A single N¹-methyladenosine on the large ribosomal subunit rRNA impacts locally its structure and the translation of key metabolic enzymes

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Supplementary Figures



Figure S1 **Rrp8 is a highly-conserved protein among eukaryotes.** Amino acid sequence alignment of Rrp8 from S. cerevisiae (Sc), C. albicans (ca), S. pombe (Sp) and Homo sapiens (Hs). All multi-alignments were performed with the open-source software T-coffee (32). EsPript was used to visualize alignments.



Figure S2 **Loss of m¹A₆₄₅ does not influence the conformation of helix 72**. Representative gel showing structure probing with DMS or SHAPE analysis with NAI in the rrp8^{G209R} loss of methylation mutant. ³²P-labeled primer (helix72_StrPrb) complementary to nucleotides 2428 to 2448 of human 28S rRNA were used for the analysis.



Figure S3. Uncropped Western blots, shown in Fig 6C

Supplementary Tables

Strain	Genotype	Reference
CEN.PK2-1C	MATa; ura3-52; his3-∆1;	Entian, KD. & Kötter, P. Yeast Genetic Strain and
	8°: SUC2	Academic Press Ltd. 36. 629–666 (2007).
CEN.PK1265-	MATa; ura3-52; his3- Δ 1;	This study
4D	leu2-3,112; trp1-289; MAL2-	
	8c; SUC2;	
	rrp8(51,1050)::loxP-natMX4-	
CEN CD6-2A	10XP MATa: ura2-52: hic2-41:	Peifer C et al Veact Brngn a novel
CEN.CF 0-2A	1000000000000000000000000000000000000	methyltransferase responsible for m1A 645 base
	8c; SUC2;	modification of 25S rRNA. Nucleic acids research 41,
	rrp8(G682C)	1151–1163 (2013).
CEN.PK1174-	MATa; ura3-52; his3-∆1;	This study
16A	leu2-3,112; trp1-289; MAL2-	
	8'; SUC2; sol3(STOP -40)6xHA-	
	kanMX4	
CEN.MB678-	MATa; ura3-52; his3- Δ 1;	Peifer, C. et al. Yeast Rrp8p, a novel
2A	leu2-3,112; trp1-289; MAL2-	methyltransferase responsible for m1A 645 base
	8c; SUC2;	modification of 25S rRNA. <i>Nucleic acids research</i> 41 ,
		1151–1105 (2015).
CEN.PK1175-	MATa; ura3-52; his3- Δ 1;	This study (from crossing CEN.MB677-1C x
1C	leu2-3,112; trp1-289; MAL2-	CEN.PK1174-2B)
	8c; SUC2;	
	rrp8(788,895)::loxP-	
	sol3(STOP -40)··6xHA-	
	kanMX4	
CEN.DH37-4C	MATa; ura3-52; his3-∆1;	This study (from crossing (CEN.CP6-2C und
	leu2-3,	CEN.PK1174-2D)
	112; trp1-289; MAL2-8c;	
	SUC2; sol3(STOP -40)···6yHA-loyP-	
	KanMX-loxP rrp8(G682C)	
	BY4741; MATa; his3Δ1;	EUROSCARF
Y00000	leu2∆0; lys2∆0; ura3∆0	
	BY4741; MATa; ura3Δ0;	EUROSCARF
	leu2 Δ 0; his3 Δ 1; met15 Δ 0;	
Y04018	YDRU83W::kanMX4	

Table S1 S. cerevisiae strains used within this work

	BY4741; MATa; ura3∆0;	EUROSCARF
	leu2Δ0; his3Δ1; met15Δ0;	
Y02857	YHR163w::kanMX4	
	MATa; ura3Δ0; leu2Δ0;	This study (from crossing Y14018 x Y02857)
	his3∆1; met15∆0;	
	YHR163w::kanMX4	
BY.PK1177-1C	YDR083w::kanMX4	
	BY4741; MATa; ura3Δ0;	EUROSCARF
	leu2Δ0; his3Δ1; met15Δ0;	
Y01971	YNL241c::kanMX4	
	MATa; ura3Δ0; leu2Δ0;	This study (from crossing Y14018 x Y01971)
	his3Δ1; met15Δ0;	
	YNL241c::kanMX4	
BY.PK1184-9B	YDR083w::kanMX4	
	BY4741; MATa; ura3∆0;	EUROSCARF
	leu2∆0; his3∆1; met15∆0;	
Y06998	YGR248w::kanMX4	
	MATa; ura3Δ0; leu2Δ0;	This study (from crossing Y14018 x Y06998)
	his3∆1; met15∆0;	
BY.PK1186-	YGR248w::kanMX4	
12A	YDR083w::kanMX4	
	MATa; ura3∆0; leu2∆0;	This study
	his3∆1; met15∆0;	
BY.DH4-1C	YDR083w::natMX4	
	MATa; ura3∆0; leu2∆0;	This study (from crossing Y02857 x Y16998)
	his3∆1;	
	met15Δ0;YHR163w::kanMX4	
BY.PK1179-5D	YGR248w::kanMX4	
	MATa; ura3Δ0; leu2Δ0;	This study (from crossing BY.PK1179-5D X BY.DH4-
	his3∆1;	1C)
	met15∆0;YDR083w::natMX4	
	YHR163w::kanMX4	
BY.DH5-3B	YGR248w::kanMX4	

 Table S2 Plasmids used within this work

Plasmids	Reference	
рРК468	Peter Kötter, unpublished data	
pPK468-CaRRP8	this study	
pPK468-SpRRP8	this study	
pPK468-NML	this study	
pPK468-nml ^{G318R}	this study	

 Table S3 Oligonucleotides used within this work

name	sequence	application
RRP8-S1	TTAACGTAGAAGGTTGGTCTATTAAGACAAAAA	Deletion of ScRRP8-ORF
	CCGTCGCCAGCTGAAGCTTCGTACGC	
RRP8-S2	CAACTTCAATAAACTTTTGTCTTCTTTCCAATTTCT	Deletion of ScRRP8-ORF
	GCCTGCATAGGCCACTAGTGGATCTG	
CaRRP8_EcoRI	GGTGAATTCATGGTGTTATTTGAAGTTAAAGG	Construction of pPK468- CaRRP8
CaRRP8_Xhol	CTACTCGAGTTACCTCCTTTTATATATACAAGG	Construction of pPK468- CaRRP8
SpRRP8_Gap_fo	ACCAAGAACTTAGTTTCGAATAAACACACATAAA	Construction of pPK468-
r	CAAACGATGTTTAAAGTAGATTGGGAC	SpRRP8
SpRRP8_Gap_re	TATAAAAAGAAAATTTATTTAAATGCAAGATTTA	Construction of pPK468-
v	AAGTAGTTATCTTCTTTTGTAAATGC	SpRRP8
HsRRP8_Gap_fo	ACCAAGAACTTAGTTTCGAATAAACACACATAAA	Construction of pPK468-NML
r	CAAACGATGTTCGAAGAGCCTGAGTGG	
HsRRP8_Gap_re	TATAAAAAGAAAATTTATTTAAATGCAAGATTTA	Construction of pPK468-NML
v	AAGTAGTCACCTGCGCTTGTAGAGAC	
NML-G318R-1	CTAGTGGTGGCTGACTTCGGCTGTCGGGATTGC	Site-directed mutagenesis
	CGCTTGGCTTCAAGTATC	NML
NML-G318R-2	GATACTTGAAGCCAAGCGGCAATCCCGACAGCC	Site-directed mutagenesis
	GAAGTCAGCCACCACTAG	NML
SOL3-6HA-1	TAATGAGGAAGCTTTTGGAAAAGTTCAAACGAA	C-terminal tagging of ScSOL3
	AACTTTTCGTACGCTGCAGGTCGAC	
SOL3-6HA-2	CTATGTCATTTTATAAAGTTTGGTATATATAACAC	C-terminal tagging of ScSOL3
	GTAATATCGATGAATTCGAGCTCG	
Oligo645	CACTCGCATAGACGTTAGACTCCTTGGTCCGTGT	Mungbean Oligo
	TTCAAGACGGGCGG	
ITS1(A2-A3)	GATTGCTGCAATGCCCAAAG	Northern hybridization
ITS2 (C1-C2)	TTGTTCGCCTAGACGCTCTC	Northern hybridization
ITS2 (E-C2)	CACTCACTACCAAACAGAAT	Northern hybridization
helix25.1_StrPr	TCACTTTCATTACGCGTATG	Primer extension
b		
Str_Prb_h25b	TCCGTGTTTCAAGACGGGCG	Primer extension
helix72_StrPrb	CTATGTCTCTTCACAATGTC	Primer extension

m ¹ A ₁₃₂₂	ACGGCGGCTTTCGTGCGAGC	Primer extension
166	AGAGGAGGCAAAUAGAGUAtt	siRNA for NML depletion
167	GAAGGGUAGUACUACAAAUtt	siRNA for NML depletion
168	CGGCAAAAGAAUAAGAGAAtt	siRNA for NML depletion
ACT1-RT-fwd	TGAAGTGTGATGTCGATGTCCG	RT-qPCR oligo
ACT1-RT-rev	CGATAGATGGACCACTTTCGTCG	RT-qPCR oligo
ASC1-RT-fwd	GATCTCCTGGAAGTTGACTGGTG	RT-qPCR oligo
ASC1-RT-rev	CCAGAGATAATCATGGAAGCCTTC	RT-qPCR oligo
RPS5-RT-fwd	GCTGGTCGTTACGCCAACAAG	RT-qPCR oligo
RPS5-RT-rev	GACAGCTTGACGTCTAGCAGC	RT-qPCR oligo
RPS10A-RT-fwd	GGGTTACGTCAAGACTCA	RT-qPCR oligo
RPS10A-RT-rev	GGTACCTGGAACAATGTG	RT-qPCR oligo
ENO2-RT-fwd	TGGGTGCTAACGCTATCTTG	RT-qPCR oligo
ENO2-RT-rev	AGCACCGTATCTCTTCTTGG	RT-qPCR oligo
PGK1-RT-fwd	GCTCACAGAGCTCACTCTTC	RT-qPCR oligo
PGK1-RT-rev	CCTTGGCACCACCTAAGATG	RT-qPCR oligo
SOL3-RT-fwd	GAGGGCTAGTTTGACCCATC	RT-qPCR oligo
SOL3-RT-rev	GTCCGTCAGTGGCACAATTC	RT-qPCR oligo