S5. Summary of modeled yeast large subunit r proteins

Protein name	Protein family	Organism	Acc.no.	Size, aa	Modeled length	Modeled range	Percent modeled, %	Template	PDB ID
rpL1	L1p	*Saccharomyces cerevisiae*	P53030	217	217	1–217	100	*Thermus thermophilus*	2HW8_A
rpL2	L2p	*Saccharomyces cerevisiae*	P05736	254	254	1–254	100	*Haloarcula marismortui*	1VQ8_A
rpL3	L3p	*Saccharomyces cerevisiae*	P14126	387	387	1–387	100	*Haloarcula marismortui*	1VQ8_B
rpL4	L4p/L4e	*Saccharomyces cerevisiae*	P10664	362	329	1–261 ; 295–362	91	*Haloarcula marismortui*	1VQ8_C
rpL5	L18p	*Saccharomyces cerevisiae*	P26321	297	297	1–297	100	*Haloarcula marismortui*	1VQ8_N
rpL6*	**L6e**	***Saccharomyces cerevisiae***	**Q02326**	**176**	**176**	**1–176**	**100**	***Sulfolobus solfataricus***	**2JOY_A**
rpL7	L30p	*Saccharomyces cerevisiae*	P05737	244	239	6–244	98	*Haloarcula marismortui*	1VQ8_W
rpL8	L7ae	*Saccharomyces cerevisiae*	P17076	256	197	60–256	77	*Haloarcula marismortui*	1VQ8_F
rpL9	L6p	*Saccharomyces cerevisiae*	P05738	191	191	1–191	100	*Haloarcula marismortui*	1VQ8_E
rpL10	L10e	*Saccharomyces cerevisiae*	P41805	221	189	33–221	86	*Haloarcula marismortui*	3CC2_H
rpL11	L5p	*Saccharomyces cerevisiae*	P0C0W9	174	168	1–168	96	*Haloarcula marismortui and Thermus thermophilus*	1VQ8_D and 2J01_G
rpL12	L11p	*Saccharomyces cerevisiae*	P17079	165	127	12–138	77	*Haloarcula marismortui*	2QA4_I
rpL13	**L13e**	***Saccharomyces cerevisiae***	**Q12690**	**199**	**169**	**14–182**	**85**	***Polyalanine***	**—**
rpL14	**L14e**	***Saccharomyces cerevisiae***	**P36105**	**138**	**138**	**1–138**	**100**	***Sulfolobus solfataricus***	**2JOY_A**
rpL15	L15e	*Saccharomyces cerevisiae*	P05748	204	193	1–193	95	*Haloarcula marismortui*	3CC2_M
rpL16	L13p	*Saccharomyces cerevisiae*	P26784	199	199	1–199	100	*Haloarcula marismortui*	1VQ8_J
rpL17	L22p	*Saccharomyces cerevisiae*	P05740	184	170	1–170	92	*Haloarcula marismortui*	1VQ8_R
rpL18	L18e	*Saccharomyces cerevisiae*	P07279	186	161	1–161	87	*Haloarcula marismortui*	1VQ8_O
rpL19	L19e	*Saccharomyces cerevisiae*	P05735	189	189	1–189	100	*Haloarcula marismortui*	1VQ8_P
rpL20	**L18ae**	***Saccharomyces cerevisiae***	**P0C2I0**	**172**	**167**	**1–167**	**97**	***Methanobacterium thermoautothropicum***	**2JXT_A**
rpL21	L21e	*Saccharomyces cerevisiae*	Q02753	160	160	1–160	100	*Haloarcula marismortui*	1VQ8_Q
rpL22	**L22e**	***Saccharomyces cerevisiae***	**P05749**	**121**	**105**	**6–110**	**87**	***Artificial gene***	**2KL8_A**
rpL23	L14p	*Saccharomyces cerevisiae*	P04451	137	131	7–137	96	*Haloarcula marismortui*	1VQ8_K
rpL24	L24e	*Saccharomyces cerevisiae*	P04449	155	73	1–73	47	*Haloarcula marismortui*	1VQ8_U
rpL25	L23p	*Saccharomyces cerevisiae*	P04456	142	122	21–142	86	*Haloarcula marismortui*	1VQ8_S
rpL26	L24p	*Saccharomyces cerevisiae*	P05743	127	123	1–123	97	*Haloarcula marismortui*	1VQ8_T
rpL27	**L27e**	***Saccharomyces cerevisiae***	**P0C2H6**	**136**	**95**	**5–99**	**70**	***Sulfolobus solfataricus***	**2JOY_A**
rpL28	L15p	*Saccharomyces cerevisiae*	P02406	149	149	1–149	100	*Haloarcula marismortui*	1VQ8_L
rpL29	**L29e**	***Saccharomyces cerevisiae***	**P05747**	**59**	**22**	**38–59**	**37**	***Oryctolagus cuniculus***	**1UTG_A**
rpL30	L30e	*Saccharomyces cerevisiae*	P14120	105	105	1–105	100	*Saccharomyces cerevisiae*	1CN7_A
rpL31	L31e	*Saccharomyces cerevisiae*	P0C2H8	113	110	1–110	97	*Haloarcula marismortui*	1VQ8_X
rpL32	L32e	*Saccharomyces cerevisiae*	P38061	130	130	1–130	100	*Haloarcula marismortui*	1VQ8_Y
rpL33	**L35ae**	***Saccharomyces cerevisiae***	**P05744**	**107**	**100**	**1–100**	**93**	***Pyrococcus furiosus***	**1SQR_A**
rpL34	**L34e**	***Saccharomyces cerevisiae***	**P87262**	**121**	**118**	**1–118**	**97**	***Rhodobacter capsulatus***	**2PPT_A**
rpL35	L29p	*Saccharomyces cerevisiae*	P39741	120	118	3–120	98	*Haloarcula marismortui*	1VQ8_V
rpL36	**L36e**	***Saccharomyces cerevisiae***	**P05745**	**100**	**77**	**24–100**	**77**	***Archeoglobus fulgidus***	**2OEB_A**
rpL37	L37e	*Saccharomyces cerevisiae*	P49166	88	88	1–88	100	*Haloarcula marismortui*	1VQ8_1
rpL38	**L38e**	***Saccharomyces cerevisiae***	**P49167**	**78**	**78**	**1–78**	**100**	***Homo sapiens***	**1WH9_A**
rpL39	L39e	*Saccharomyces cerevisiae*	P04650	51	51	1–51	100	*Haloarcula marismortui*	1VQ8_2
rpL40	**L40e**	***Saccharomyces cerevisiae***	**P14796**	**52**	**40**	**13–52**	**77**	***Sulfolobus solfataricus***	**2AYJ_A**
rpL41	**L41e**	***Saccharomyces cerevisiae***	**P05746**	**25**	**25**	**1–25**	**100**	**de novo**	**—**
rpL42	L44e	*Saccharomyces cerevisiae*	P02405	106	106	1–106	100	*Haloarcula marismortui*	1VQ8_3
rpL43	L37ae	*Saccharomyces cerevisiae*	P49631	92	92	1–92	100	*Haloarcula marismortui*	3CC2_Z
rpP0	L10p	*Saccharomyces cerevisiae*	P05317	312	257	1–257	82	*Pyrococcus horikoshii and Methanocaldococcus janaschii*	3A1Y_G and 3JSY_A
rpP1	L12p	*Saccharomyces cerevisiae*	P05318	106	58	5–62	54	*Pyrococcus horikoshii*	3A1Y_E
rpP2	L12p	*Saccharomyces cerevisiae*	P05319	106	58	1–58	55	*Pyrococcus horikoshii*	3A1Y_F

*Bold rows indicate newly localized r proteins in this study, compared to the bacterial and archaeal X-ray structures.

Table S6. Localization of unassigned eukaryotic 80S r proteins

Protein family	*Triticum aestivum* name	*Saccharomyces cerevisiae* name	Localization basis
L6e	L6	rpL6	(*i*) Cross-linking and accessibility to proteolysis (1). (*ii*) Comparison of *S. cerevisiae* and *T. aestivum* cryo-EM maps (which contain L6e) with the *Haloarcula marismortui* 50S X-ray structure and the *Pyrococcus furiosus* 70S cryo-EM structure (which lack L6e). (*iii*) The N terminus of L6e was assigned based on differences between the density and the sequence of *T. aestivum* and *S. cerevisiae*.
L13e	L13	rpL13	(*i*) Cross-linking and accessibility to proteolysis (1). (*ii*) Heterogeneous distribution in archaea.
L14e	L14	rpL14	(*i*) Fold search and secondary structure prediction. (*ii*) Length differences between *S. cerevisiae* and *T. aestivum* L14e sequences, i.e., C terminus is longer in *T. aestivum* L14e and N terminus is longer in *S. cerevisiae*.
L18ae	L18a	rpL20	(*i*) The difference between archaea and eukaryotes, namely, that majority of this density existed only on eukaryotic ribosomes. (*ii*) Fold search revealed that the protein consists of two domains with a distinct LX motif.
L22e	L22	rpL22	(*i*) Cross-linking and accessibility to proteolysis (1). (*ii*) Comparison of *S. cerevisiae* and *T. aestivum* cryo-EM maps (which contain L22e) with the *H. marismortui* 50S X-ray structure and the *P. furiosus* 70S cryo-EM structure (which lack L22e).
L27e	L27	rpL27	(*i*) Cross-linking and accessibility to proteolysis (1). (*ii*) Comparison of *S. cerevisiae* and *T. aestivum* cryo-EM maps (which contain L27e) with the *H. marismortui* 50S X-ray structure and the *P. furiosus* 70S cryo-EM structure (which lack L27e).
L28e	L28	—	(*i*) Difference map between *S. cerevisiae* 80S cryo-EM map with *T. aestivum* 80S cryo-EM maps, because L28e does not exist in *S. cerevisiae*, but is present in *T. aestivum* (2). (*ii*) Cross-linking and accessibility to proteolysis (1).
L29e	L29	rpL29	(*i*) Cryo-EM reconstruction of ΔL29e-80S ribosome at 20.5 Å and comparison with yeast 80S ribosome from wild-type strain (Fig. 1 *F–H*).
L34e	L34	rpL34	Based on the fact that it exists in Eukarya and Archaea, on the fold of the model, and the fact that this was the only major density left unassigned.
L35ae	L35a	rpL33	(*i*) Cross-linking and accessibility to proteolysis (1). (*ii*) Heterogeneous distribution in Archaea.
L36e	L36	rpL36	(*i*) Cross-linking and accessibility to proteolysis (1). (*ii*) Comparison of *S. cerevisiae* and *T. aestivum* cryo-EM maps (which contain L36e) with the *H. marismortui* 50S X-ray structure and the *P. furiosus* 70S cryo-EM structure (which lack L36e).
L38e	L38	rpL38	(*i*) Cryo-EM reconstruction of ΔL38e-80S ribosome at 21 Å and comparison with yeast 80S ribosome from wildtype strain (Fig. 1 *C–E*).
L40e	L40	rpL40	(*i*) Fold and the size of the protein.
L41e	L41	rpL41	(*i*) Size and density features: L41e is only 25 amino acids. (*ii*) Location: isolated density that is unlikely to be an RNA or an r-protein extension.
S4e	S4	rpS4	(*i*) Cross-linking (3). (*ii*) Structural information from *Thermoplasma acidophilum* PDB 3KBG.
S7e	S7	rpS7	(*i*) Immuno-EM (4).
S17e	S17	rpS17	(*i*) Cross-linking (3). (*ii*) Localization: All remaining density on the head of the small subunit was already assigned. (*iii*) Structural information (5).
S19e	S19	rpS19	(*i*) Location: Assembly precursors indicate S19e to be associated with the head of the small subunit (6). (*ii*) Structural information (7). (*iii*) Subsequently localized in the fungi 80S ribosome (8).

Protein family	*Triticum aestivum* name	*Saccharomyces cerevisiae* name	Localization basis
S21e	S21	rpS21	(*i*) Immuno-EM (4).
S24e	S24	rpS24	(*i*) Immuno-EM (4).
S25e	S25	rpS25	(*i*) Cross-linking to IRES elements (9).
S26e	S26	rpS26	(*i*) Cross-linking to mRNA (10).
S27e	S27	rpS27	(*i*) Structural information (11).
S28e	S28	rpS28	(*i*) Cross-linking to mRNA (10). (*ii*) Structural information (12).
S30e	S30	rpS30	(*i*) Cross-linking to mRNA (13, 14).

IRES, internal ribosome entry site.

1 Marion MJ, Marion C (1987) Localization of ribosomal proteins on the surface of mammalian 60S ribosomal subunits by means of immobilized enzymes. Correlation with chemical cross-linking data. *Biochem Biophys Res Commun* 149:1077–1083.

2 Lecompte O, et al. (2002) Comparative analysis of ribosomal proteins in complete genomes: An example of reductive evolution at the domain scale. *Nucleic Acids Res* 30:5382–5390.

3 Gross B, Westermann P, Bielka H (1983) Spatial arrangement of proteins within the small subunit of rat liver ribosomes studied by cross-linking. *EMBO J* 2:255–260.

4 Bommer UA, Lutsch G, Stahl J, Bielka H (1991) Eukaryotic initiation factors eIF-2 and eIF-3: Interactions, structure and localization in ribosomal initiation complexes. *Biochimie* 73:1007–1019.

5 Wu B, et al. (2008) The solution structure of ribosomal protein S17E from *Methanobacterium thermoautotrophicum*: A structural homolog of the FF domain. *Protein Sci* 17:583–588.

6 Ferreira-Cerca S, et al. (2007) Analysis of the in vivo assembly pathway of eukaryotic 40S ribosomal proteins. *Mol Cell* 28:446–457.

7 Gregory LA, et al. (2007) Molecular basis of Diamond-Blackfan anemia: Structure and function analysis of RPS19. *Nucleic Acids Res* 35:5913–5921.

8 Taylor DJ, et al. (2009) Comprehensive molecular structure of the eukaryotic ribosome. *Structure* 17:1591–1604.

9 Nishiyama T, Yamamoto H, Uchiumi T, Nakashima N (2007) Eukaryotic ribosomal protein RPS25 interacts with the conserved loop region in a dicistroviral intergenic internal ribosome entry site. *Nucleic Acids Res* 35:1514–1521.

10 Pisarev AV, et al. (2008) Ribosomal position and contacts of mRNA in eukaryotic translation initiation complexes. *EMBO J* 27:1609–1621.

11 Herve du Penhoat C, et al. (2004) The NMR solution structure of the 30S ribosomal protein S27e encoded in gene RS27_ARCFU of *Archaeoglobus fulgidis* reveals a novel protein fold. *Protein Sci* 13:1407–1416.

12 Aramini JM, et al. (2003) Solution NMR structure of the 30S ribosomal protein S28E from *Pyrococcus horikoshii*. *Protein Sci* 12:2823–2830.

13 Bulygin K, et al. (2005) The first position of a codon placed in the A site of the human 80S ribosome contacts nucleotide C1696 of the 18S rRNA as well as proteins S2, S3, S3a, S30, and S15. *Biochemistry* 44:2153–2162.

14 Takahashi Y, Mitsuma T, Hirayama S, Odani S (2002) Identification of the ribosomal proteins present in the vicinity of globin mRNA in the 40S initiation complex. *J Biochem* 132:705–711.