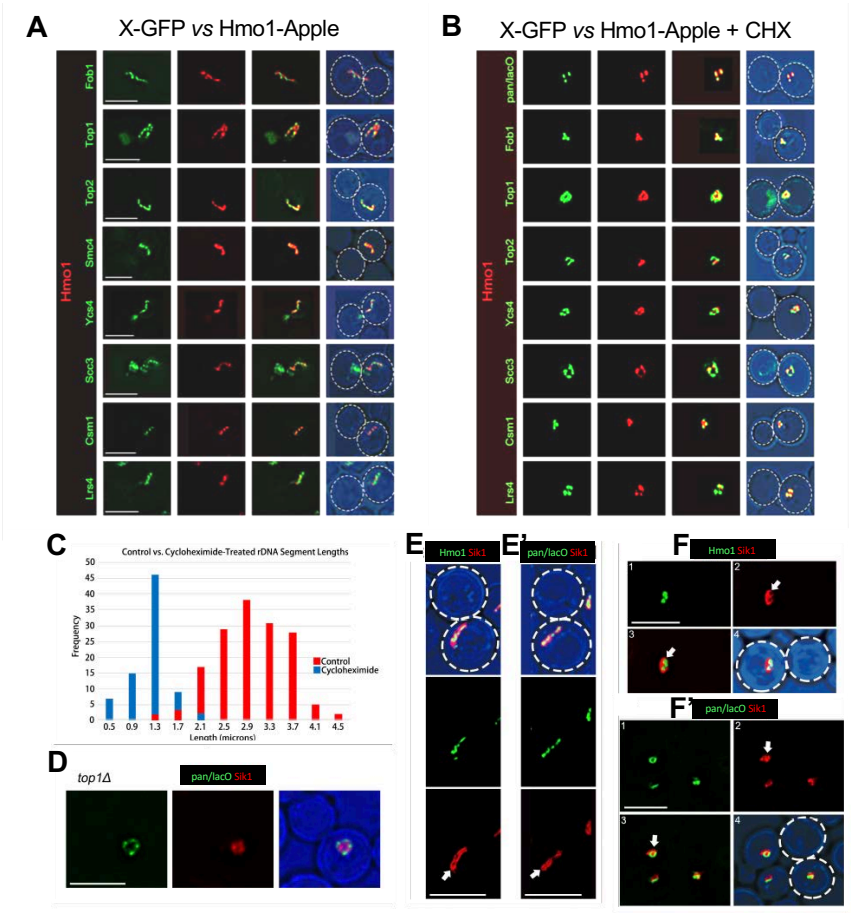

Figure S1 Distributions of Proteins that Associate with the rDNA Axis (Metaphase^{Cdc20} Cells). Related to Figure 1.

A) Images of arrested cells that express Hmo1-Apple as well as single GFP-tagged rDNAPs (X-GFP). Note that the GFP-tagged proteins align with Hmo1-Apple in the mother. Single planes are illustrated. Strains: ATY10682, ATY10653, ATY10638, ATY10627, ATY10645, ATY10641, ATY10620, ATY10654, ATY10623.

B) Equivalent to (A) after exposure to 0.1 mg/ml cycloheximide for 30 min. Note the condensed distribution of each rDNAP and their continued proximity to Hmo1-Apple.

C) Quantitation of the impact of cycloheximide on the dimensions of pan-lacO rDNA as detected in projected images (n = 75-100). After arrest in medium with methionine, cells were incubated +/- cycloheximide for 30 min. For each cell, we measured the distance between the two furthest separated

elements of the tagged rDNA. These data were then pooled by 0.4 μ intervals. Thus, the 0.5 μ bar represents the % of cells for which the distance was between 0.1-0.5 μ etc. Strain: ATY10688.

D) Distribution of pan-lacO and Sik1-mRFP in cells that lack Top1. Deletion of Top1 reduces the area occupied by rDNA. As shown, the pan-lacO signal continues to contact the snoRNP marker, Sik1-mRFP. Strain: ATY10795.

E) Localization of Hmo1-GFP and pan-lacO compared to Sik1-mRFP. Strains: ATY10342, ATY747.

F) Impact of cycloheximide on the distribution of Hmo1-GFP (E) and pan-lacO (E') compared with Sik1-mRFP. Panels 1-4 include only GFP, only mRFP, or both colors as well as cell outlines. As shown, both GFP markers contact the Sik1-positive domain. Corresponding control images are in Figure 1H/H'. Strains: ATY10342, ATY10747.

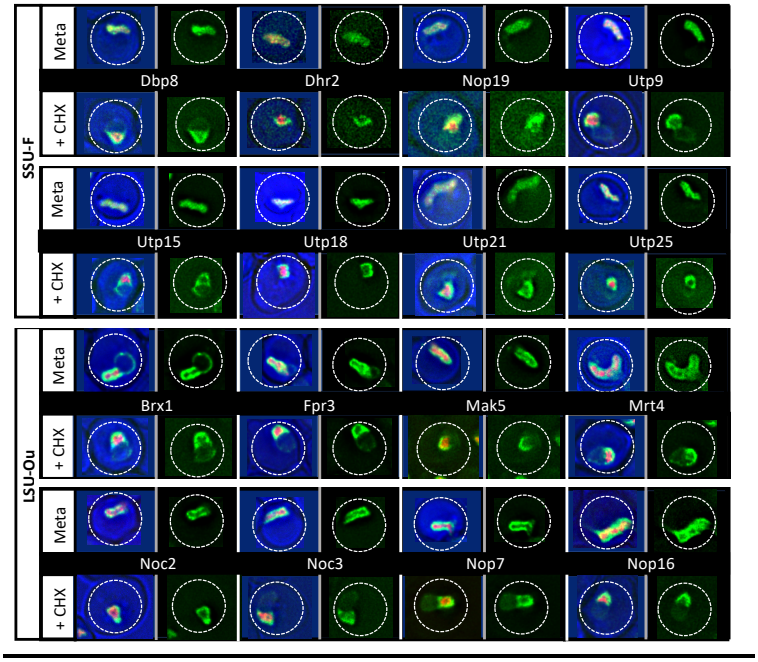

Figure S2 Examples of the Localization of SSU-F AFs and LSU-Ou AFs. Related to Figure 3.

As for Figure 3B, each cell type was either exposed to cycloheximide (+ CHX) or studied after depletion of Cdc20 (Meta). The names of the GFP-tagged proteins are designated. All strains also express Sik1-mRFP.

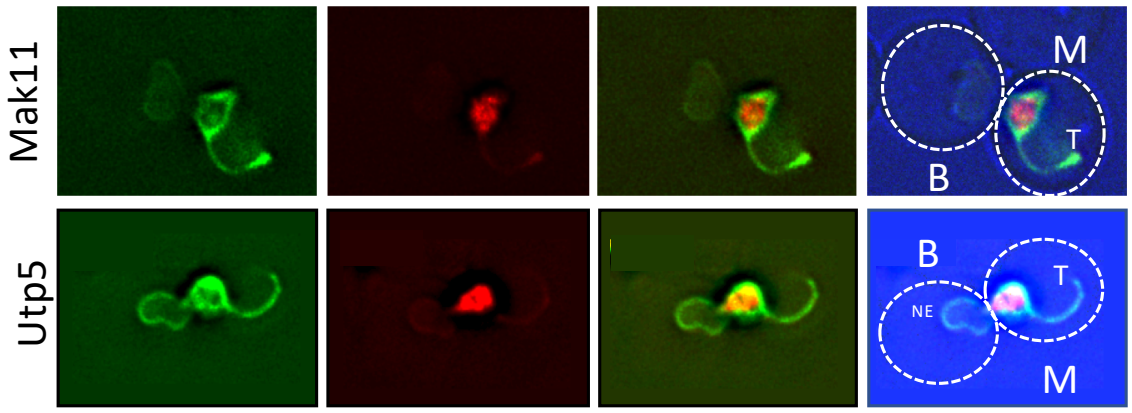

Figure S3 Distributions of Assembly Factors in Arrested Cells Treated with Cycloheximide. Related to Figure 3.

Metaphase^{Cdc20} cells expressing a LSU AF (Mak11-GFP) or a SSU AF (Utp5-GFP), as well as Sik1-mRFP, were arrested. Cycloheximide was then added for 30 min before imaging. Note the characteristic strong outer layer signal of the GFP-tagged proteins, surrounding the red inner layer. The elaboration of NE signal into the Bud (B) is most obvious for Utp5. The “tail” (T) extending into the mother (M) is presumably the remnant of the elongated NE that is found in arrested cells. Equivalent behavior is seen for many dozens of SSU-F and LSU-Ou AFs that we have studied. Strains: ATY10473, ATY10387.

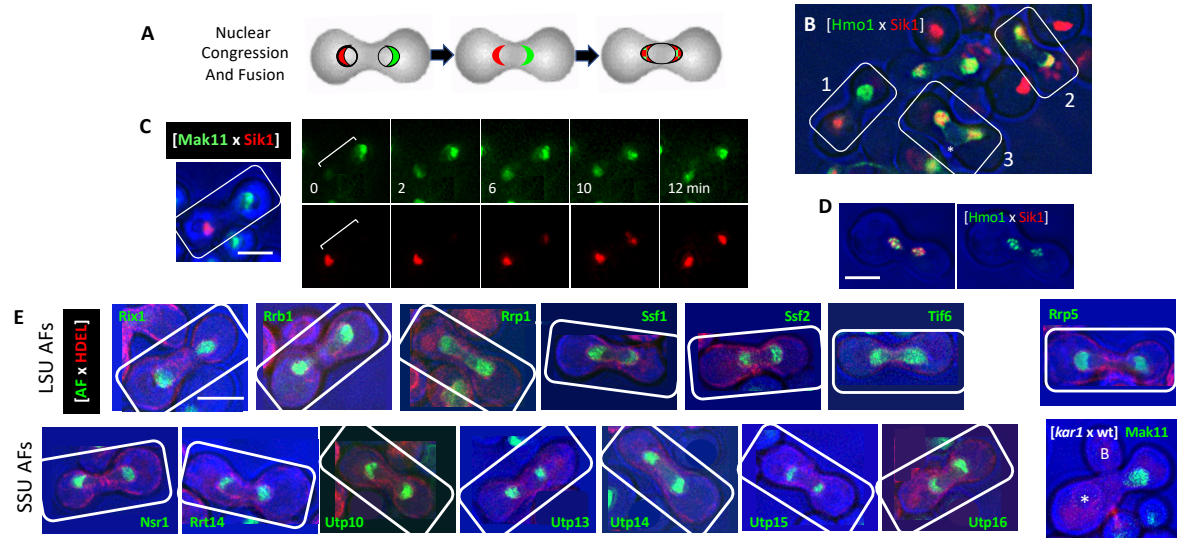

Figure S4 Nucleolar Assembly Factors Shuttle within the Nucleus. Related to Figure 5 and Table S2.

A) Model of intranuclear shuttling of two nucleolar proteins (red, green). In the panel at the left the two cells have fused. In the middle panel the two nuclei contact each other. In the third panel the two nuclei have fused, the nucleoli have not yet fused, but the tagged proteins have equilibrated.

B) Stages of shuttling of Mak11-GFP and Sik1-mRFP, each of which was contributed by one of the parental cells. Note in zygote (1) that the two labels have not intermixed, while in zygote (2), after karyogamy, they have fully intermixed, although the nucleoli are separate from each other. In zygote (3), the NE (asterisk) is stretching toward the incipient bud, but the nucleoli have still not fused with each other.

C) Kinetics of exchange of Mak11-GFP and Sik1-mRFP. Each protein was initially present in only one of the parental cells (image at the left, including cell outlines). The brackets indicate the positions of both nucleoli. Strains: ATY1513, ATY8007.

D) Evidence of exchange of Hmo1-GFP and Sik1-mRFP, each of which was synthesized by one of the parental cells. Cells were imaged 2 hr after being mixed. Strains: ATY10024, ATY1513.

E) Each GFP-tagged AF was expressed by a cell that was crossed with a partner that expressed the ER/NE marker, mRFP-HDEL. Unbudded zygotes were then imaged after 2 hr. Top row: LSU AFs.

Bottom Row: SSU AFs. Note the extensive transfer of the GFP-tagged protein in each case. Right, upper: Rrp5-GFP. Right, lower: To control for the possibility that nucleolar AFs were detected in the second nucleolus of zygotes due to a) new synthesis, or b) export to the cytoplasm followed by reimport, 14 strains, each expressing a GFP-tagged AF, were crossed with a *kar1Δ15* mutant for 2.5 hr at room temperature (see Table S2). In such [*kar1Δ 15* x wt] zygotes, nuclear fusion is delayed ($t_{1/2} \approx 5$ hr, by comparison to wt zygotes for which $t_{1/2}$ is < 2 hr [73]. Labeling of the second nucleolus was detected in < 5% of unbudded zygotes. Thus, signal transfer occurs after nuclear fusion, and neither new synthesis nor shuttling *via* the cytoplasm can account for the transfer of label between nucleoli. The image illustrates the example of Mak11-GFP crossed with a strain that expressed mRFP-HDEL. B: Bud. Strains: ATY6618, ATY8007.

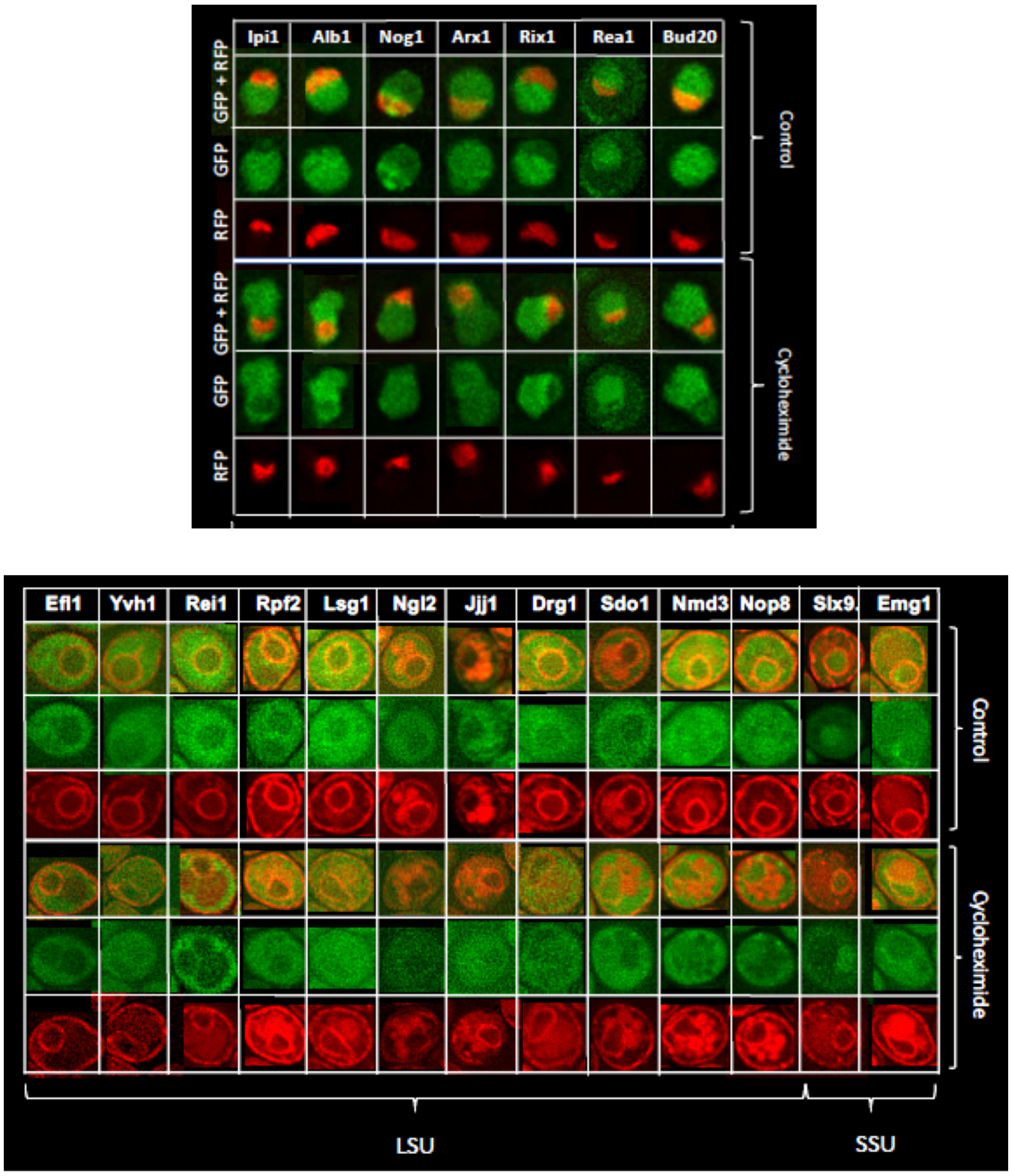

Figure S5 Nucleoplasmic and Cytoplasmic AFs. Related to Figures 2 and 3D.

Upper - Nucleoplasmic AFs: The distributions of GFP-tagged AFs are illustrated +/- Sik1-mRFP. Nog1 is visible in the nucleoplasm but is less visible than the others. After cycloheximide, we see no indication that these proteins relocate. Strains: ATY8105, ATY8073, ATY8703, ATY1033, ATY8280, ATY7833, ATY8077.

Lower - Cytoplasmic AFs: The distributions of GFP-tagged AFs are illustrated +/- mRFP-HDEL to label the NE/ER. (The vacuole often is also bright.) After cycloheximide, we see no indication that Drg1, Efl1, Jjj1, Lsg1, Ltv1, Ngl1, Nmd3, Nop8, Rei1, Rio2, Rpf2, Slk9, Sdo1 and Yvh1 relocate to the nucleus. Strains: ATY10064, ATY10062, ATY9962, ATY9954, ATY9959, ATY9961, ATY9955, ATY10027, ATY9949, ATY9951, ATY10063, ATY9953.

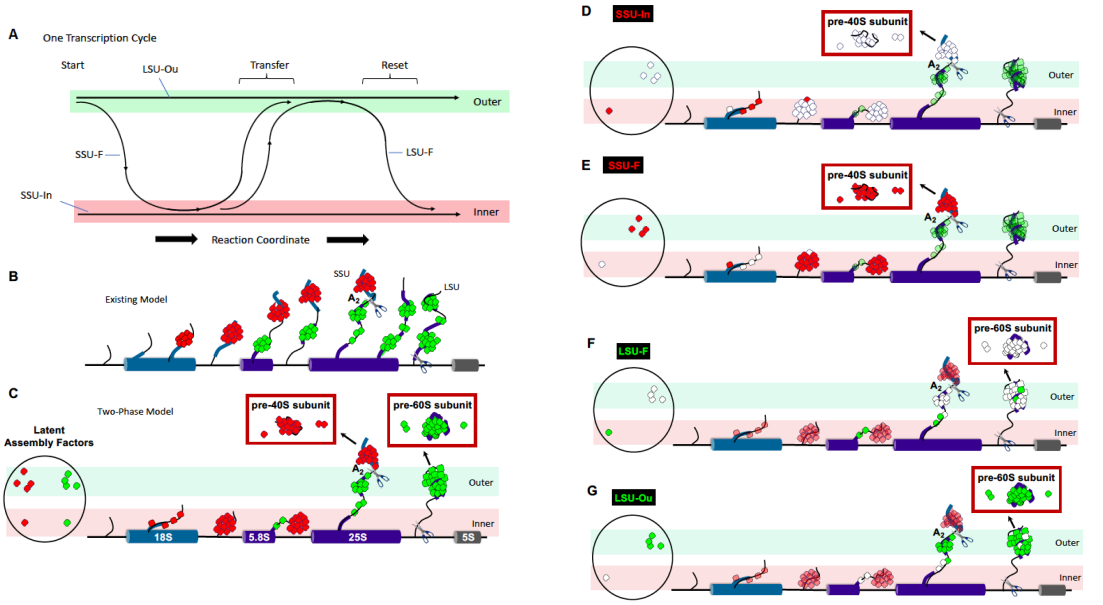

Figure S6 Overviews of Relocation of Assembly Factors During a Single Cycle. Related to Figure 5.

A) Schematic of the flux of AFs, showing that two subsets remain in the layers where they begin (SSU-In, LSU-Ou), while two others (SSU-F, LSU-F) redistribute in anti-parallel fashion.

Panels (B-G) illustrate localizations of AFs in the context of ongoing transcription. (A) represents the classical view of elongation and assembly. (B) Modifies this scheme, indicating the distributions of the latent forms of AFs (at the left) and the proposed distribution of both latent and operative AFs between the two layers, as is described in the text. Panels (D-G) trace the distributions of each of the four indicated subsets of AF. In each case, the subset of principal interest is illustrated in bright color, while other AFs contributing to the same subset are empty and those pertaining to the other subset are pale.

	Name (motif)	**rRNA sequences needed for association** [58, 63].
SSU-In	Dbp4 (DExD/H-box)	SSU RNA 5'-domain
	Efg1	SSU RNA 5'-domain
	Nop6 (RRM)	SSU RNA 3'-major domain
	Nop9 (RNA binding)	SSU RNA 3'-major domain
	Nsr1 (RRM)	SSU RNA central domain
snoRNP Proteins	Gar1 (β-barrel)	SSU RNA central domain
	Nhp2	SSU RNA central domain
	Nop1	rRNA 5'-ETS
	Nop5/Nop58 (Nop1 domain, WD domain)	rRNA 5'-ETS
	Nop10	SSU RNA central domain
	Sik1/Nop56 (Nop1 domain, WD domain)	rRNA 5'-ETS
Other	Dbp3 (DExD/H-box)	Required for SSU & LSU
	Prp43 (DExD/H-box)	Required for SSU & LSU
	Rnt1 (nuclease)	Cleaves site B_o

Table S1 Binding Specificities of Assembly Factors. Related to Figure 5.

This table lists published information on RNA binding sites of AFs.

SSU	LSU	snoRNP	RNA-BP	rDNAPs
Dbp8	Bmt2	Gar1	Nop19	Brn1
Dhr1	Brx1	Nop1	Rcl1	Cdc14
Dhr2	Bud20	Nop10	Rnt1	Csm1
Efg1	Cic1*	Nop5/58	Rrp5	Fob1*
Enp1	Drs1	Sik1/Nop56*		Hmo1*
Fal1	Fpr3			Lrs4
Fyv7	Fpr4			Net1
Krr1	Mak11*			Smc4
Nop6	Noc1*			Ycs4
Nop9*	Noc3			
Nsr1*	Noc4			
Rrt14	Nog1			
Sgd1	Nop12			
Utp1	Nop15			
Utp10*	Nop2			
Utp13	Nop7			
Utp14*	Npa1*			
Utp15	Npa2*			
Utp16	Nsa2			
Utp18*	Nug1			
Utp19	Puf6			
Utp22*	Rcm1			
Utp25	Rea1			
Utp5	Rix1			
Utp8*	Rix7			
	Rrp1			
	Rrp17			
	Ssf1*			
	Ssf2			
	Tif6			
	Tri1			

Table S2 Nucleolar Assembly Factors Show Intranuclear Shuttling. Related to Figure S4.

All strains were studied in crosses with wt and showed evidence of shuttling in these crosses. Strains designated with (*) were also studied in crosses with a *kar1Δ15* strain that expressed mRFP-HDEL to label the ER/NE. None of these crosses showed signs of shuttling.