Appendix information for

Structure of a eukaryotic cytoplasmic pre-40S ribosomal subunit

Alain Scaiola[1], Cohue Peña[1], Melanie Weisser, Daniel Böhringer, Marc Leibundgut, Purnima Klingauf-Nerurkar, Stefan Gerhardy, Vikram Govind Panse*, Nenad Ban*

[1]equal contribution

*Correspondence to Vikram Govind Panse (vpanse@imm.uzh.ch) and Nenad Ban (ban@mol.biol.ethz.ch)

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Appendix Figures S1-S4

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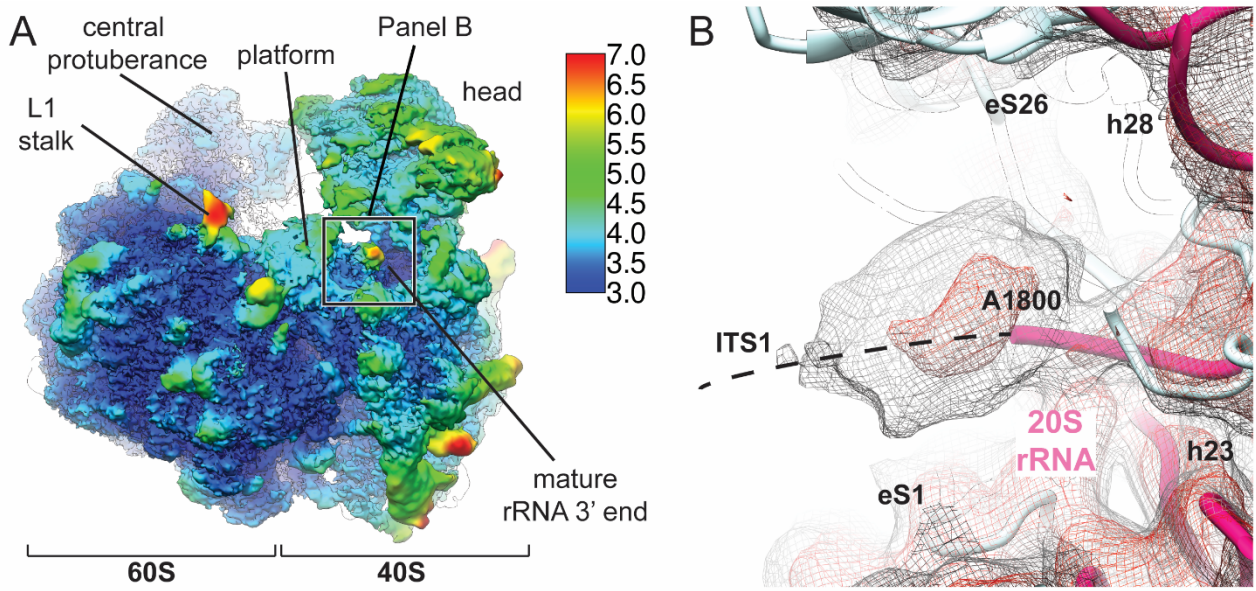
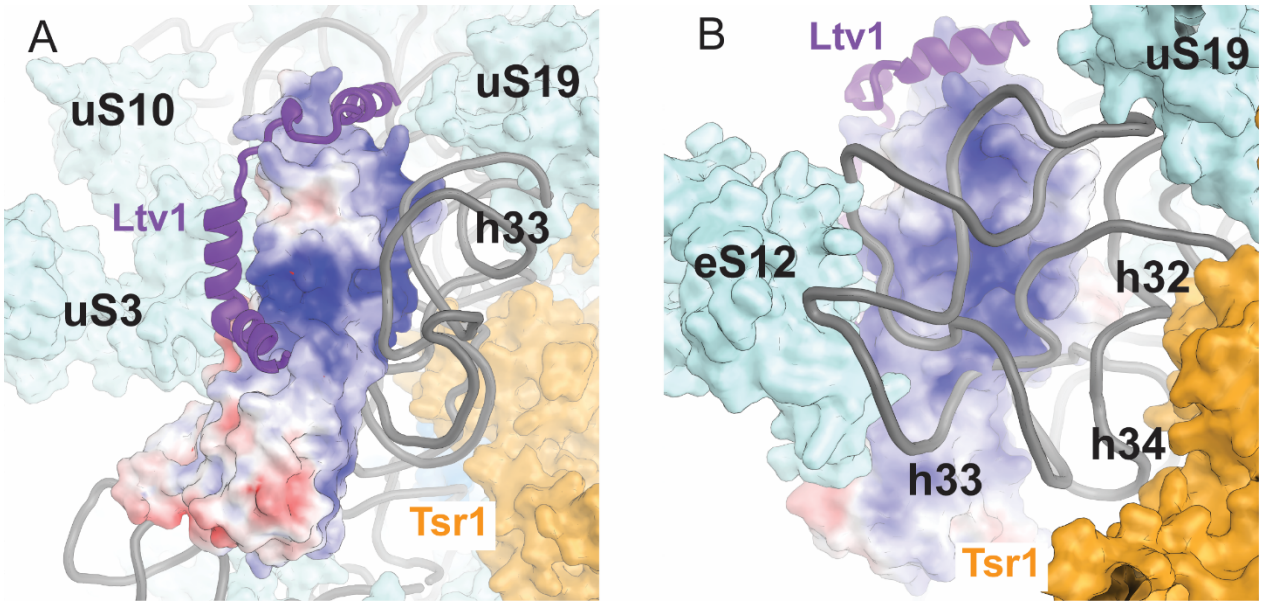


Appendix Figure S1: 3D reconstruction of the Nob1-D15N 80S ribosome

A. Local resolution heat-map of the reconstruction of the 80S ribosome subclass, which reached an overall resolution of 3.1 Å. The local resolution varies between 2.8 Å for the core of the subunits and 7 Å for the flexible rRNA stalks. A low local resolution (6-7 Å) density can be observed protruding from the 40S subunit (Panel B). "relion_postprocess" from Relion 2.1 (Kimanius et al., 2016) was used to estimate the local resolution.

B. Close-up view of the cryo-EM map, low-pass filtered to 6 Å for clarity, shown at two different contour levels (red and gray mesh) around the 3' end of the 18S rRNA. The model of the mature 40S (Ben-Shem et al., 2011) (r-proteins in light blue, 18S rRNA in pink) was rigid-body fitted into the density using UCSF Chimera (Pettersen et al., 2004) and the nucleotides 1799 and 1800, absent in the original PDB, were added into the density using COOT (Emsley et al., 2010). The extra density following the final nucleotide 1800 corresponds to the ITS1 that was not cleaved by the catalytically inactive Nob1-D15N.

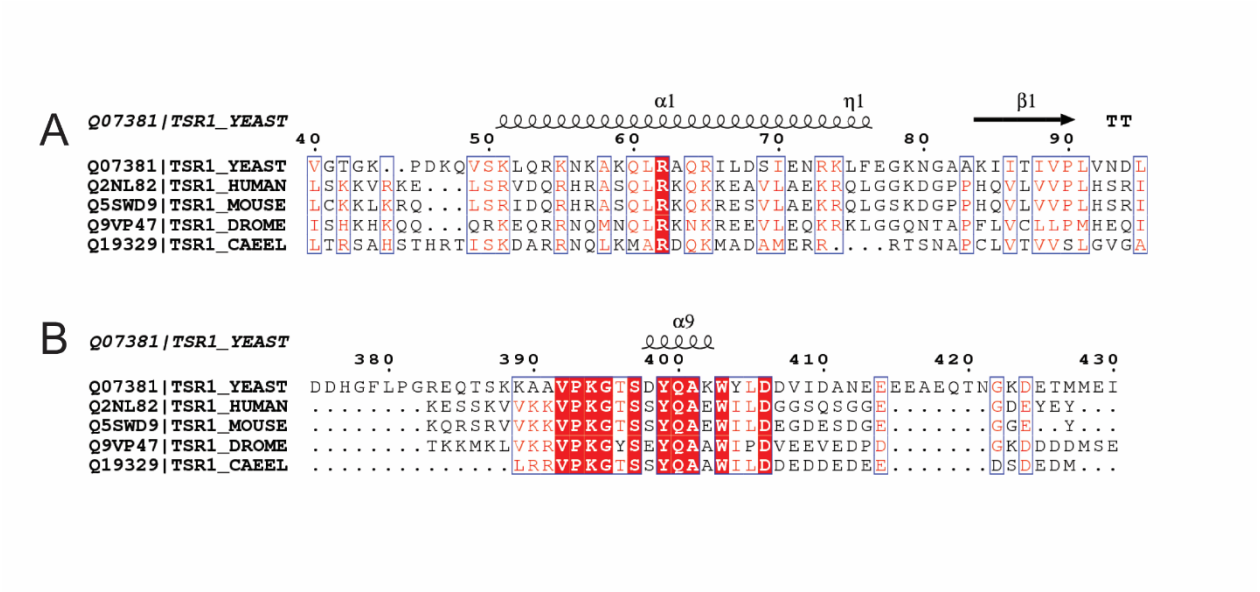


Appendix Figure S2: Surface representation of electrostatic surface charges of Enp1.

A-B. Surface representation of Enp1 colored by electrostatic potential in context of the cytoplasmic pre-40S rRNA (gray), the r-proteins uS10, uS3, uS19 and eS12 (light blue), Tsr1 (orange), and Ltv1 (purple). The large positively charged surface (blue) is directly interacting with the backbone of the three-way junction of helices 32, 33 and 34.

A. Front view from the tip of the beak. eS12 is hidden for clarity.

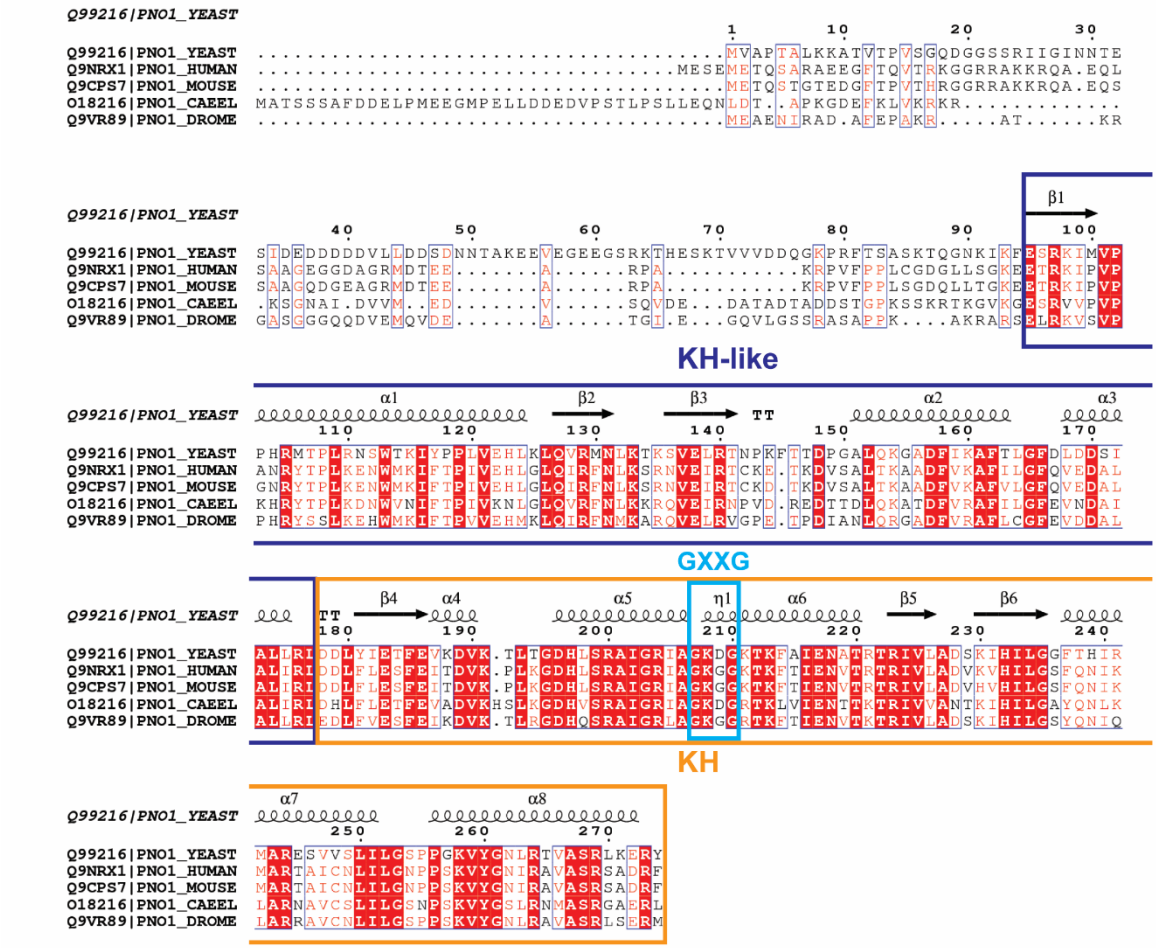
B. Side view from the intersubunit space.



Appendix Figure S3: Multiple sequence alignments, conservation and secondary structure of parts of assembly factor Tsr1.

A. Multiple sequence alignment of the N-terminus of Tsr1. Residues 51 to 75 (in *S. cerevisiae*) had not been resolved in the previously reported crystal structure of Tsr1 (McCaughan et al., 2016), but appear as an α-helix in our cryo-EM map of the cytoplasmic pre-40S particle (as shown in Fig. 4C). The helix contains conserved positively charged residues (e.g. R62 and R73) that make contacts with the rRNA backbone of helices 5, 11 and 44.

B. Multiple sequence alignment of the previously unresolved loop of Tsr1 (comprising residues 380 to 409 in *S. cerevisiae*). The cryo-EM map of the cytoplasmic pre-40S particle reveals that the conserved residues 392-408 specifically contact the displaced helix 31, as well as ribosomal proteins uS13 and uS19 and that residues 398-404 form a short α-helix (Fig. 4E).



Appendix Figure S4: Multiple sequence alignment, conservation and secondary structure predictions of assembly factor Pno1.

Multiple sequence alignment of Pno1 to highlight the high conservation of the KH-like (squared in blue) and KH domain (squared in orange). The poorly conserved N-terminal region was not resolve in our cryo-EM density. The conservation of Pno1 surface is shown in Figure 5C.

Appendix Table S1: Yeast strains used in this study

Strain name	Genotype	Origin
BY4741	*MATa ura3 his3 leu2 met15 TRP1*	Euroscarf
P_{GAL1}-*NOB1*	*MATa ura3 his3 leu2 met15 TRP1* P_{Gal1}-*NOB1::natNT2*	This study
P_{GAL1}-*HRR25*	*MATa ura3 his3 leu2 met15 TRP1* P_{Gal1}-*HRR25::natNT2*	This study
Enp1-GFP	*MATa ura3 leu2 TRP1 ENP1-GFP::HIS3MX*	Open biosystems
Tsr1-GFP	*MATa ura3 leu2 TRP1 TSR1-GFP::HIS3MX*	Open biosystems

Appendix Table S2: Plasmids used in this study

Plasmid	Gene and Information	Source
pRS315-*NOB1*	*NOB1 LEU2 AMP*	This study
pRS315-pA-TEV-FLAG-*NOB1*	*ProteinA-TEV-FLAG-NOB1 LEU2 AMP*	(Pertschy et al., 2009)
pRS315-pA-TEV-FLAG-*nob1-D15N*	*nob1 D15N LEU2 AMP*	This study
pRS315-*HRR25*	*HRR25 LEU2 AMP*	This study
pRS315-*hrr25-K38A*	*hrr25 K38A LEU2 AMP*	This study
YEP351-P_{Gal1}-*hrr25-K38A*	P_{Gal1}-*hrr25 K38A LEU2 AMP*	This study

Appendix Table S3: Local resolution of the factors based on their atom position

Factor	Chain ID	Resolution (relion_postprocess)
Pno1	H	3.6
Enp1	I	4.1
Ltv1	J	4.2
Tsr1	K	3.7
Rio2	L	4.0
Dim1	Not deposited	6.1

Appendix Table S4: Initial PDB templates used for docking and modeling

Structure	Template	Organism of the template
40S ribosome	4V88	*Saccharomyces cerevisiae*
Pno1	3AEV	*Pyrococcus horikoshii*
Enp1	5WWO	*Saccharomyces cerevisiae*
Ltv1	5WWO	*Saccharomyces cerevisiae*
Tsr1	5IW7	*Saccharomyces cerevisiae*
Rio2	1ZAO	*Archaeoglobus fulgidus*
Dim1	1ZQ9	*Homo sapiens*

References for the appendix figures and tables:

Ben-Shem A, Garreau de Loubresse N, Melnikov S, Jenner L, Yusupova G, Yusupov M (2011) The structure of the eukaryotic ribosome at 3.0 A resolution. *Science* 334: 1524-9

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McCaughan UM, Jayachandran U, Shchepachev V, Chen ZA, Rappsilber J, Tollervey D, Cook AG (2016) Pre-40S ribosome biogenesis factor Tsr1 is an inactive structural mimic of translational GTPases. *Nat Commun* 7: 11789

Pertschy B, Schneider C, Gnadig M, Schafer T, Tollervey D, Hurt E (2009) RNA helicase Prp43 and its co-factor Pfa1 promote 20 to 18 S rRNA processing catalyzed by the endonuclease Nob1. *J Biol Chem* 284: 35079-91

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