Supplemental Material

Supplementary Figures:

Figure S1

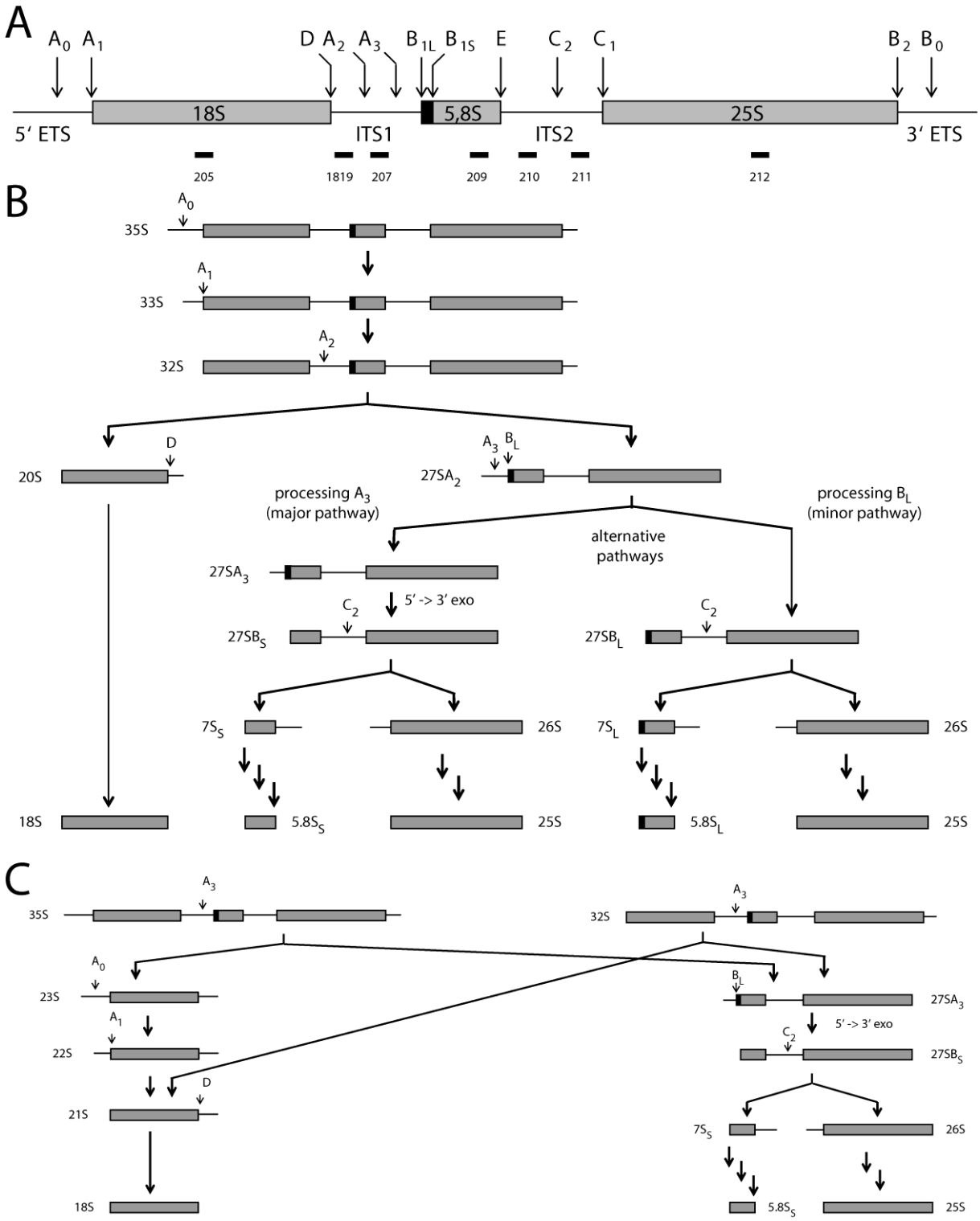

Fig. S1 rRNA processing in *S. cerevisiae*

A) The 35S rRNA gene contains the sequences of 18S, 5.8S and 25S rRNAs separated by two internal transcribed spacer (ITS1, ITS2) regions and flanked by external transcribed spacer regions (5'-ETS, 3'-ETS). Processing sites of precursor rRNAs are indicated (A0, A1, etc.). Positions of antisense oligo probes (o205, etc.) used for Northern hybridisation and primer extension analyses are indicated with bars.

B) Canonical pre-rRNA processing pathways

Processing of the 35S pre-rRNA at sites A0 and A1 generates 32S rRNA, which contains the 5' end of the 18S rRNA. Subsequent cleavage at site A2 separates 20S pre-rRNA from 27SA2 pre-rRNA, which further on maturate independently. The 27SA2 pre-rRNA is processed via two mutually exclusive pathways. Processing at site A3 and subsequent 5'-> 3' exonucleolytic trimming to site $B1_S$ yields the $27SB_S$ pre-rRNA, having the 5' end of the major $5.8S_S$ rRNA. Alternatively, processing at site $B1_L$ yields $27SB_L$ pre-rRNA, having the 5' end of the minor $5.8S_L$ rRNA. Processing of $27SB_L$/$27SB_S$ pre-rRNAs at site C2 separates the precursors of 25S and $5.8S_L$ or $5.8S_S$, respectively, which are subsequently converted into the mature species by exonucleolytic trimming. Processing at site D converts the 20S precursor into 18S rRNA.

C) Alternative pre-rRNA processing pathways bypassing A2-site processing

In mutants, in which processing at site A2 is impaired, but also to a low extent in normal yeast cells, separation of the precursors of small subunit and large subunit components can occur by processing 35S or 32S pre-rRNAs at site A3, resulting in 27SA3 and 23S or 21S pre-rRNAs, respectively. Processing at sites A0 and A1 converts 23S into 21S pre-rRNA which can possibly be processed at site D to yield 18S rRNA.

Figure S2

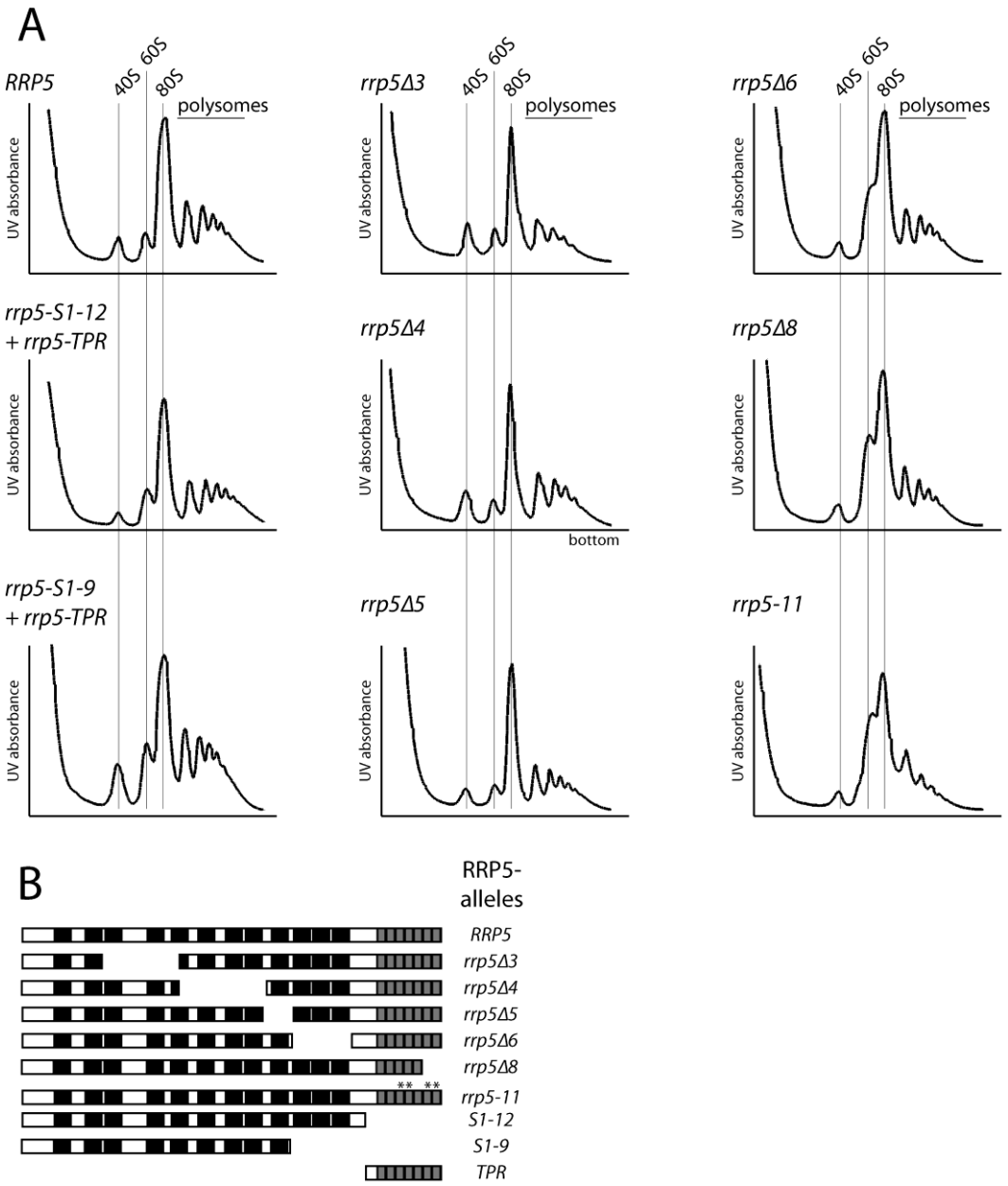

Fig. S2 Polysome gradients of *RRP5* mutant strains

A) An *RRP5* deletion strain (Y3762) was complemented with the indicated *RRP5* alleles (see panel B) expressed from the respective pJV314-ProtA plasmids (see Fig. S4) and grown in YPD at 30°C to OD 0.5 –0.8. Corresponding cell lysates were applied to 10-50% sucrose gradients, centrifuged for 16h at 27 000rpm and UV profiles were recorded at 254. The peaks of free 40S and 60S ribosomal subunits, 80S monosomes and polysomes are indicated. B) Overview of the *rrp5* alleles analysed in (A) and schematic presentation of the corresponding protein variants. Black bars illustrate the S1 RNA binding motifs, grey bars the tetratricopeptide repeats, respectively (adapted from (7)). Point mutations in the temperature sensitive rrp5-11 allele are indicated (*).

Figure S3

name	TY	genotype	origin
BSY420	1	mat A, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1	sup. reference (2)
BY4741	206	mat A, his31, leu20, met150, ura30	Euroscarf
BSY420 NOC1-TAP	483	mat A, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1:: NOC1-TAP-TRP1	this study, derivate of TY1
CG379	543	mat alpha, ade5-1, his7-2, leu2-3,-112, trp1-289, ura3-52	sup. reference (3)
CG379 NOC2-TAP	577	mat alpha, ade5, his7-2, leu2-112, trp1-289, ura3-52, noc2:: NOC2-TAP-TRP1	this study, derivate of TY543
RRP5-TAP	615	mat alpha, ADE2, ADE3, his3, leu2, trp1, ura3, rrp5::RRP5-TAP-TRP1	this study
RRP5 shuffle (YJV148)	616	mat A, ade2, his3, leu2, trp1, ura3, rrp5::HIS3, pURA3 RRP5 (URA3)	sup. reference (4)
NOC1-Shuffle	772	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1::HIS3, YCplac33-pNOC1-NOC1 (URA3)	sup. reference (2)
NOC2-Shuffle	773	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc2::HIS3, YCplac33-pNOC2-NOC2 (URA3)	sup. reference (2)
pGAL-NOC1	775	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1::HIS3, YCplac22-pGAL-NOC1 (TRP1)	this study, derivate of TY772
CG379 UTP4-TAP	1540	mat alpha, ade5-1, his7-2, leu2-3,-112, trp1-289, ura3-52, utp4::UTP4-TAP-URA3	this study, derivate of TY543
CG379 NOG2-TAP	1965	mat alpha, ade5-1, his7-2, leu2-3,-112, trp1-289, ura3-52, nog2::NOG2-TAP-URA3	this study, derivate of TY543
pGAL-RRP5	2299	mat A, ade2, his3, leu2, trp1, ura3, rrp5::HIS3, YCplac22-pGAL-RRP5 (TRP1)	this study, derivate of TY616
pGAL-RRP5 NOC2-TAP	2301	mat A, ade2, his3, leu2, trp1, ura3, rrp5::HIS3, noc2::NOC2-TAP-URA3, YCplac22-pGAL-RRP5 (TRP1)	this study, derivate of TY2299
RRP5 shuffle NOC1-TAP	2302	mat A, ade2, his3, leu2, trp1, ura3, rrp5::HIS3, noc1::NOC1-TAP-TRP1, pURA3 RRP5 (URA3)	this study, derivate of TY616
RRP5 shuffle NOC2-TAP	2303	mat A, ade2, his3, leu2, trp1, ura3, rrp5::HIS3, noc2::NOC2-TAP-TRP1, pURA3 RRP5 (URA3)	this study, derivate of TY616
pGAL-RRP5 NOC1-TAP	2343	mat A, ade2, his3, leu2, trp1, ura3, rrp5::HIS3, noc1::NOC1-TAP-URA3, YCplac22-pGAL-RRP5 (TRP1)	this study, derivate of TY2299
NOC1-shuffle UTP22-TAP	2417	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1::HIS3, utp22::UTP22-TAP-kanMX, YCplac33-pNOC1-NOC1 (URA3)	this study, derivate of TY772
pGAL-NOC1 UTP22-TAP	2418	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1::HIS3, utp22::UTP22-TAP-kanMX, YCplac22-pGAL-NOC1 (TRP1)	this study, derivate of TY775
BY4741 Rpb2-TEV-ProtA	2424	mat A, his31 leu20 met150 ura30 rpb2::RPB2-TEV-ProtA-kanMX6	this study, derivate of TY206
BY4741 A135-TEV-ProtA	2423	mat A, his31 leu20 met150 ura30 rpa135::RPA135-TEV-ProtA-kanMX6	this study, derivate of TY206
NOC1-Shuffle RRP5-TAP	2499	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1::HIS3, rrp5::RRP5-TAP-kanMX, YCplac33-pNOC1-NOC1 (URA3)	this study, derivate of TY772
NOC2-Shuffle RRP5-TAP	2500	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc2::HIS3, rrp5::RRP5-TAP-kanMX, YCplac33-pNOC2-NOC2 (URA3)	this study, derivate of TY773
pGAL-NOC1 RRP5-TAP	2501	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc1::HIS3, rrp5::RRP5-TAP-kanMX, YCplac22-pGAL-NOC1 (TRP1)	this study, derivate of TY2499
pGAL-NOC2 RRP5-TAP	2502	mat alpha, ade2-1, can1-100, his3200, leu2-3,112, trp1-1, ura3-1, noc2::HIS3, rrp5::RRP5-TAP-kanMX, YCplac22-pGAL-NOC2 (TRP1)	this study, derivate of TY2500
Noc1-TAP	Y3572	Mat alpha, ADE2, ADE3, leu2, trp1, his3, ura3, noc1::NOC1-TAP-HIS3	This study
Noc1, Rrp5 shuffle	Y3668	Mat alpha, ade2, his3, leu2, trp1, ura3, noc1::HIS3, rrp5::HIS3, pURA3-RRP5, YCplac33-NOC1	This study
Noc3, Rrp5 shuffle	Y3715	ade2, his3, leu2, trp1, ura3, noc3::HIS3, rrp5::HIS3, pURA3 RRP5, YCplac33 Noc3	This study
Noc1-TAP, Rrp5 shuffle	Y3716	ade2, his3, leu2, trp1, ura3, rrp5::HIS3, noc1::NOC1-TAP-natNT2, pURA3-RRP5	This study
Rrp5 shuffle	Y3762	Mat alpha, ADE2, ADE3, LYS2, his3, leu2, trp1, ura3, rrp5::HIS3, pURA3-RRP5	This study
Noc2, Rrp5 shuffle	Y4207	MAT a, LYS2, ADE2, ADE3,ura3, his3, trp1, leu2, noc2::KanMX, rrp5::HIS3, pURA3-RRP5, pRS316-NOC2	This study

Fig. S3 Yeast strains used in this study

Figure S4

name	TK	features	origin	generation
YCplac22GAL-NOC2	11	CEN4 ARS1 TRP1 pGAL-NOC2	this study	yeast NOC2 was amplified by PCR using oligos o56 and o57 and cloned into V48 via SalI/PstI
YCplac22GAL-NOC1	37	CEN4 ARS1 TRP1 pGAL-NOC1	this study	yeast NOC1 was amplified by PCR using oligos o34 and o39 and cloned into V48 via XbaI/HindIII
pUCDM	1127	Ori-R6Kg ChlR loxP p10 polh	sup. reference (5)	
pSPL	1129	Ori-R6Kg SpecR loxP p10 polh	sup. reference (6)	
pFL	1130	Ori-ColE1 AmpR GentR loxP Tn7L/R p10 polh	sup. reference (6)	
pSPL-6xHis	1211	Ori-R6Kg SpecR loxP p10 polh	this study	Primer annealing of oligos o1966 and o1967, cloning into TK1129 via BamHI/SalI
pFL-Flag-TEV	1212	Ori-ColE1 AmpR GentR loxP Tn7L/R p10 polh	this study	Primer annealing of oligos o1964 and o1965, cloning into TK1130 via BamHI/SalI
pFL-Flag-TEV-Noc2	1213	Ori-ColE1 AmpR GentR loxP Tn7L/R p10 polh-NOC2	this study	yeast NOC2 was amplified by PCR using oligos o1968 and o1969 and cloned into TK1212 via SalI/PstI
pUCDM-Noc1	1230	Ori-R6Kg ChlR loxP p10 polh-NOC1	this study	yeast NOC1 was amplified by PCR using oligos o1974 and o1975 and cloned into TK1127 via SalI/XbaI
pFL-Flag-TEV-Noc2-pUCDM-Noc1	1232	Ori-ColE1 Ori-R6Kg AmpR GentR ChlR loxP Tn7L/R p10 polh-NOC2, polh-NOC1	this study	in vitro cre-lox recombination of plasmid TK1230 and TK1213
pSPL-6xHis-Rrp5	1259	Ori-R6Kg SpecR loxP p10 polh-RRP5	this study	yeast RRP5 was amplified by PCR using oligos o1972 and o1973 and cloned into TK1211 via BamHI/PstI
pFL-Flag-TEV-Noc1	1476	Ori-ColE1 AmpR GentR loxP Tn7L/R p10 polh-NOC1	this study	yeast NOC1 subcloned from TK1230 into TK1212 via SalI/XbaI
pMA-RQ-3xHA-Thrombin	1500	Ori-ColE1 AmpR	Geneart	gene synthesis by Geneart
pSPL-3xHA-ThrombinSS	1502	Ori-R6Kg SpecR loxP p10 polh	this study	sequence encoding START-codon-3xHA-tag-Thrombin-recognition-site subcloned from TK1500 (BglII/PstI) in TK1129 (BamHI/PstI)
pSPL-3xHA-Rrp5	1503	Ori-R6Kg SpecR loxP p10 polh-RRP5	this study	yeast RRP5 subcloned from TK1259 into TK1502 via BamHI/PstI
pFL-Flag-TEV-Noc2-pSPL-3xHA-Rrp5-pUCDM-Noc1	1504	Ori-ColE1 Ori-R6Kg AmpR GentR ChlR SpecR loxP Tn7L/R p10 polh-NOC1, polh-NOC2, polh-RRP5	this study	in vitro cre-lox recombination of plasmid TK1230, TK1213 and TK1503
YCplac22GAL-RRP5	1517	pGAL-RRP5 ARS1 CEN4 TRP1	this study	yeast RRP5 subcloned from TK1503 into V48 via BamHI/PstI
pFL-Flag-TEV-NOC1-pSPL-3xHA-RRP5	1658	Ori-ColE1 Ori-R6Kg AmpR GentR SpecR loxP Tn7L/R p10 polh-NOC1, polh-RRP5	this study	in vitro cre-lox recombination of plasmid TK1476 and TK1503
pFL-Flag-TEV-NOC2-pSPL-3xHA-RRP5	1677	Ori-ColE1 Ori-R6Kg AmpR GentR SpecR loxP Tn7L/R p10 polh-NOC2, polh-RRP5	this study	in vitro cre-lox recombination of plasmid TK1212 and TK1503
pSPL-3xHA-rrp5-S1-9+N	1698	Ori-R6Kg SpecR loxP p10 polh-rrp5-S1-9	this study	truncated allele of yeast RRP5 was generated by PCR using oligos o1972 and o2901 and cloned into TK1502 via BamHI/PstI
pSPL-3xHA-rrp5-S1-6+N	1699	Ori-R6Kg SpecR loxP p10 polh-rrp5-S1-6	this study	truncated allele of yeast RRP5 was generated by PCR using oligos o1972 and o2900 and cloned into TK1502 via BamHI/PstI
pSPL-3xHA-rrp5-S1-6	1700	Ori-R6Kg SpecR loxP p10 polh-rrp5-S1-6-dN	this study	truncated allele of yeast RRP5 was generated by PCR using oligos o2897 and o2900 and cloned into TK1502 via BamHI/PstI
pSPL-3xHA-rrp5-S10-TPR	1703	Ori-R6Kg SpecR loxP p10 polh-rrp5-S10-TPR	this study	truncated allele of yeast RRP5 was generated by PCR using oligos o2905 and o1973 and cloned into TK1502 via BamHI/PstI
pSPL-3xHA-rrp5-S1-9+N-pFL-Flag-NOC1	1727	Ori-ColE1 Ori-R6Kg AmpR GentR SpecR loxP Tn7L/R p10 polh-NOC1, polh-rrp5-S1-9	this study	in vitro cre-lox recombination of plasmid TK1476 and TK1698
pSPL-3xHA-rrp5-S1-6+N-pFL-Flag-NOC1	1728	Ori-ColE1 Ori-R6Kg AmpR GentR SpecR loxP Tn7L/R p10 polh-NOC1, polh-rrp5-S1-6	this study	in vitro cre-lox recombination of plasmid TK1476 and TK1699
pSPL-3xHA-rrp5-S1-6-pFL-Flag-NOC1	1729	Ori-ColE1 Ori-R6Kg AmpR GentR SpecR loxP Tn7L/R p10 polh-NOC1, polh-rrp5-S1-6-dN	this study	in vitro cre-lox recombination of plasmid TK1476 and TK1700
pSPL-3xHA-rrp5-S10-TPR-pFL-Flag-NOC1	1732	Ori-ColE1 Ori-R6Kg AmpR GentR SpecR loxP Tn7L/R p10 polh-NOC1, polh-rrp5-S10-TPR	this study	in vitro cre-lox recombination of plasmid TK1476 and TK1703
YCplac22GAL	V48	CEN4 ARS1 TRP1 pGAL1/10	gift from Markus Künzler	GAL1/10 sequence cloned into YCplac22 via BamHI/EcoRI
pURA3-RRP5		CEN6 ARSH4 URA3 pRRP5-RRP5	sup. reference (4)	
pUN100 Noc2		CEN4 ARS1 LEU2 pNOC2-NOC2	sup. reference (2)	
pRS315 noc2-1		CEN6 ARSH4 LEU2 pNOC2-noc2-1	this study	yeast noc2-1 allele including promotor (-450bp) and terminantor (+350bp) region was amplified by PCR and cloned into pRS315 via SacI/XhoI
pNOPPA1L NOC3		CEN6 ARSH4 LEU2 pNOP1-PA-NOC3	this study	internal BglII fragment of non complementing pNOPPA1L-NOC3 (Milkereit et al., 2001) was replaced by BglII Fragment of YEP13 NOC3 (Baßler et al, 2006)
pNOPPA1L noc3-1		CEN6 ARSH4 LEU2 pNOP1-PA-noc3-1	sup. reference (2)	
pNOPPA1L noc3-2		CEN6 ARSH4 LEU2 pNOP1-PA-noc3-2	O.Gadal unpublished	generated analogously as pNOPPA1L-noc3-1 (Milkereit et al, 2001)
pNOPPA1L NOC1		CEN6 ARSH4 LEU2 pNOP1-PA-NOC1	sup. reference (2)	
pNOPPA1L noc1-1		CEN6 ARSH4 LEU2 pNOP1-PA-noc1-1	sup. reference (2)	
pNOPPA1L noc1-3		CEN6 ARSH4 LEU2 pNOP1-PA-noc1-3	O. Gadal unpublished	generated analogously as pNOPPA1L-noc1-1 (Milkereit et al, 2001)
pJV314 protA RRP5		CEN6 ARSH4 TRP1 pRRP5-PA-RRP5	sup. reference (4)	
pJV314 protA rrp5-11		CEN6 ARSH4 TRP1 pRRP5-PA-rrp5-11	sup. reference (7)	
pJV314 protA rrp5Δ3		CEN6 ARSH4 TRP1 pRRP5-PA-rrp5-Δ3	sup. reference (7)	
pJV314 protA rrp5Δ4		CEN6 ARSH4 TRP1 pRRP5-PA-rrp5-Δ4	sup. reference (7)	
pJV314 protA rrp5Δ5		CEN6 ARSH4 TRP1 pRRP5-PA-rrp5-Δ5	sup. reference (7)	
pJV314 protA rrp5Δ6		CEN6 ARSH4 TRP1 pRRP5-PA-rrp5-Δ6	sup. reference (7)	
pJV314 protA rrp5Δ8		CEN6 ARSH4 TRP1 pRRP5-PA-rrp5-Δ8	sup. reference (7)	
pRS315 RRP5-GFP		CEN6 ARSH4 LEU2 pRRP5-RRP5-GFP	this study	yeast RRP5 promotor was amplified by PCR and cloned into pRS315 Rpl25-GFP (Gadal et al, 2001) via NotI/PstI; subsequently, yeast RRP5 without stop codon was amplified by PCR and inserted via PstI/BamHI
pRS315 rrp5 S10-TPR-GFP		CEN6 ARSH4 LEU2 pRRP5-rrp5-S10-TPR-GFP	this study	yeast RRP5 promotor was amplified by PCR and cloned into pRS315 Rpl25-GFP (Gadal et al, 2001) via NotI/PstI; subsequently, yeast rrp5-S10-TPR without stop codon was amplified by PCR adding a start codon and inserted via PstI/BamHI
pRS314 rrp5 S1-9-GFP		CEN6 ARSH4 TRP1 pRRP5-rrp5-S1-9-GFP	this study	yeast RRP5 promotor was amplified by PCR and cloned into pRS314 Rpl25-GFP (Gadal et al, 2001) via NotI/PstI; subsequently, yeast rrp5-S1-9 codon was amplified by PCR and inserted via PstI/BamHI
YCplac33 NOC1		CEN4 ARS1 URA3 pNOC1-NOC1	sup. reference (2)	
pRS316 NOC2		CEN6 ARSH4 URA3 pNOC2-NOC2	O.Gadal unpublished	yeast NOC2 including promotor and terminantor region was amplified by PCR and cloned into pRS316 via PstI/SacI
YCplac33 NOC3		CEN4 ARS1 URA3 pNOC3-NOC3	sup. reference (2)	

Tn7L/R: Tn7 transposition sequences; p10, polh: baculoviral promoters; loxP: Cre-lox recombination site; Ori-ColE1, Ori-R6Kg: bacterial replication origins;
AmpR, GentR, ChlR, SpecR: resistance marker for Ampicillin, Gentamycin, Chloramphenical, Spectinomycin

Fig. S4 Plasmids used in this study

Figure S5

Primer for tagging

oligo	sequence
177	ATCTGCCGACGATTATGCTCAATATTTAGATCAAGATTCAGACTCCATGGAAAAGAGAAG
178	TAATTTACAACACCGAAGTGTTTAGTTAATGTATTATTATTTTTACGACTCACTATAGGG
287	GCTACTGAGTATGTCGCTAGCCATGAATCTCAAAAAGCAGACGAACGTACGCTGCAGGTCGAC
288	AACTTAGCCATTTATATTACTTTACAGTTAAAAATCCATCAGGAAATCGATGAATTCGAGCTCG
621	AAGTGATGATGACAACGAAGATGTTGAAATGTCAGACGCT TCCATGGAAAAGAGAAG
622	CTATTGAATTCAAGACAAAAAATCAAATCTTGCTGAGTTGTACGACTCACTATAGGG
1813	ACTTTTCACTCCAAACAAAAGGCGTTTATTCAACCAAAGTTAGTGTTTTCCATGGAAAAGAGAAG
1814	GCCTTTTAATAGCATCTCTCTATTCTTCGGTATGTTGACTTAAATTAATACGACTCACTATAGGG
2161	AGGAAGGGGAAGAAAAACCAAAGAAGAAAGAAGTTGAGAAGACGGCATCCATGGAAAAGAGAAG
2162	TCACTTGTAATCTTAAATTAATATTATACACCGGTTGTCCGTTTTTACCTTACGACTCACTATAGGG
2261	GAGATTGCTGCATTCGGGAATGACATGGTTATAAATTTTGAGACAGATTCCATGGAAAAGAGAAG
2262	TTTAATATTATACAGATACTTCTAAAAGTTATGATTTTGTTGTTTATTTACGACTCACTATAGGG
2952	ATGAGATTGCTGCATTCGGGAATGACATGGTTATAAATTTTGAGACAGATCGTACGCTGCAGGTCGAC
2953	GCTGCTTTAATTATTTAATATTATACAGATACTTCTAAAAGTTATGATTTTGTTGTTTATT ATCGATGAATTCGAGCTCG
3179	GATGGTACCCTGAGCTATCCGCAATGGGTATAAGATTGCGTTATAATGTAGAGCCCAAACGTACGCTGCAGGTCGAC
3180	CTACCGCGGCCAAGCCTTCATTTACCATTCTATATCAATTTGGAAAGAAGGGTATTTCTATCGATGAATTCGAGCTCG
3225	ATGAACATTACACCACGTTTATATACCGATCGTTCGAGAGATTTT CGT ACG CTG CAG GTC GAC
3226	AATGTTTTTTATTATTTTACTTTCTTAGAGTTACAACATTATTTC ATC GAT GAA TTC GAG CTC G

Primer for cloning

oligo	sequence
34	TTTTTTAAGCTTTTGTCGCTACAAATGTGCCTATAT
39	TTTTTTTCTAGAATGAGTGAGAACAACGGCAATCCGC
56	TTTTTTCTGCAGGAAATCATATATCTTGTACTAAATTTTGCG
57	TTTTTTGTCGACATGGGTAAAGTTTCTAAATCGACCAAG
1964	TCGACGAGGGATCCGGCGCCCTGAAAATACAGGTTTTCCAGGATCTTATCGTCGTCATCCTTGTAATCCATA
1965	GATCTATGGATTACAAGGATGACGACGATAAGATCCTGGAAAACCTGTATTTTCAGGGCGCCGGATCCCTCG
1966	GATCTATGCATCACCATCACCATCACGATTACGGATCCCTCG
1967	TCGACGAGGGATCCGTAATCGTGATGGTGATGGTGATGCATA
1968	TTTTTTGTCGACATGGGTAAAGTTTCTAAATC
1969	TTTTTTCTGCAGTTAAGCGTCTGACATTTCAA
1972	TTTTTTGGATCCATGGTAGCTTCCACCAAAAG
1973	TTTTTTCTGCAGTTATTCGTCTGCTTTTTGAG
1974	TTTTTTGTCGACATGAGTGAGAACAACGGCAA
1975	TTTTTTTCTAGATTAGTCTGAATCTTGATCTA
2897	TTTTTTTT GGATCC ATG CTAATTGAACATGTCAACTTTAAAACG
2900	TTTTTTTT CTGCAG TTA TTTAGCGTCTTTAATCAGAGAAC
2901	TTTTTTTT CTGCAG TTA AACTTTAATTGTGGACATATTTATGTC
2905	TTTTTTTT GGATCC ATG TCCACAATTAAAGTTGGTGATGAA
2906	TTTTTTTT GGATCC ATG ACTGTGGATCAACTGGAAAAG

Primer for qPCR:

oligo	sequence	amplicon
613	CATGATCAGATGGGGCTTGA	9
614	ACCGGTGGTAGCGACTCTGT	
710	TGGAGCAAAGAAATCACCGC	7
711	CCGCTGGATTATGGCTGAAC	
712	GAGTCCTTGTGGCTCTTGGC	3
713	AATACTGATGCCCCCGACC	
920	GCCATATCTACCAGAAAGCACC	8
921	GATTGCAGCACCTGAGTTTCG	
969	TCATGGAGTACAAGTGTGAGGA	1
970	TAACGAACGACAAGCCTACTC	
2011	CTTGGATGTGGTAGCCGTTT	2
2012	TCGACCCTTTGGAAGAGATG	
2429	AAAGAAGACCCTGTTGAGCTTGA	6
2430	GTATTTCACTGGCGCCGAA	
2481	GGTGGTAAATTCCATCTAAAGCTAAATATT	5
2482	CACGTACTTTTTCACTCTCTTTTCAAA	
2864	GCATGCCTGTTTGAGCGTC	4
2865	CGACCGTACTTGCATTATACC	

Probes for Northern Blot:

oligo	sequence
202	CCAACTTGTCAGACTGCCATT
205	CATGGCTTAATCTTTGAGAC
207	TGTTACCTCTGGGCCC
209	TTTCGCTGCGTTCTTCATC
210	GGCCAGCAATTTCAAGTTA
211	GAACATTGTTCGCCTAGA
212	CTCCGCTTATTGATATGC
1819	GTAAAAGCTCTCATGCTCTTGCC
2474	TTAACTACAGTTGATCGG

Fig. S5 Oligonucleotides used in this study

Figure S6

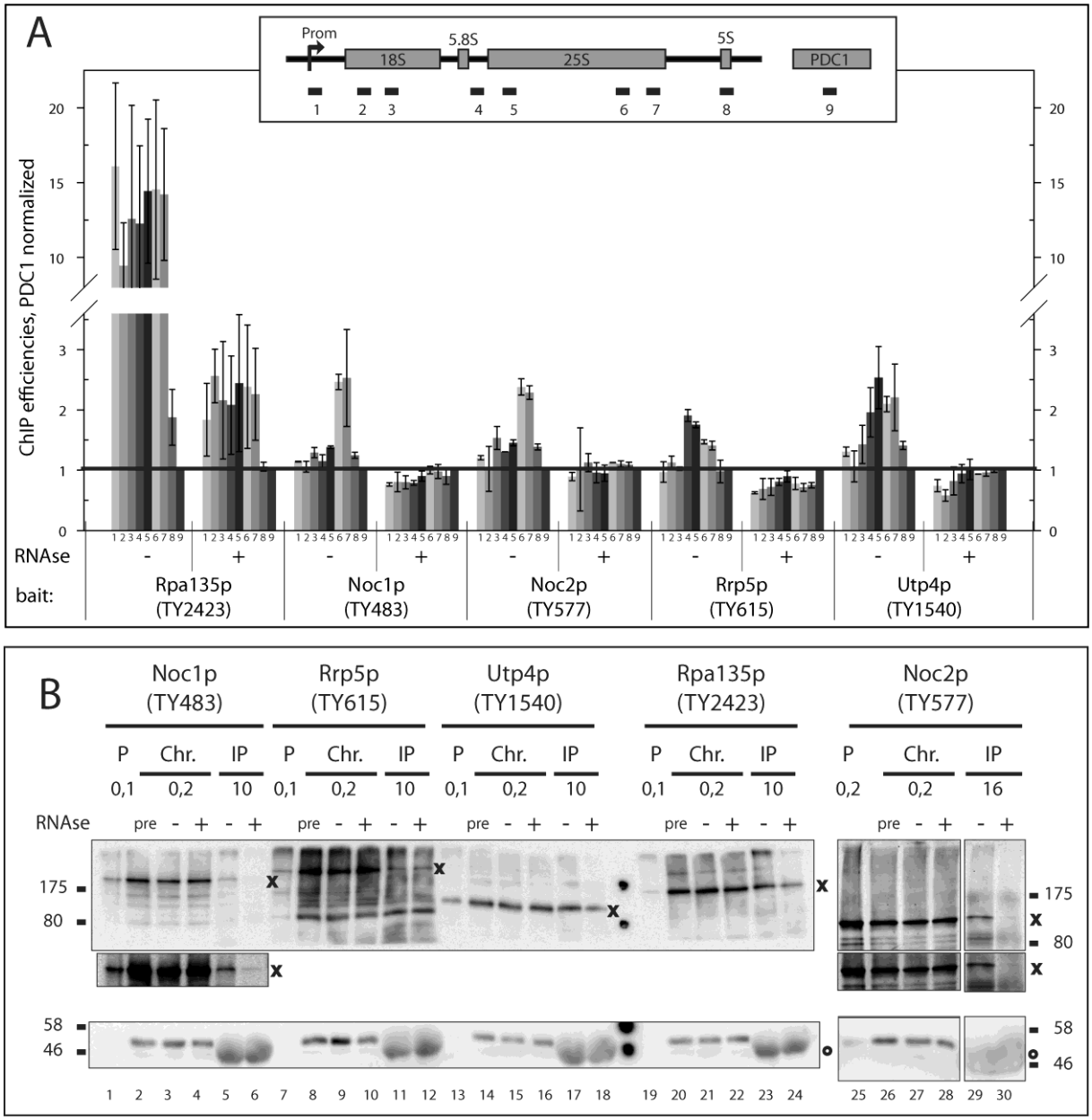

Fig. S6: Comparison of co-transcriptional recruitment of ribosome biogenesis factors and Pol-I in the presence and absence of RNAse treatment using ChIP

A) ChIP experiments using the same primer pairs as described in Figure 7 with and without RNAse A and T1 treatment before immunoprecipitation were performed as described in the supplementary methods. The amounts of specific DNA fragments present in the input and retained on the beads were determined by qPCR. In each experiment the precipitation efficiencies (% IP (rDNA)) for the respective amplified DNA regions were calculated and normalised to the PDC1 precipitation efficiencies (% IP (rDNA) / % IP (PDC1)). The graph shows the average of two biological replicates including standard deviations. A black line depicts the internal background as a result of the normalisation to the precipitated PDC1 DNA.

B) Yield of immunoprecipitated Pol-I and biogenesis factors in ChIP experiments without and with RNase treatment using Western blotting. Relative amounts to the chromatin input per IP of the insoluble material after sonication (P), the soluble chromatin fraction (Chr) without (pre) or after incubation at 25°C with (+) or without (-) RNAses, and the precipitated material (IP) from RNAse treated (+) or untreated (-) chromatin are indicated. Upper panel: The TAP-tag fusion proteins were detected with PAP detection reagent (Sigma, P1291) (left) or with anti-ProtA antibody (P3775, Sigma-Aldrich) and a fluorophor coupled secondary antibody (LICOR, 926-32211) (right; TY577). The bands of the TAP-tagged proteins are marked (x). Insets show a digitally enhanced view of the same blot. Lower panel: Tubulin was detected as a loading control. Rabbit IgG chains detected in the IP lanes by cross reaction of the secondary antibody are indicated (°).

Comments:

In two independent experiments, a significant amount of Pol-I was still associated with the Pol-I promoter and the 35S rDNA after RNase treatment of the chromatin fraction. In contrast, neither Noc1p, Noc2p, Rrp5p nor Utp4p were more enriched at the Pol-I-transcribed rDNA locus than at the 5S or PDC1 genes (background control) after RNase treatment, indicating that their rDNA association is mediated through interaction with RNA. Surprisingly, the absolute amount of rDNA associated Pol-I dropped also after RNase treatment. This could be due to formation of Pol-I - rDNA aggregates after RNase treatment, which are not accessible anymore for immunoprecipitation. We note that for unknown reasons also purification efficiencies of tagged Noc1p and Noc2p were reproducibly reduced after RNAse treatment of the chromatin fraction.

Proteome analysis of Pol-I associated chromatin (according to Fig. 6), which was isolated including or not treatment with RNAse, supported the assumption that a subgroup of ribosome biogenesis factors is associated with rDNA chromatin in an RNA dependent manner. The average iTRAQ ratio for Pol-I peptides was 1.13, for putative contaminants like translation factors, ribosomal proteins or heat shock proteins 1.23, and for peptides of SSU processome components and early LSU biogenesis factors 1.39, when Pol-I associated chromatin from mock treated and RNAse treated samples was compared.

Figure S7

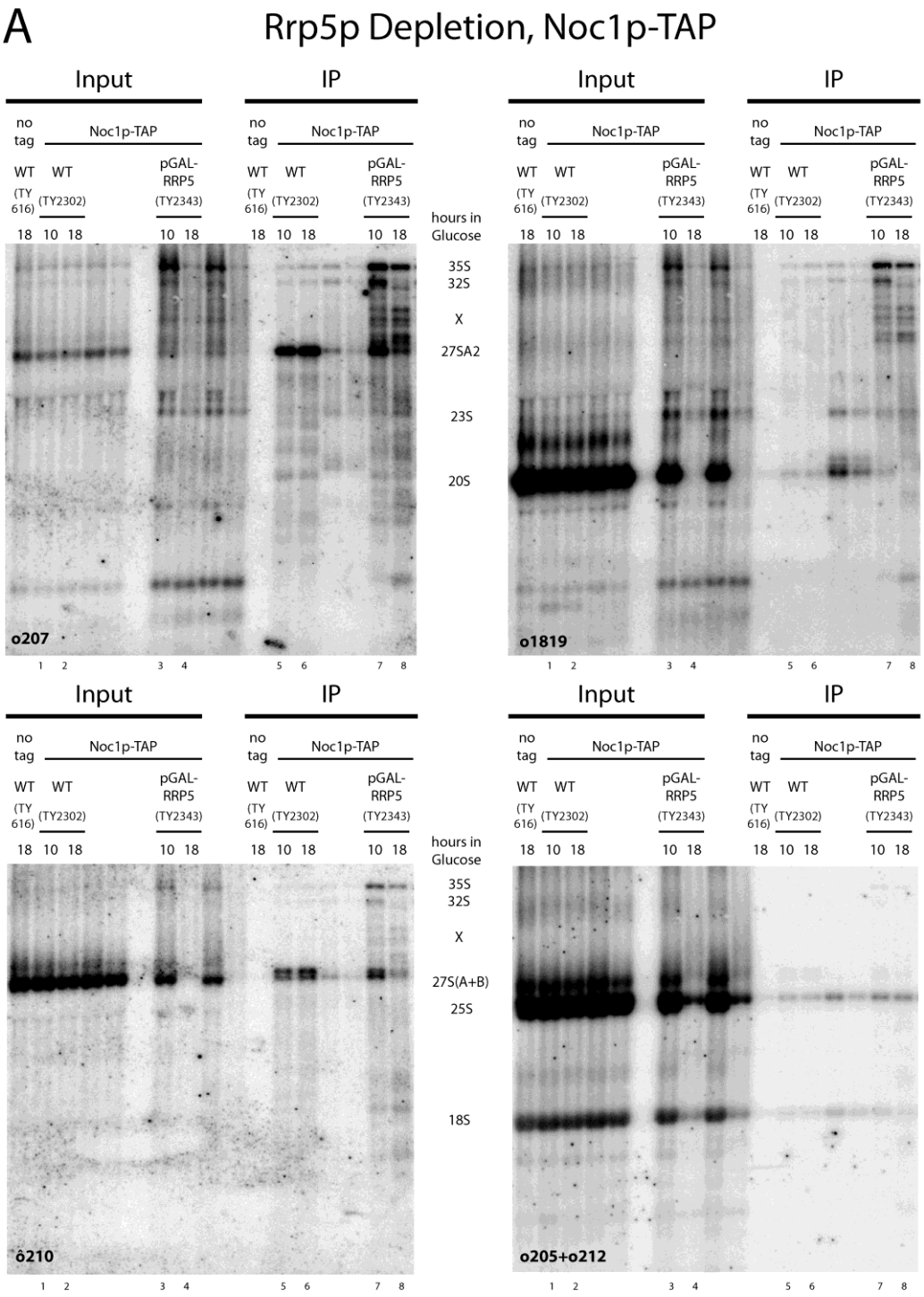
A
Rrp5p Depletion, Noc1p-TAP
Input
IP
Input
IP
no tag
Noc1p-TAP
WT (TY 616)
WT (TY2302)
pGAL-RRP5 (TY2343)
18 10 18 10 18
hours in Glucose
35S
32S
X
27SA2
23S
20S
o207
o1819
1 2 3 4 5 6 7 8
Input
IP
Input
IP
27S(A+B)
25S
18S
ô210
o205+o212

Figure S7

B Rrp5p Depletion, Noc2p-TAP

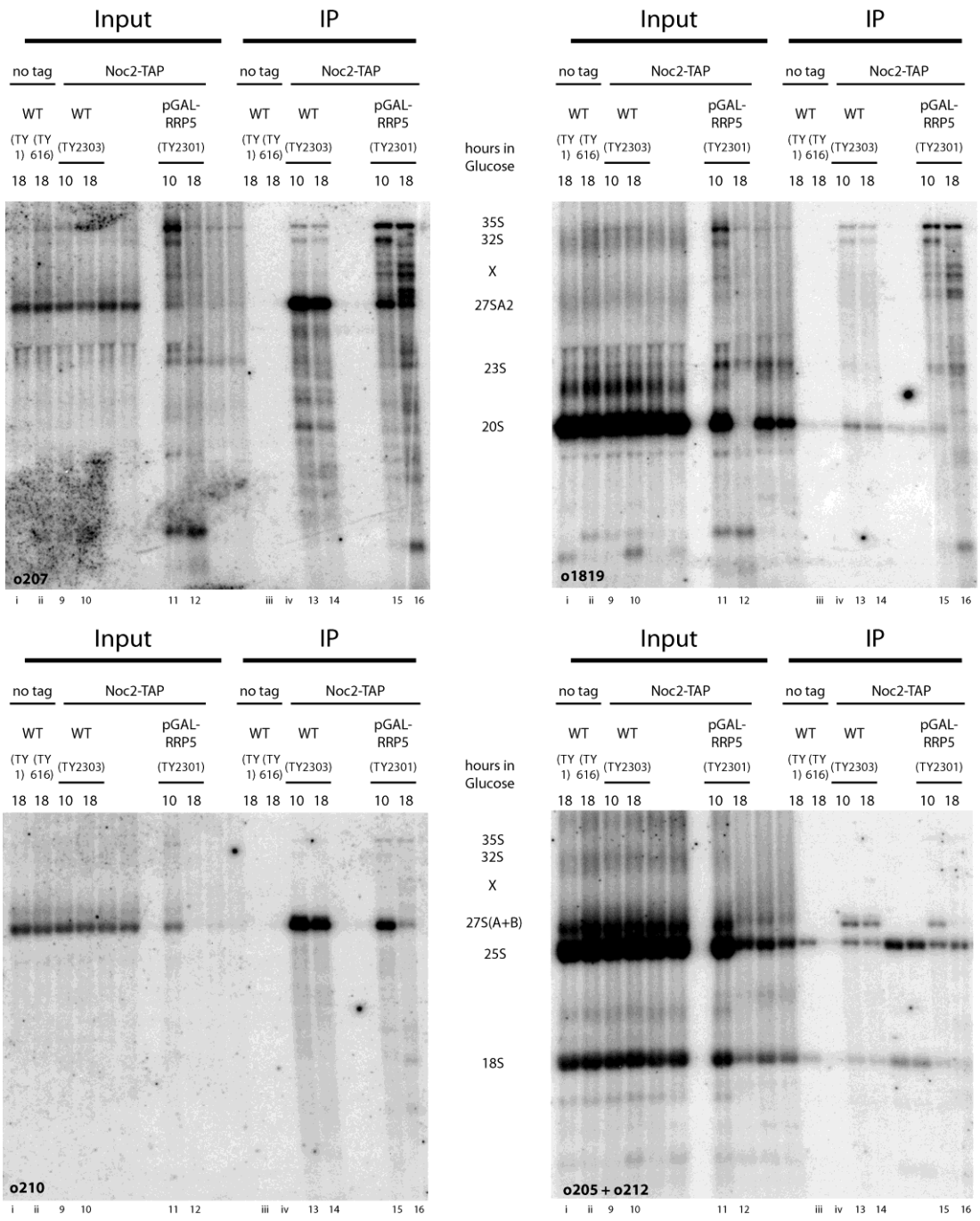

Figure S7

C Noc2p Depletion, Rrp5p-TAP

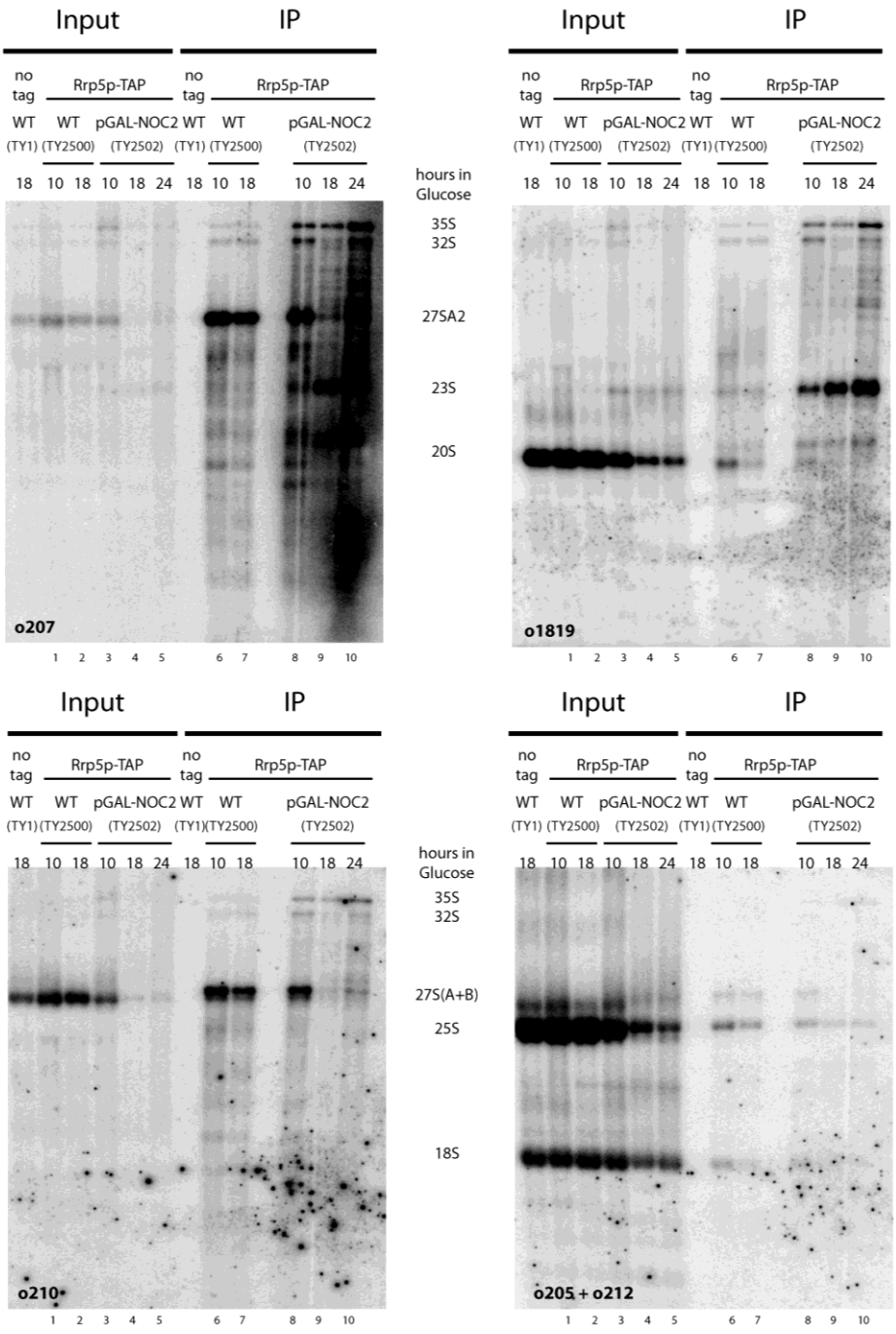

Figure S7

D Noc1p Depletion, Rrp5p-TAP

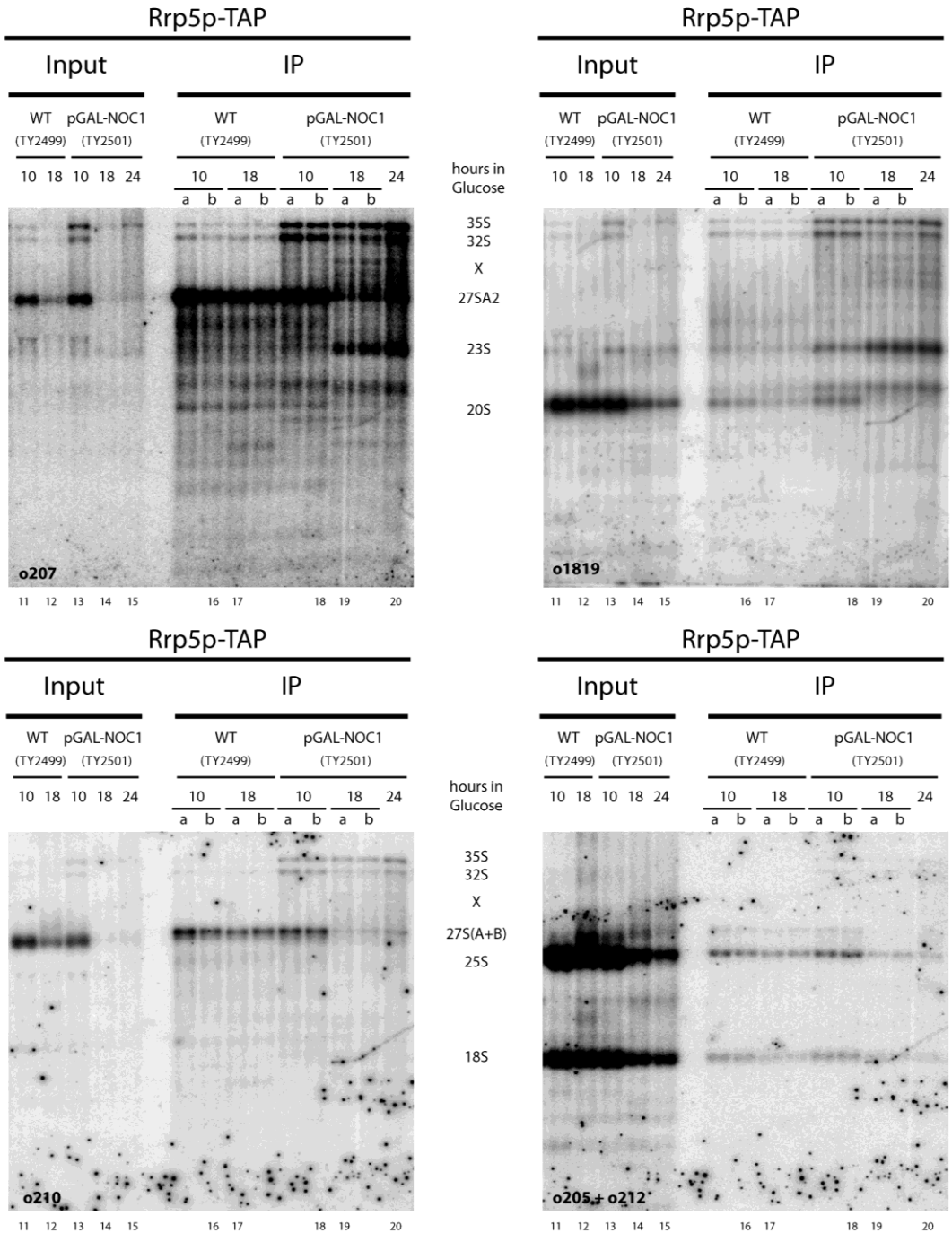

Figure S7

E Noc1p Depletion, Utp22p-TAP

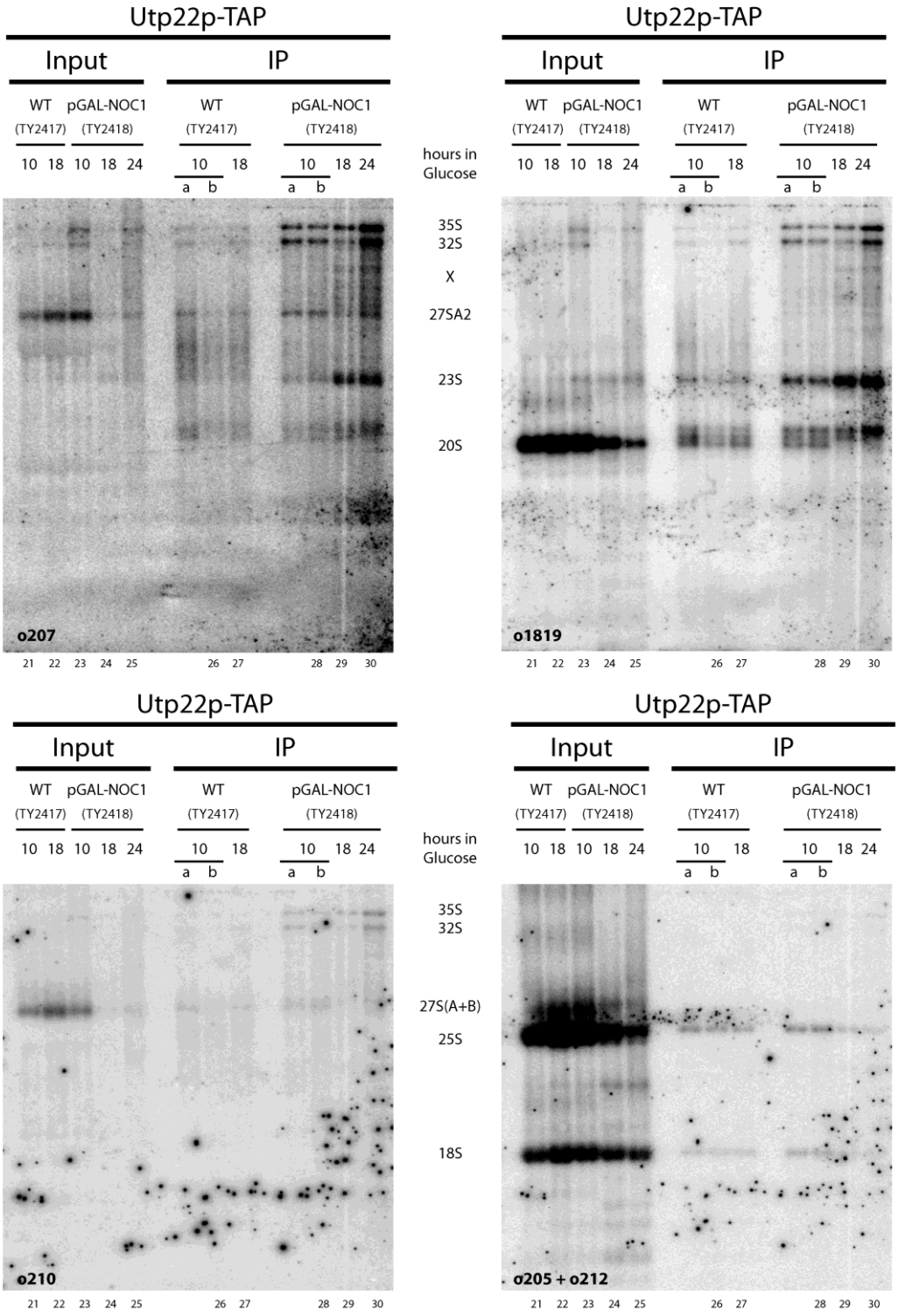

Fig. S7 Analysis of the binding hierarchy of biogenesis factors to pre-ribosomal particles

Shown are the whole Northern blots derived from agarose gels, from which the panels for the composite Figure 8 were excised. The probes used for hybridization are indicated in the lower left corners of the blots (see Fig. S1A for binding sites of the probes). Positions of major (pre-) rRNA species are marked, as are positions of aberrant pre-rRNA fragments resulting from depletion of biogenesis factors which are still co-purified with other factors (X). Labelling and numbering of the lanes correspond to the ones in Fig. 8 A-D. See legend to Fig. 8 for details. Unlabelled lanes are derived from unrelated experiments.

A) Noc1p-TAP purification after *in vivo* depletion of Rrp5p corresponding to Fig. 8A. B) Noc2p-TAP purification after *in vivo* depletion of Rrp5p corresponding to Fig. 8A. C) Rrp5p-TAP purification after *in vivo* depletion of Noc2p corresponding to Fig. 8B. D) Rrp5p-TAP purification after *in vivo* depletion of Noc1p corresponding to Fig. 8C. a, b) IPs were performed in duplicate from the same cell extract. E) Utp22p-TAP purification after *in vivo* depletion of Noc1p corresponding to Fig. 8C. a, b) IPs were performed in duplicate from the same cell extract.

Supplementary Methods:

Sucrose Density Gradient Centrifugation

Isolation of ribosomes under low salt conditions by sucrose gradient centrifugation was performed as described (1).

Chromatin immunoprecipitation (ChIP) and PCR based analysis of co-purified DNA after RNase treatment

ChIP experiments after RNase treatment of the chromatin fractions were performed according to (8). Briefly, yeast strains in which Noc1p (TY483), Noc2p (TY577), Rrp5p (TY615), Utp4p (TY1540) or Rpa135p (TY2423) are expressed as ProteinA or TAP-tag fusion proteins were grown in 120 ml rich medium at 30°C to exponential phase (OD600 = 0.5-0.7), subsequently cross linked using formaldehyde (final concentration 1%) for 15’ at 30°C and harvested in two aliquots of 50 ml each. From each cell pellet, the chromatin fraction was prepared as described in the Material and Methods section. Chromatin fractions from the same strains were pooled, and two aliquots of each pooled fraction were treated either with 10 U of RNase A and 400 U of RNase T1 (RNase A/T1 cocktail, Ambion) or an equivalent volume of lysis buffer. After incubating at 25°C for 40 min, input samples of the RNase treated and untreated chromatin were taken and immunopurification of the remaining solution was performed as described in the Material and Methods section. After the last washing step, an aliquot of the precipitated material (10% or 20%) was prepared for Western blot analysis. Samples for Western blot analysis were incubated in 1x Laemmli protein sample buffer (9) at 95°C for 15’ to reverse the formaldehyde cross link.

Supplementary references:

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