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Supplemental Information

Visualizing the Assembly Pathway of Nucleolar Pre-60S Ribosomes

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Table S1. Plasmids used in this Study. Related to STAR methods plasmid constructs and yeast strains

Name	Relevant information	Source
pFA6a-TAP-klURA3	CBP-TEVcleavage site-ProtA, T*ADH1*, for genomic C-terminal tagging	This study
pFA6a-Flag-natNT2	Flag, T*CYC1*, for genomic C-terminal tagging	This study
pnatNT2 P_{YTM1} Flag	P_{YTM1}, Flag tag, for genomic N-terminal tagging	This study
YCplac111-TAP-Flag-*YTM1*	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-term. ProtA-TEVcleavage site-CBP-Flag tag	(Thoms et al., 2016)
YCplac111-TAP-Flag-*ytm1* ΔUBL	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-term. ProtA-TEVcleavage site-CBP-Flag tag	This study
YCplac111-TAP-Flag-*ytm1* E80A	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-term. ProtA-TEVcleavage site-CBP-Flag tag	This study
YCplac111-pA-TEV-$(His)_6$-*RPF1*	CEN, *LEU2*, P*NOP53*, T*ADH1*, N-term. ProtA-TEVcleavage site-$(His)_6$ tag	This study
pET15b-*Ct*Rrp1-$(His)_6$ *	Amp^r, T7 promoter, *lac* operator	(Bassler et al., 2017)
pET15b-Nsa1-$(His)_6$ *	Amp^r, T7 promoter, *lac* operator	This study

E.coli expression plasmids are marked with asterisk.

Table S2. Yeast Strains used in this Study. Related to STAR methods plasmid constructs and yeast strains

Name	Relevant genotype	Source
W303	wild-type, MAT	(Thomas and Rothstein, 1989)
DS1-2b *	wild-type	(Nissan et al., 2002)
***NSA1*-TAP Flag-*YTM1* ***	*NSA1*-TAP::klURA3, $P_{Flag\text{-}Ytm1}$-*YTM1*::natNT2	This study
***RIX1*-TAP *RPF2*-Flag ***	*RIX1*-TAP::*TRP1*, *RPF2*-Flag::natNT2	This study
***BRX1*-HTpA**	*BRX1*-HTpA::His3MX4	This study
pA-TEV-(HIS)$_6$-RPF1	*rpf1::HIS3MX4*, *YCplac111 pA-TEV-(HIS)$_6$-RPF1*	This study

Strains marked with an asterisk were derived from DS1-2b (Nissan et al., 2002)

Table S3: Data collection and refinement statistics for *Sc*Nsa1. Related to STAR methods protein production, crystallization and structure determination

Data collection	
Wavelength (Å)	1.0
Space group	P 21 21 21
Cell dimensions	
a, b, c (Å)	54.72 81.1 87.57
α, β, γ (°)	90, 90, 90
Resolution (Å)	59.5 - 2.4 (2.49 - 2.4)
R_{merge}	0.04384(0.1126)
I/σ(*I*)	34.78 (14.02)
Reflections total	111874 (8692)
Reflections unique	15750 (1543)
Multiplicity	7.1 (5.6)
Refinement	
R_{work}	0.1592 (0.1724)
R_{free}	0.2027 (0.2799)
No. atoms	
Protein	2955
Water	194
B-factors	
Protein	20.70
Water	22.80
R.m.s deviations	
Bond lengths (Å)	0.011
Bond angles (º)	1.41
Ramachandran plot	
Most favoured (%)	97
Disallowed	0.55

Table S4: Data collection and refinement statistics for *Ct*Rrp1. Related to STAR methods protein production, crystallization and structure determination

	Native 1 (*C*2)	Native 2 ($P6_322$)	Pt-derivative
Data collection			
Wavelength (Å)	1.03323		1.07156
Space group	*C*2	$P6_322$	$P6_322$
Cell dimensions			
a, b, c (Å)	131.72, 74.67, 68.79	124.26, 124.26, 124.79	214.75, 214.75, 125.95
α, β, γ (°)	90, 90.52, 90	90, 90, 120	90, 90, 120
Resolution (Å)	47.36 – 2.65 (2.74 –2.65)	49.22 – 2.24 (2.32 – 2.24)	47.72 – 3.3 (3.41 – 3.3)
R_{pim}	0.093 (0.3138)	0.021 (0.3559)	0.114 (1.935)
/σ(*I*)	6.88 (1.67)	24.62 (2.16)	12.61 (1.18)
Reflections total	219854 (17947)	1632833 (166862)	1010978 (96718)
Reflections unique	19400 (1827)	80956 (7988)	26210 (2572)
Multiplicity	11.3 (9.8)	20.2 (20.9)	38.6 (37.6)
Refinement			
R_{work}	0.2052 (0.2967)	0.1836 (0.2855)	
R_{free}	0.2342 (0.3679)	0.2090 (0.2918)	
No. atoms			
Protein	3746	3723	
Water	117	212	
B-factors			
Protein	40.42	69.65	
Water	38.82	68.03	
R.m.s deviations			
Bond lengths (Å)	0.003	0.003	
Bond angles (º)	0.52	0.68	
Ramachandran plot			
Most favoured (%)	98.66	98.66	
Disallowed	0.45	0.45	

Table S5. Cryo-EM model and refinement statistics. Related to STAR methods model building and refinements

	State C	State E
Data collection		
Particles	157,184	115529
Pixel size (Å)	1.084	1.084
Defocus range (μm)	0.9 – 3.5	0.9 – 3.5
Voltage (kV)	300	300
Electron dose ($e^{-}A^{-2}$)	27	27
Model Refinement		
Model composition		
Non-hydrogen atoms	88243	135041
Protein residues	5800	9709
RNA bases	1943	2680
Refinement		
Resolution for refinement (Å)	3.6	3.3
Map sharpening B-factor ($Å^2$)	-144	-123
Average B-factor ($Å^2$)	174.8	101.1
$FSC_{average}$	0.845	0.805
R.m.s. deviations		
Bond lengths (Å)	0.0129	0.0068
Bond angles (°)	1.35	1.17
Validation & Statistics		
Validation		
Molprobity score	2.86	2.4
Clashscore, all atoms	8.08	6.53
Good rotameres (%)	89.06	95.29
Ramachandran Plot		
Favored (%)	86.66	90.64
Outliers (%)	3.56	1.33
Validation (RNA)		
Correct sugar puckers (%)	97.1	97.1
Good backbone conformations (%)	72.1	66.7