Supplementary Information

Dominant Rio1 kinase/ATPase catalytic mutant induces trapping of late pre-40S biogenesis factors in 80S-like ribosomes

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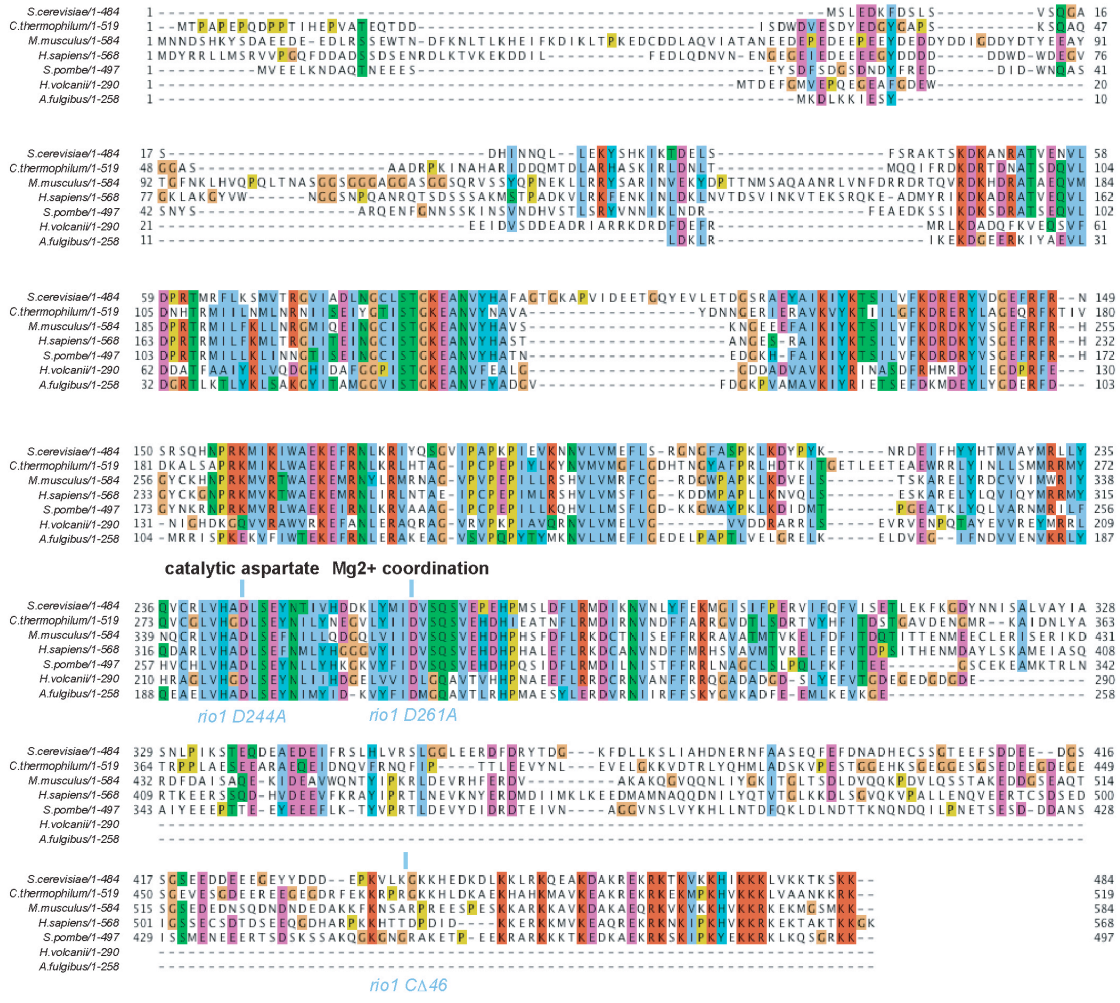
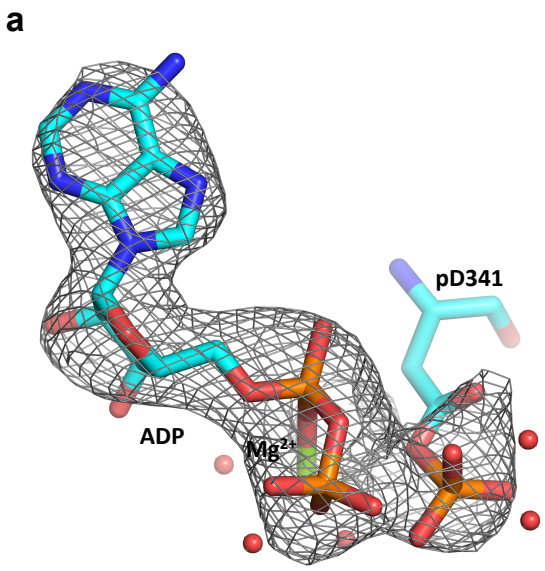
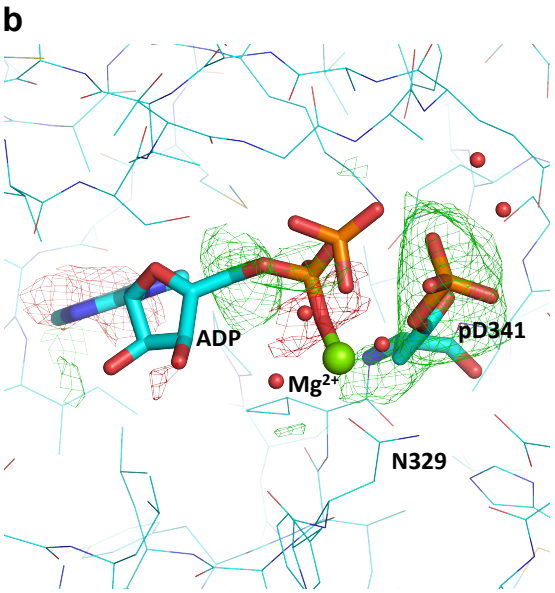
Supplementary Figure 1 & 2

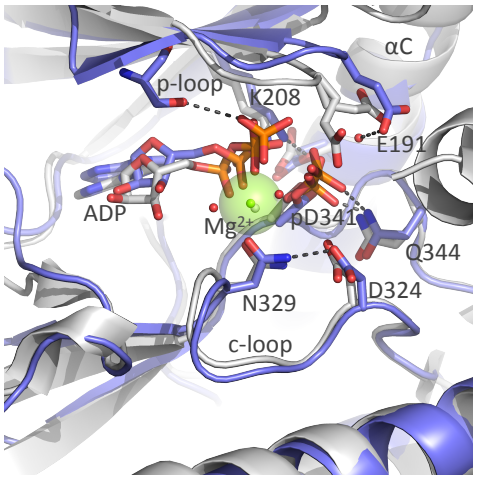
Supplementary Table 1 & 2



Supplementary Figure 1 | Multiple sequence alignment of eukaryotic Rio1 in comparison to archaeal Rio1 orthologs. Sequence alignment of eukaryotic *ct*Rio1 (*Chaetomium thermophilum;* XP_006692618.1), *sc*Rio1 (*Saccharomyces cerevisiae;* NP_014762.3), *mm*Rio1 (*Mus musculus;* NP_648489.1), *hs*Rio1 (*Homo sapiens;* NP_113668.2), *sp*Rio1 (*Saccharomyces pombe*; NP_593261.2) in comparison to archaeal *af*Rio2 (*Archaeoglobus fulgidus;* NP_070631.1) and *hv*Rio1 (*Haloferax volcanii;* YP_003534210.1) using T-Coffee multiple sequence alignment (http://www.ebi.ac.uk/Tools/msa/tcoffee) and Jalview. Yeast Rio1 point and deletion mutations are indicated in blue.







d

atom	ATP	ADP/PHD
O1B	107.76	106.11
O2B	112.82	107.90
O3B	137.14	109.47
PB	110.31	109.23
O1G/OP1	170.04	120.85
O2G/OP2	171.93	117.65
O3G/OP3	170.23	117.82
PG/P	169.66	109.92
AVG*	170.47	116.56

Supplementary Figure 2 | Electron density maps and fit comparisons for ADP/phosphoaspartate in the structure of human Rio1. a. Composite omit map calculated for the entire asymmetric unit, contoured at 3σ and carved at 3 Å around the ADP and the phosphate of pAsp 341. **b.** Difference density (Fo-Fc) calculated with omission of transferred phosphate only (green-positive; red-negative). Positive and negative density near ribose and base moieties may be due to slight repositioning of the nucleotide with refinement in the absence of the γ-phosphate. **c.** Superposition of the active sites of human Rio1 (blue) and *C. thermophilum* Rio2 (grey). Catalytic residues, including phosphoaspartate, are shown as sticks, and numbered according to hRio1 sequence. **d.** B-factors (in $Å^3$) for the β- and γ-phosphates (PB and PG respectively) when ATP is fit compared to the ADP β-phosphate and the pAsp phosphate (PB and P). * denotes average of the B-factors for the γ-phosphate of the ATP and the corresponding phosphate of the pAsp for comparison. Occupancies were fixed at 1.00 for all atoms. Lower B-factors, and shape of density, were used to determine the best fit ligand was ADP and phosphoaspartate.

Supplementary Table 1 | List of strains used in this study.

Strain	Genotype	Plasmid	Origin
BY4741/WT	Mat a; his3Δ; leu2Δ; met15Δ; ura3Δ		Euroscarf
Rio1Δ /RIO1 diploid	BY4743 Mat a/α; his3Δ/his3Δ; leu2Δ/leu2Δ; LYS2/ lys2Δ; MET15/met15Δ; ura3Δ/ura3Δ; YOR119c/ YOR119c::kanMX4		Euroscarf
RIO1 shuffle strain	Mat a; his3Δ; leu2Δ; lys2Δ; met15Δ; ura3Δ; YOR119c::kanMX4	Ycplac33-RIO1	this work: derived from *rio1Δ/RIO1* diploid
GAL::RIO1	Mat a; his3Δ; leu2Δ; met15Δ; ura3Δ; NatMX6::P_{GAL}-*3xHA-RIO1*		this work; derived from BY4741

Supplementary Table 2 | List of plasmids used in this study.

Plasmid	Features	Origin
Ycplac33-Rio1	*CEN*; URA3; *RIO1* ORF including 400nt before ATG and 220nt after stop codon	this study
P_{ADH1}-ProtA-Tev-Flag-Nob1	*CEN*; *LEU2*;	(1)
P_{ADH1}-ProtA-Tev-Flag-Rio1	*CEN*; *LEU2*;	this study derived from P_{ADH1}-Flag-Tev-ProtA-Nob1
P_{ADH1}-ProtA-Tev-Flag-*rio1* D244A	*CEN*; *LEU2*;	this study derived from P_{ADH1}-Flag-Tev-ProtA-Nob1
P_{ADH1}-ProtA-Tev-Flag-*rio1*CΔ46	*CEN*; *LEU2*;	this study derived from pADH1-Flag-Tev-ProtA-Nob1
P_{ADH1}-ProtA-Tev-Flag-*rio1*CΔ80	*CEN*; *LEU2*;	this study derived from P_{ADH1}-Flag-Tev-ProtA-Nob1
P_{ADH1}-Rio2-Flag-Tev-ProtA	*CEN*; *LEU2*;	this study derived from P_{ADH1}-Flag-Tev-ProtA-Nob1
pET24a-*ct*Rio1	Kanamycin	this study
pET24a-*ct*Rio1 D244A	Kanamycin	this study
pET24a-*ct*Rio1 D261A	Kanamycin	this study

pET24a-*ctRio1*CΔ80	Kanamycin	this study
pDEST527-*hs*Rio1(143-494)	Ampicillin	this study
YEP352-*GAL::RIO1*	2μ; URA3; *RIO1* ORF under *GAL* promoter	this study
YEP352-*GAL::RIO1 D244A*	2μ; URA3; *RIO1 D244A* ORF under *GAL* promoter	this study, derived from YEP352-*GAL::RIO1*
YEP352-*GAL::RIO1* CΔ46	2μ; URA3; *RIO1* CΔ46 ORF under *GAL* promoter	this study
YEP352-*GAL::RIO1 D244A* CΔ46	2μ; URA3; *RIO1 D244A* CΔ46 ORF under *GAL* promoter	this study
YEP352-*GAL::ProtA-TEV-Flag-RIO1*	2μ; URA3; *RIO1* ORF under *GAL* promoter	this study
YEP352-*GAL::ProtA-TEV-Flag-rio1 D244A*	2μ; URA3; *RIO1* ORF under *GAL* promoter	this study

1. Pertschy, B., Schneider, C., Gnadig, M., Schafer, T., Tollervey, D. and 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**, 35079-35091.