Viewing pre-60S maturation at a minute's timescale

Supplemental information

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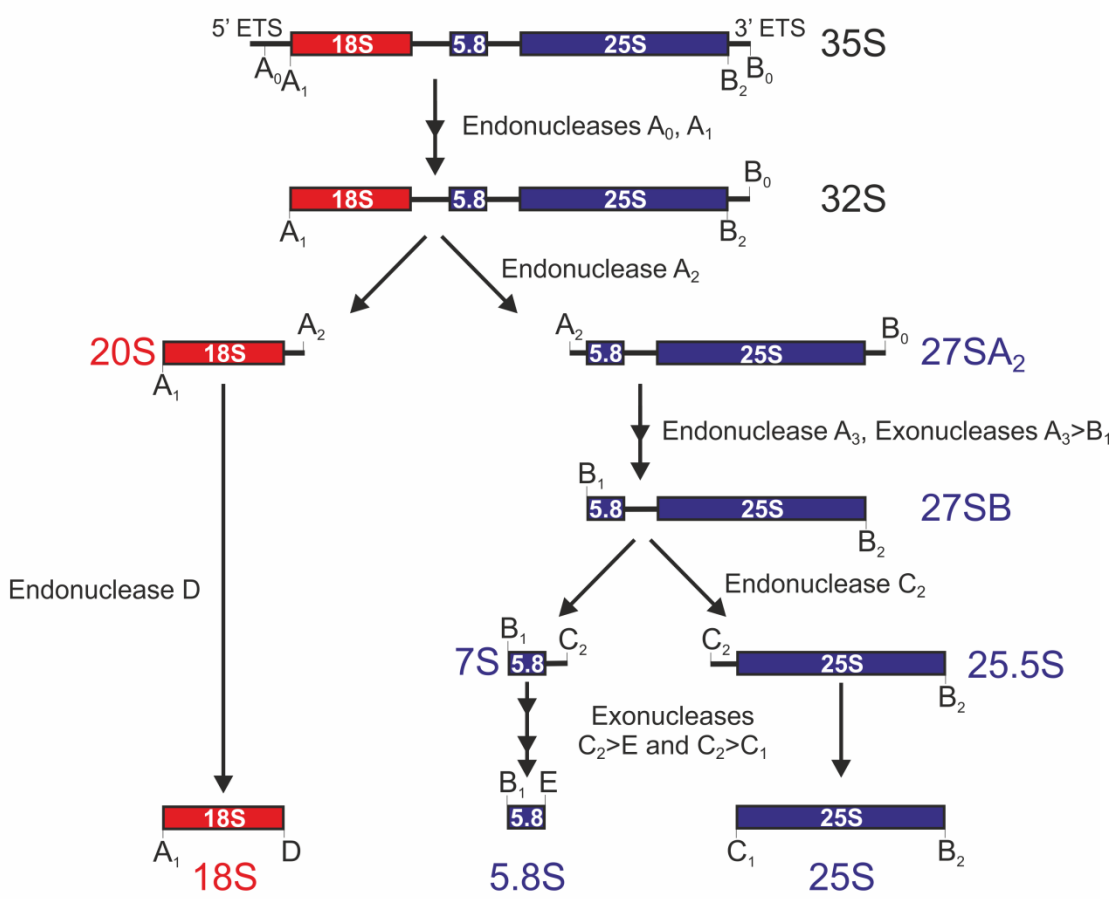

Fig. S1: Processing scheme of pre-rRNA to the mature 25S, 5.8S and 18S rRNA. Only the major steps are indicated. The 5S rRNA maturation occurs separately and is not shown.

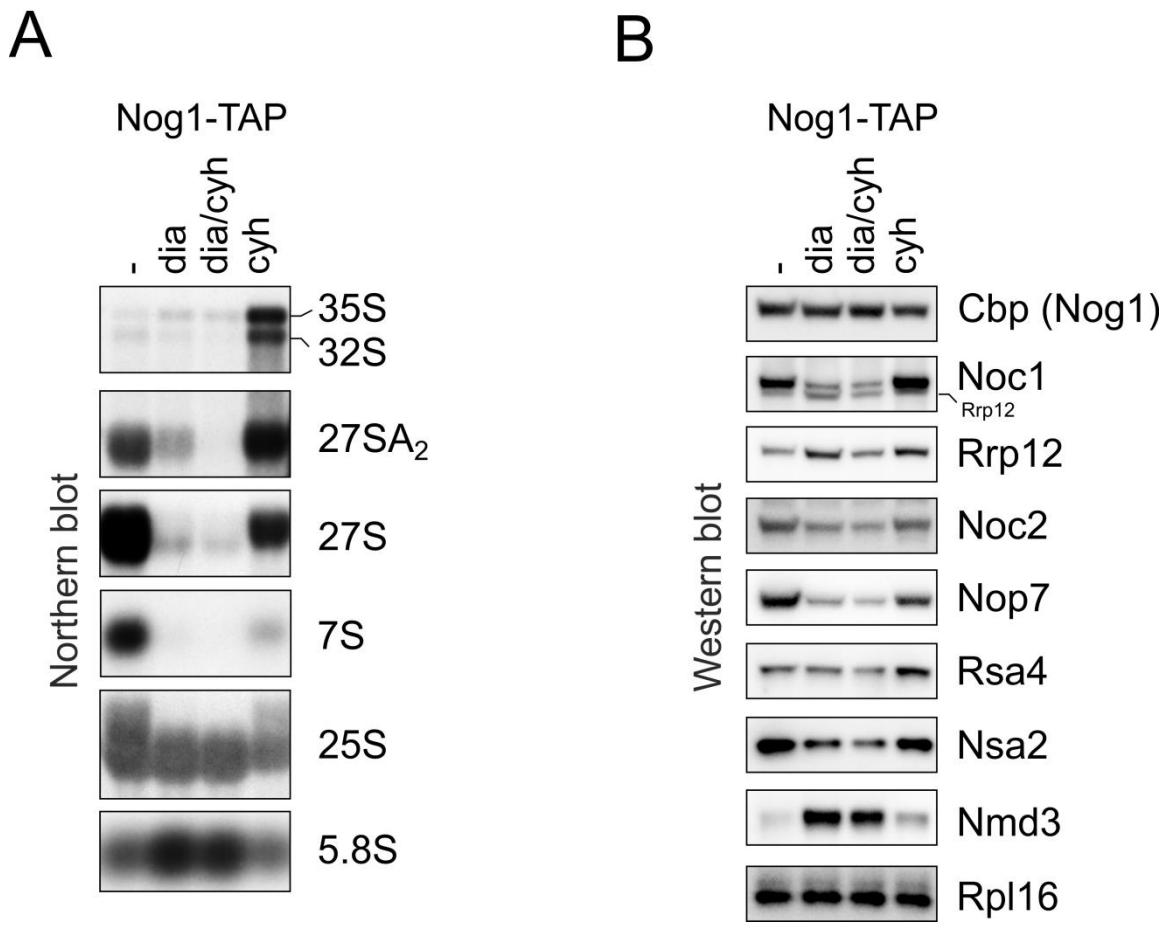

Fig. S2: Incorporation of de novo synthesized Nog1-TAP is responsible for co-purification of $27SA_2$ pre-rRNA and early pre-60S particles after longer diazaborine treatment. Pre-60S particles were purified from cells treated with diazaborine (dia), diazaborine and cycloheximide (dia/cyh) or cycloheximide (cyh) alone for 30 minutes using Nog1-TAP as bait protein. Particles were analyzed for co-purified pre-rRNA and proteins using Northern blotting **(A)** and Western blotting **(B)**, respectively.

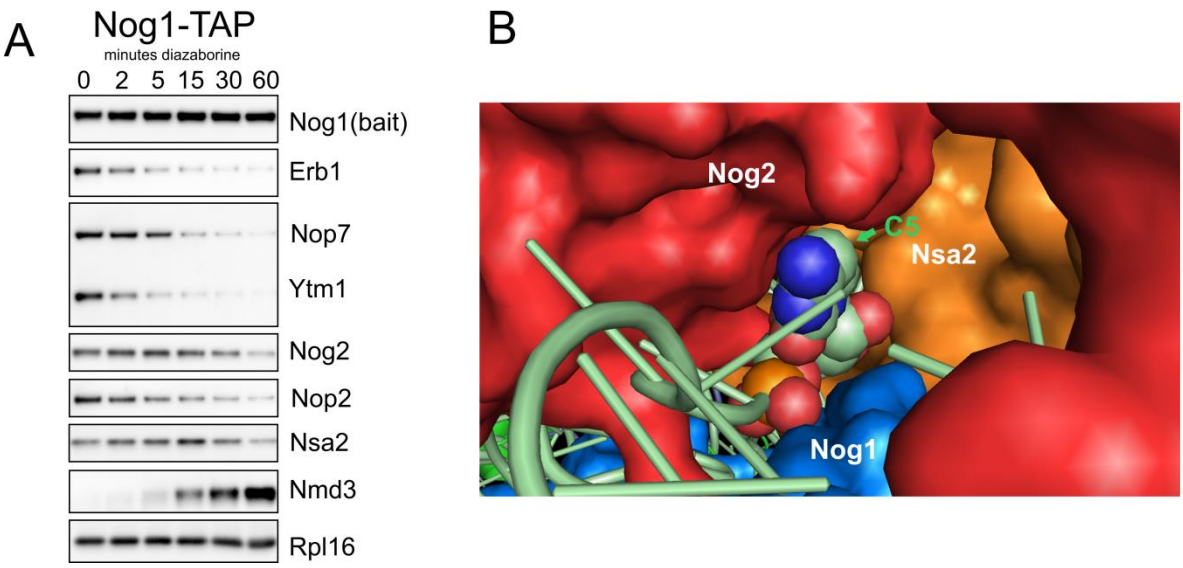

Fig. S3: **Analysis of selected proteins for their joining and leaving behavior on pre-60S particles. (A)** pre-60S particles were purified from cells after different time periods of treatment with diazaborine using Nog1-TAP as bait protein and analyzed by Western blotting for Ytm1/Erb1/Nop7 protein complex members and Nop2, Nog2 and Nsa2 proteins. Samples represent a different biological replicate as shown in Figure 2. To facilitate analysis of the Ytm1/Erb1/Nop7 complex, an antiserum simultaneously recognizing Ytm1 and Nop7 was used [1]. Western blots against the bait protein and the export factor Nmd3 are shown as controls. **(B)** Structural detail of the pre-60S particle around nucleotide C2870. Nucleotide C2870 methylated at C5 by the RNA:m5C-methyltranferase Nop2 is shown in the spheres mode and C5 highlighted by an arrowhead. Proteins associated with this region are Nog1 (blue), Nog2 (red) and Nsa2 (orange) and are shown as surface representation. 25S rRNA is shown in green. Based on data from [2].

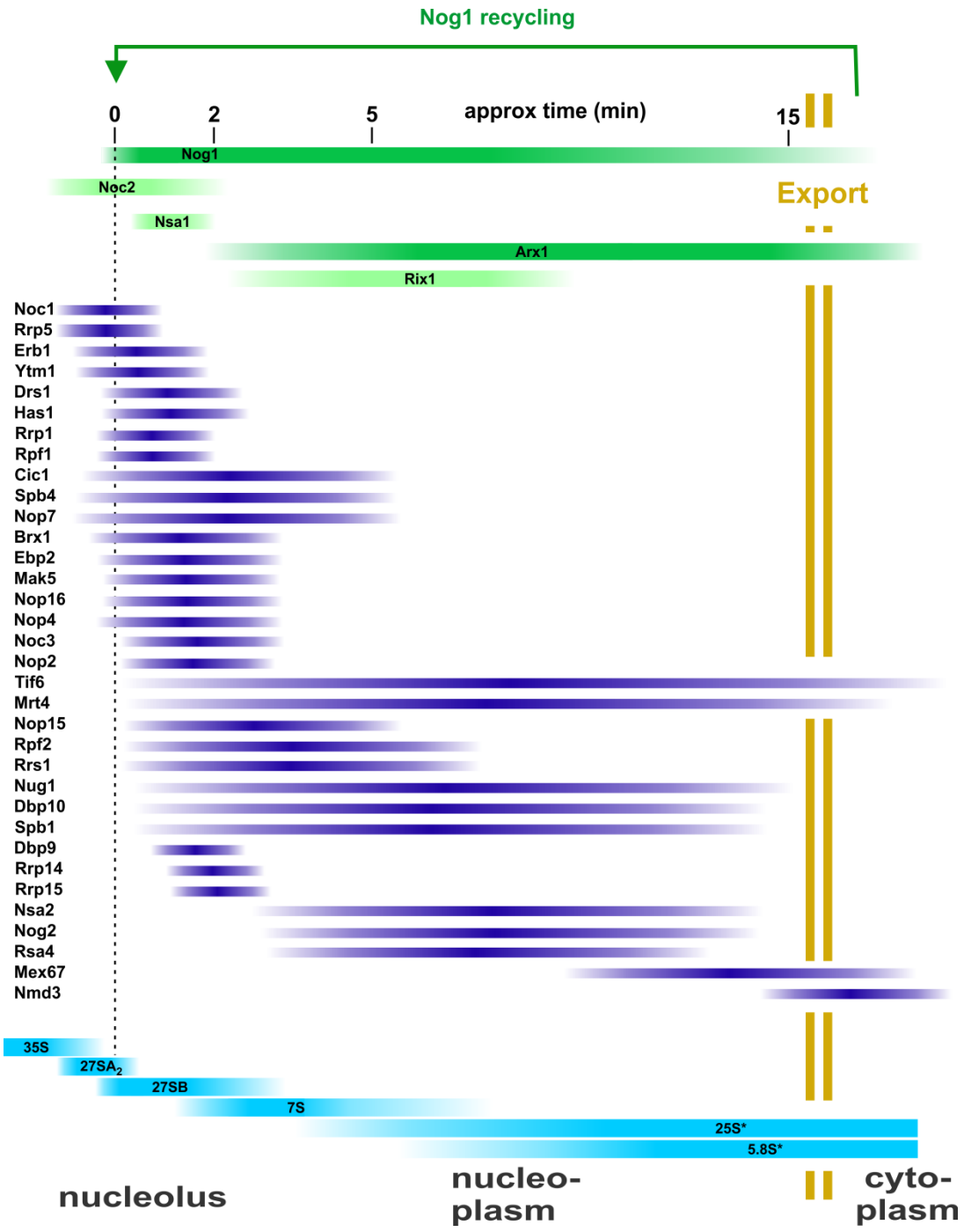

Fig. S4: **Scheme showing joining and release of individual pre-60S maturation factors.** Binding characteristics of bait proteins used in this study are symbolized by green bars. The approximate association period of selected maturation factors as inferred by western blotting and iTRAQ data is indicated by purple bars, while the co-purifying pre-rRNAs are symbolized by blue bars. The two brown vertical bars indicate the nuclear membrane.

Supplemental Table 1: *Saccharomyces cerevisiae* strains used in this study

***S.cerevisiae* strain**	**Genotype**	**Source**
Nog1-GFP Hho1-mCherry	*MATα leu2 ura3 his3 met15 NOG1-GFP::HIS3MX6 HHO1-mCherry::hphNT1*	this study
Nog1-GFP Nop58- mCherry	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 NOG1-GFP::HIS3MX6 NOP58-mCherry::hphNT1*	this study
Nog1-GFP Nic96-mCherry	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 NOG1-GFP::HIS3MX6 NIC96-mCherry::hphNT1*	this study
Rix1-GFP Nic96-mCherry	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 RIX1-GFP::HIS3MX6 NIC96-mCherry::hphNT1*	this study
Bud20-GFP Nic96-mCherry	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 BUD20-GFP::HIS3MX6 NIC96-mCherry::hphNT1*	this study
Noc2-TAP	*MATa his3 leu2 ura3 lys2 Noc2-TAP::URA3*	[3]
Nsa1-TAP	*MATα his3 leu2 ura3 trp1 Nsa1-TAP::TRP*	[4]
Rix1-TAP	*MATα his3 leu2 ura3 trp1 Rix1-TAP::TRP*	[5]
Arx1-TAP	*MATα his3 leu2 ura3 trp1 Arx1-TAP::TRP*	[6]
Nog1-TAP	*MATα his3 leu2 ura3 trp1 ade2 Nog1-TAP::TRP*	[6]

Supplemental Table 2: Oligonucleotides used for Northern blotting

Oligo designation	Sequence	Recognized pre-rRNAs
Probe A2/A3	5'-TGTTACCTCTGGGCCC-3'	$27SA_2$, 35S
Probe E/C2	5'-GGCCAGCAATTTCAAGTTA-3'	27S, 7S, 35S
Probe 5.8S	5'-GCGTTCTTCATCGATGC-3'	5,8S, 7S, 27S, 35S
Probe 25S	5'-CTCCGCTTATTGATATGC-3'	25S, 27S, 35S
Probe 18S	5'-GCATGGCTTAATCTTTGAGAC-3'	18S, 20S, 35S
Probe 5S	5'-GGTCACCCACTACACTACTCGG-3'	5S

Supplemental Table 3: Proteomic analysis of Nog1-TAP after 15 minutes of diazaborine treatment

		Replicate 1		Replicate 2				
function/localization	**protein§**	**labeling 1**	**labeling 2**	**labeling 1**	**labeling 2**	**mean**	**sd***	**%sd***
shuttling	Tif6	1,35	1,49	0,85	0,81	1,12	0,30	26,39
shuttling	**Nog1**	**1,00**	**1,00**	**1,00**	**1,00**	**1,00**	**0,00**	**0,00**
	Rrp12		0,76	1,03	0,92	0,90	0,11	12,51
shuttling	Arx1	0,83	1,00	0,82	0,83	0,87	0,07	8,49
	Mak5	0,85	0,88	0,86	0,87	0,86	0,01	0,99
	Nop13	0,91	0,90	0,87	0,74	0,86	0,07	7,75
late nucleoplasmic	Nog2	0,79	1,06	0,75	0,79	0,85	0,12	14,48
shuttling	Rlp24	1,10		0,72	0,70	0,84	0,18	21,81
shuttling	Mrt4	1,00	1,01	0,66	0,68	0,84	0,17	19,76
	Nop12	0,88	1,06	0,55		0,83	0,21	25,48
late nucleoplasmic	Rsa4	0,88	1,04	0,65	0,52	0,77	0,20	26,24
	Jip5	0,58	0,59	0,90	0,82	0,72	0,14	19,67
	Puf6	0,81	0,75	0,65	0,65	0,72	0,07	9,46
	Pwp1	0,81	0,80	0,62	0,61	0,71	0,09	13,23
	Ssf1	0,59	0,48	0,83	0,66	0,64	0,13	19,75
	Nsr1	0,88		0,54	0,49	0,64	0,17	27,05
late nucleoplasmic	Rix1	0,34	0,81	0,57	0,76	0,62	0,18	29,62
5S binding	Rpf2	0,51	0,56	0,66	0,63	0,59	0,06	10,07
5S binding	Rrs1	0,63	0,61	0,50	0,53	0,57	0,05	9,38
late nucleoplasmic	Nug1	0,57	0,65	0,47	0,54	0,56	0,06	11,41
late nucleoplasmic	Rea1	0,52	0,75	0,53	0,42	0,56	0,12	21,84
	Noc2	0,66		0,44	0,46	0,52	0,10	19,12
	Brx1	0,58	0,59	0,46	0,43	0,52	0,07	13,44
	Nip7	0,58	0,65	0,36	0,34	0,48	0,13	27,78
	Rrp15	0,55		0,37	0,51	0,47	0,08	16,48
	Drs1	0,40	0,51	0,45	0,54	0,47	0,06	11,70
	Rrp5	0,47		0,49	0,45	0,47	0,02	3,39
	Nop4	0,53		0,40	0,45	0,46	0,05	11,54
	Has1	0,35	0,53	0,43	0,48	0,45	0,07	14,76
	Nop2	0,54	0,50	0,42	0,32	0,44	0,08	18,78
	Fpr3	0,58	0,56	0,29	0,34	0,44	0,13	29,50
	Spb4	0,45	0,43	0,34	0,46	0,42	0,05	11,25
ITS2 binding	Nop53	0,35	0,36	0,47	0,47	0,41	0,06	14,65
	Rpf1	0,47		0,31	0,38	0,38	0,07	17,01
Nop7 loading	Ytm1	0,40	0,40	0,33	0,40	0,38	0,03	8,41
ITS2 binding	Nsa3	0,35	0,41	0,34	0,41	0,38	0,03	8,71
ITS2 binding	Nop7	0,37	0,52	0,34	0,26	0,37	0,09	24,77
	Rrp1	0,48	0,44	0,30	0,26	0,37	0,09	25,03
	Ebp2	0,42	0,42	0,29	0,31	0,36	0,06	16,82
ITS2 binding	Rlp7	0,40	0,39	0,33	0,28	0,35	0,05	14,24
Nop7 loading	Erb1	0,39	0,47	0,26	0,25	0,34	0,09	27,00
ITS2 binding	Nop15	0,31	0,37	0,34	0,30	0,33	0,02	7,51
	Nop16	0,40	0,32	0,25	0,26	0,31	0,06	20,50
	Nsa1	0,36	0,29	0,24	0,27	0,29	0,04	15,38

sd*: standard deviation

§ only factors identified in at least 3 independent labeling experiments and exhibiting less than 30% standard deviation were considered

SUPPLEMENTAL REFERENCES

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