Supplementary Information

Structural basis of highly conserved ribosome recycling in eukaryotes and archaea

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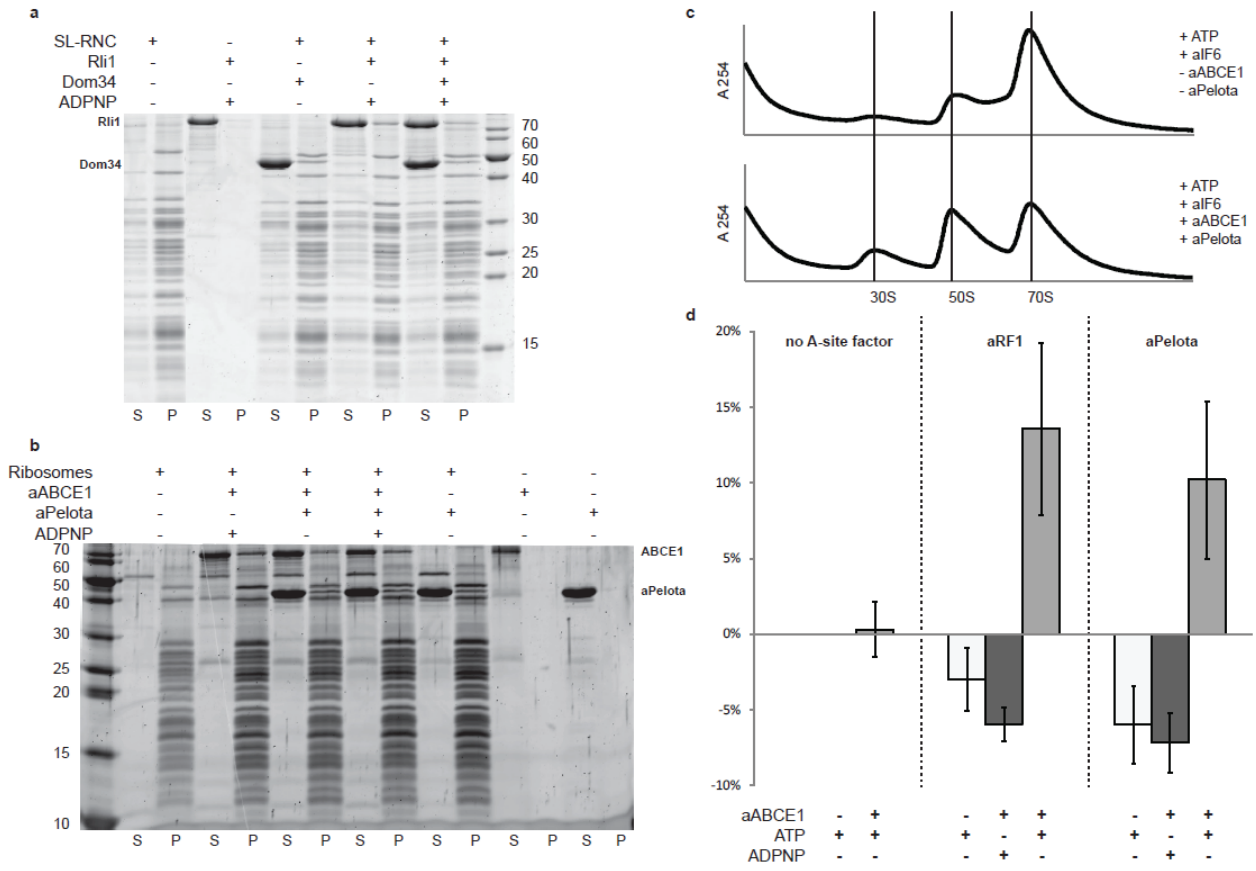
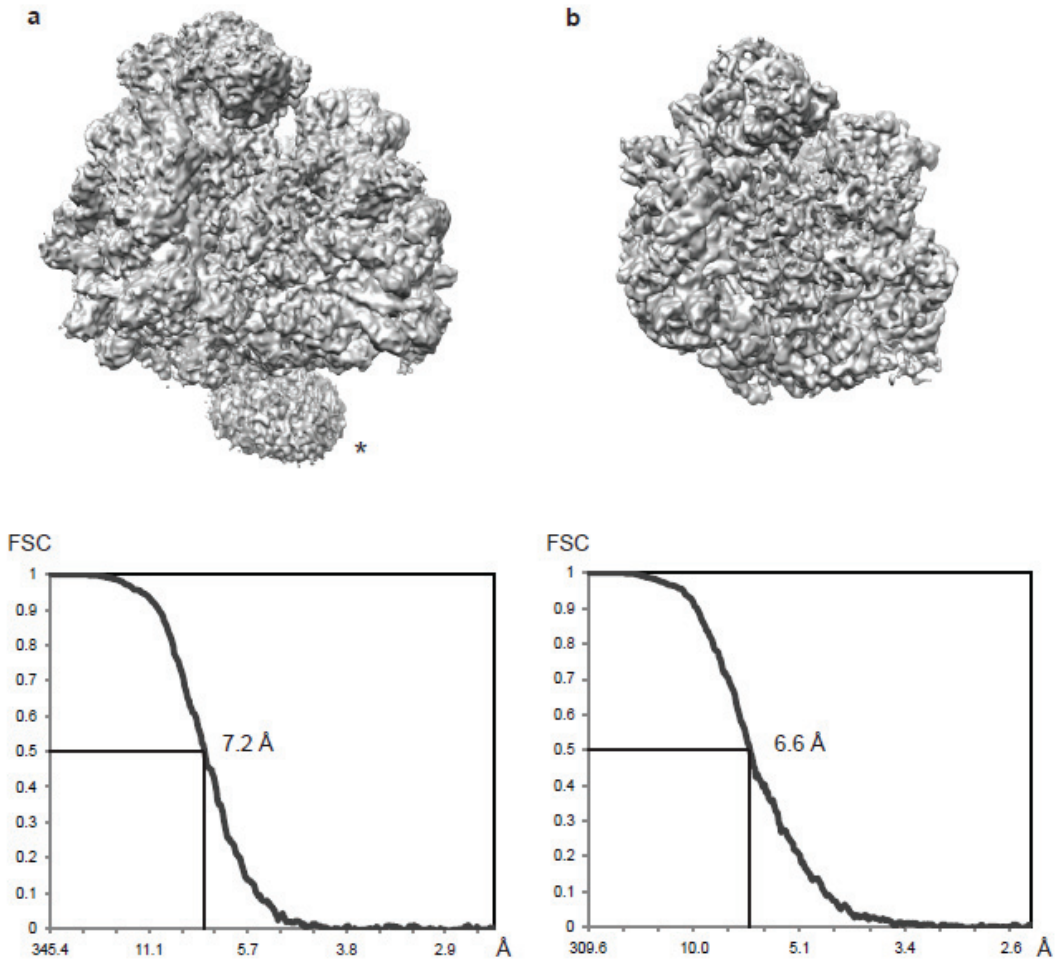
Supplementary Information



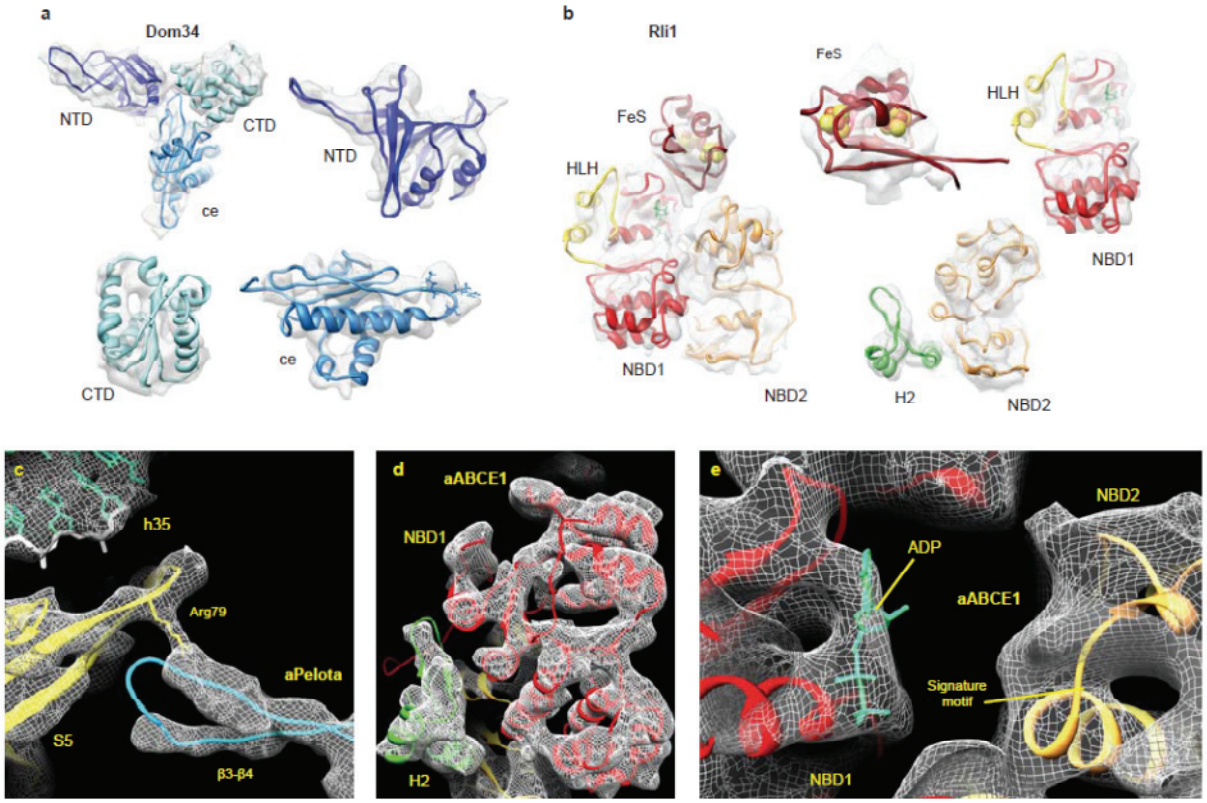
Suppl. Fig. 1: *In vitro* ribosome binding of Pelota and ABCE1 and ribosomal splitting.

(**a**) Purified Dom34 and Rli1 were bound to yeast SL-RNCs in the presence of ADPNP. (**b**) aPelota and aABCE1 were bound to archaeal 70S ribosomes. Reactions were spun through a sucrose cushion. Supernatant (S) and pellet (P) fractions were analyzed by SDS-PAGE and stained with SYPRO Orange. Binding of Pelota and ABCE1 alone as well as in combination is detectable in both, *S. cerevisiae* and *P. furiosus*. (**c**) Dissociation by aABCE1, aPelota and aIF6 compared with factor-independent dissociation of 70S archaeal ribosomes assayed by sucrose density centrifugation. (**d**) Splitting efficiency of aABCE1, aRF1 or aPelota and different nucleotides for archaeal 70S ribosomes. Error bars indicate +/- standard deviation. aIF6 was added to prevent reassembly of free subunits.



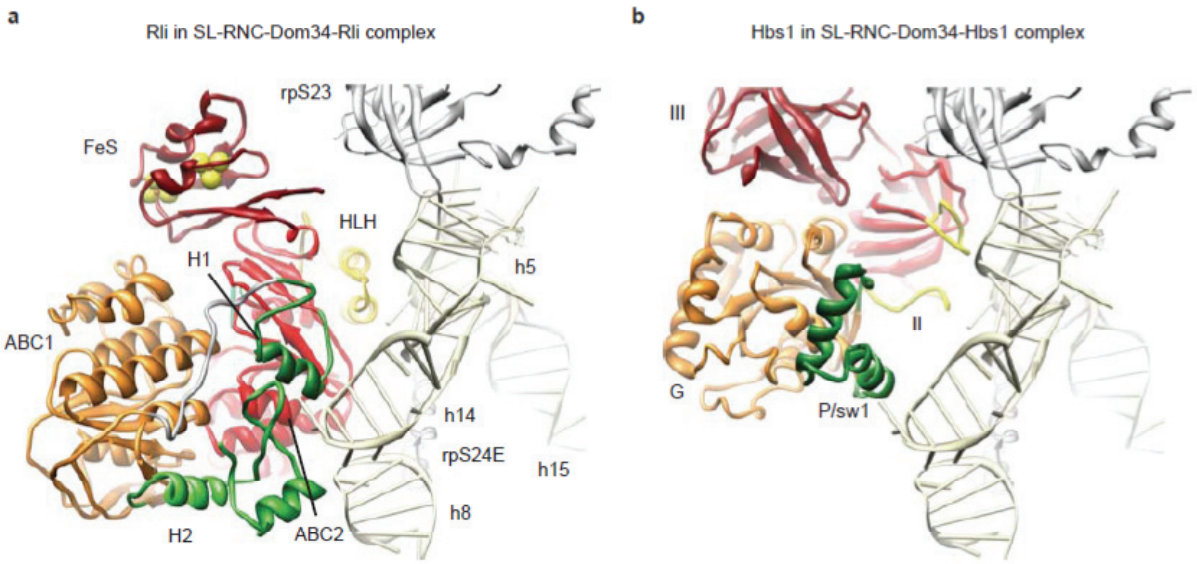
Suppl. Fig. 2: Cryo-EM reconstructions and resolution.

Cryo-EM maps of the yeast SL-RNC-Dom34-Rli1 complex (**a**) and the archaeal 70S aPelota-aABCE1 complex (**b**) at a resolution of 7.2 and 6.6 Å, respectively, according to a FSC at a cutoff value of 0.5. For the yeast SL-RNC complex mammalian Sec61 (mSec61; indicated with an asterisk) is visible at the exit site. mSec61 was used to prevent orientational bias of the particles on the cryo-EM grid.



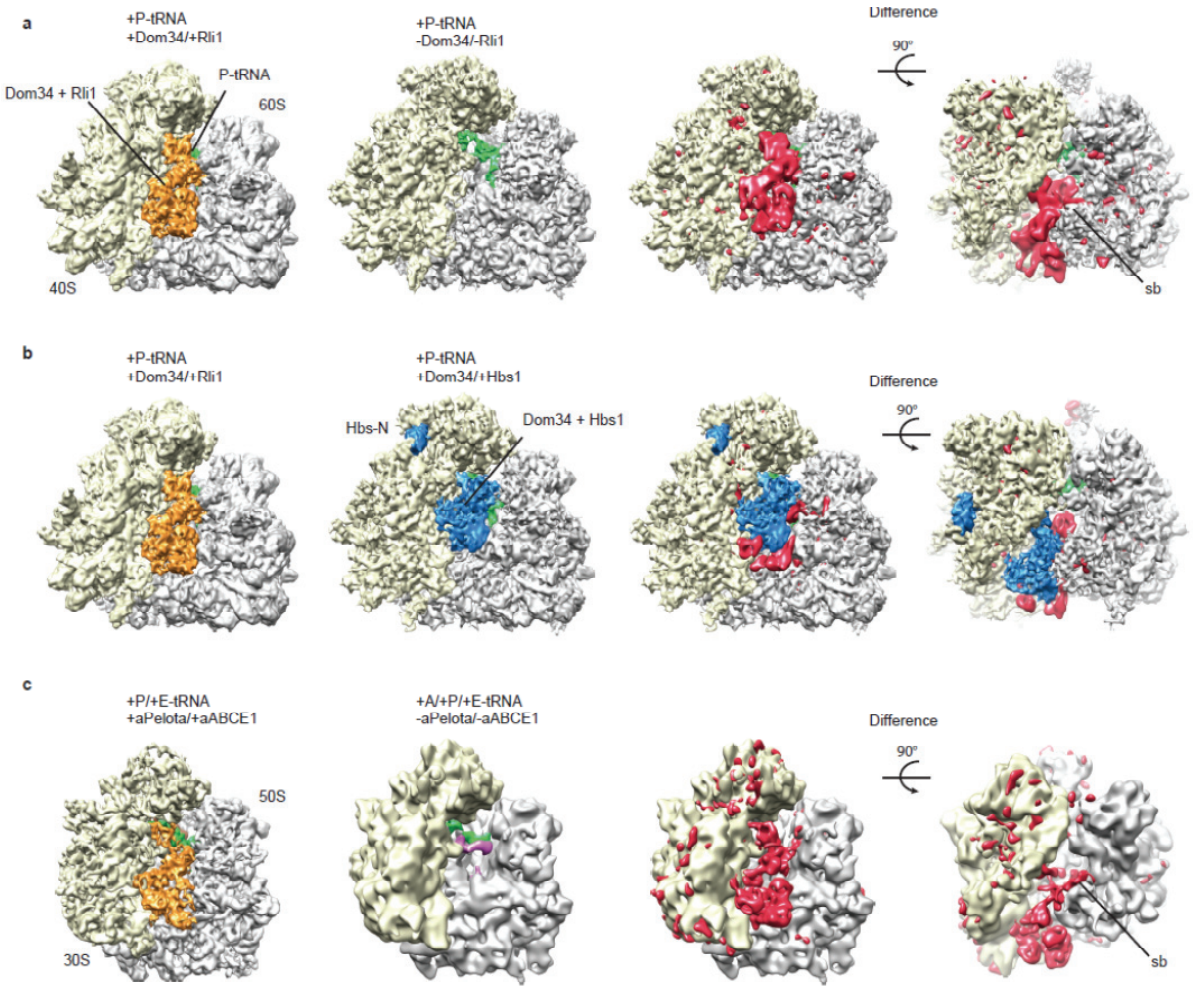
Suppl. Fig. 3: Fitting of Pelota and ABCE1 domains.

(**a, b**) Homology models of *S. cerevisiae* Dom34 and Rli1 fitted into isolated cryo-EM densities (transparent mesh). (**c**) Homology models for *T. kodakarensis* aPelota, rRNA and ribosomal protein S5 fitted into isolated cryo-EM densities showing the contact between aPelota and S5 formed by Arg79 (NTD blue, S5 yellow, rRNA green-white). (**d, e**) Crystal structure of aABCE1 fitted into isolated cryo-EM density (NBD1 red, hinge 2 domain green, NBD2 yellow)[6]. (d) View focusing on NBD1 and hinge domain 2 (H2). (**e**) View focusing on the nucleotide binding site of NBD1 (ADP green).



Suppl. Fig. 4: Comparison of Rli1 binding with Hbs1 binding to the 40S subunit.

The Rli1 helix-loop-helix motif (HLH; yellow) binds to rRNA helices h5 and h15 that are bound by domain II of Hbs1[19] (red; residues interacting with rRNA in yellow). The Rli1 hinge domain (H1, H2; green) binds to the rRNA h8-h14 junction that is contacted by the P-loop/switch1-loop region of Hbs1 (P/sw1; green). The color code of Rli1 is as in **Fig. 1c**, Hbs1 G-domain is shown in orange, domain II in red and domain III in dark red, rRNA in pale yellow and ribosomal proteins in grey.



Suppl. Fig. 5: Difference maps.

(**a**) Cryo-EM maps for the yeast SL-RNC-Dom34-Rli1 complex, a vacant SL-RNC (middle) and the difference map (side and top views) overlaid upon the SL-RNC map. (**b**) Cryo-EM maps for the SL-RNC-Dom34-Rli1 complex, the SL-RNC-Dom34-Hbs1 complex[19] and the difference map (side and top view). (**c**) Cryo-EM maps for the archaeal 70S-aPelota-aABCE1 complex, the 70S ribosome with A-, P- and E-site tRNA and the difference map (side and top view). Note that the stalk base (sb) is moved inwards upon Dom34-Rli1/aPelota-ABCE1 binding as observed for binding of Dom34-Hbs1. The small ribosomal subunits are colored in pale yellow, large subunits in grey, Dom34-Rli1/aPelota-aABCE1 density in orange, Dom34-Hbs1 density in blue, P-site tRNA density in green, A-site tRNA density in purple and difference densities in red.

Suppl. Table 1: Contacts between Dom34 and Rli1 with the ribosome and with each other. Known mutations of or in direct neighborhood to contacting residues in Dom34 and Rli1 and their effects are given.

Ribosome			Dom34		Mutation	
	Protein	**Residue**	**Domain**	**Residue**	**Residue**	**Effect ***
LSU	rpL12/L11	24-26	CTD	311-312		
SSU	rpS30e	7-8	NTD	77-78		

Ribosome			Dom34		Mutation	
	RNA	**Nucleotide**	**Domain**	**Residue**	**Residue**	**Effect ***
LSU	H43	1242	CTD	345		
	H44	1270	CTD	373-375		
	H69	2259	ce	255-256		
	H69	2265	NTD	113		
	H71	2285	ce	175	174KKK176 to 174AAA176[58,59]	+
	H89	2850-2852	ce	232, 235, 243		
		2839-2840	ce	237, 241		
		2842	ce	286		
	H92	2926-2927	ce	218	G217Y[32], 216PGF218 to 216AAA218[58]	+, +
	H95	3027	CTD	288-293		
SSU	h18	575-578	NTD	47, 49-52, 103-104	F47A[32], Δ(F47-T60)[32], 49SKLDf53 to 49AAAAA53[58]	++, +
	h28	1634	NTD	56-57	Δ(F47-T60)[32]	++
	h31	1182-1185	NTD	9-11		
		1179, 1188-1189	NTD	91-92, 94-95	(D90-P100)A[32], 87TVTDES92 to 87AAAAAA92[58]	+, +
	h34	1272-1274	NTD	100-101	(D90-P100)A[32]	+
	h44	1756-1757	NTD	113		

tRNA		Dom34		Mutation	
Position	**Residue**	**Domain**	**Residue**	**Residue**	**Effect ***
p-site	1, 71	ce	177, 186-187	174KKK176 to 174AAA176[58,59] 184FDEKT188 to 184AAAAA188[58]	+,-
	65, 67	ce	174, 178, 183, 186	174KKK176 to 174AAA176[58,59], 184FDEKT188 to 184AAAAA188[58]	-

Rli1		Dom34		Mutation	
Domain	**Residue**	**Domain**	**Residue**	**Residue**	**Effect ***
FeS	63-64	CTD	292-293		
FeS	33	CTD	300-302	Y300A[32,59]	+
FeS	27-28	CTD	384-386		

	Ribosome		Rli1		Mutation	
	Protein	**Residue**	**Domain**	**Residue**	**Residue**	**Effect ***
LSU	rpL9	96-97	NBD2	516		
	rpP0	145,147	NBD2	432, 435, 439		
SSU	rpS6e[23]	19, 107	H2	587-592, 595	S588E[7]	++
	rpS24e	132-134	NBD1	262		

	Ribosome		Rli1		Mutation	
	RNA	**Nucleotide**	**Domain**	**Residue**	**Residue**	**Effect ***
LSU	H95	3021-3025	NBD2	445-446, 508-512		
		3019-3020	NBD1	337-338		
SSU	h5	51	HLH	148-151	R148A[7]	-
	h8	155-157	H2	582-585, 587-589	S588E[7]	++
	h14	416-417	H1/H2	311, 580-582, 515	R311E[7]	++
	h15	429-430, 438-440	HLH	146, 153-154	L152E[7]	-

	Dom34		Rli1		Mutation	
	Domain	**Residue**	**Domain**	**Residue**	**Residue**	**Effect ***
	CTD	292-293	FeS	63-64	C65A[7,9]	++
	CTD	300-302	FeS	33		
	CTD	384-386	FeS	27-28	C29A[7,9]	-

* Effect reads as: – no effect + mild effect ++ strong effect

Suppl. Table 2: Contacts of aPelota and aABCE1 with the ribosome and with each other. Known mutations of or in direct neighborhood to contacting residues in aPelota and aABCE1 and their effects are given.

Ribosome			aPelota		Mutation	
	Protein	**Residue**	**Domain**	**Residue**	**Residue ****	**Effect *****
LSU	L10e	100-106	ce	174-176	174KKK176 to 174AAA176[58,59] (174-176)	+
	L11	18-24	CTD	283-285	Y300A[32,59] (283)	+
SSU	S5	79	NTD	48-49	49SKLDf53 to AAAAA[58] (47-51)	+

Ribosome			aPelota		Mutation	
	RNA	**Nucleotide***	**Domain**	**Residue**	**Residue ****	**Effect *****
LSU	H43	1205-1207	CTD	325-326		
	H44	1234	CTD	353,354		
	H69	2038-2040	ce	239-240, 243		
	H72	2070-2071	ce	207-208, 231-233	G217Y[32] (208), 216PGF218 to 216AAA218[58] (207-209)	+, +
		2087	ce	235-236		
	H89	2595	ce	223		
	H95	2776	CTD	272-273, 276		
SSU	h18	470-472, 483-484	NTD	42, 63-64, 68, 94-96	(D90-P100)A[32] (90-104)	+
	h28	1357	NTD	46	F47A[32] (45), 49SKLDf53 to 49AAAAA53[58] (47-51)	++, +
	h31	916	NTD	9		
	h34	1004-1006, 1156	NTD	46, 94-96	F47A[32] (45), 49SKLDf53 to 49AAAAA53[58], (47-51), (D90-P100)A[32] (90-104)	++, +
	h44	1449	NTD	60		

tRNA		aPelota		Mutation	
Position	**Residue**	**Domain**	**Residue**	**Residue ****	**Effect *****
P site	1	ce	172-174	174KKK176 to 174AAA176[58,59] (174-176)	+
	68	ce	163-164		
	73	ce	172		

ABCE1		aPelota		Mutation	
Domain	**Residue**	**Domain**	**Residue**	**Residue ****	**Effect *****
FeS	30-31, 33-34, 58	CTD	283, 285, 344-345	Y300A[32,59] (283)	+

	Ribosome		aABCE1		Mutation	
	Protein	**Residue**	**Domain**	**Residue**	**Residue ****	**Effect *****
LSU	L14	132	H1	324-327		
	L9	96	NBD2	434-435		

	Ribosome		aABCE1		Mutation	
	RNA	**Nucleotide***	**Domain**	**Residue**	**Residue ****	**Effect *****
SSU	h5	51	NBD1	149	L152A^{r} (150)	-
	h8	144-145	H2	576-579, 582	S588E^{r} (576)	++
	h14	339-341	H1	302, 304	R311E^{r} (304)	++
			H2	563, 572, 575	R573E^{r} (563)	++
	h15	363-364	NBD1	146-149	R148A^{r}, L152A^{r} (146, 149)	-,-

aPelota		aABCE1		Mutation	
Domain	**Residue**	**Domain**	**Residue**	**Residue ****	**Effect *****
CTD	283, 285, 344-345	FeS	30-31, 33-34, 58	C29S/A^{7,9}, C54S^{8}, ΔFeS^{8}, (29, 58, 1-78)	-,-,++

* *P. furiosus* numbering

** Numbers in brackets indicate the corresponding residues for aPelota (*T. kodakarensis*) and aABCE1 (*P. furiosus*)

*** Effect reads as: - no effect + mild effect ++ strong effect

Supplementary Movies

Movie 1: Domain movement of Pelota central domain stabilized by ABCE1 - yeast

The movie shows the crown view on the 60S ribosomal subunit of *S. cerevisiae* with isolated densities of P-site tRNA, Dom34, Hbs1 and Rli1 and the fitted molecular models. After dissociation of Hbs1 and binding of Rli1 the central domain of Dom34 is stabilized in a rotated conformation contacting the P- site tRNA.

Movie 2: Domain movement of Pelota central domain stabilized by ABCE1 - archaea

The movie shows the crown view on the 50S ribosomal subunit of *P. furiosus* with isolated densities of P-site tRNA, E-site tRNA aPelota and aABCE1 and the fitted molecular models. After dissociation of aEF1α and binding of aABCE1 the central domain of aPelota is stabilized in a rotated conformation contacting the P-site tRNA.

Movie 3: Conformational transition of ABCE1

The movie shows the morphing of the aABCE1 conformations starting with the open (ADP-bound) conformation via the intermediate ribosome-bound conformation into the closed (ATP-bound) conformation.

Supplementary References

58 Passos, D. *et al.* Analysis of Dom34 and Its Function in No-Go Decay. *Mol. Biol. Cell* **20**, 3025-3032 (2009).

59 van den Elzen, A. M. G. *et al.* Dissection of Dom34-Hbs1 reveals independent functions in two RNA quality control pathways. *Nat. Struct. Mol. Biol.* **17**, 1446-U1474 (2010).