

Supplementary Tables and Table Legends

| Table S1. Subsets of Nucleolar Assembly Factors | | | | | | |
|--|-------------------------------|----------------------------|----------------------------|------------------------------|--|---|
| Subset * | Operative Distribution | Latent Distribution | Suggested Role | Fraction Essential ** | Non-Essential AFs ^x | Orthologs Implicated in Disease ^{+ °} |
| SSU-In | Inner layer | Inner layer | Initiator ? | 2/5 | Efg1, Nop6, Nsr1 | |
| SSU-F | Inner layer | Outer layer | SSU knob formation | 50/52 | Fyv7, Rrt14 | BUD23 [Bud23] Cirhin [Utp4] DHX37 [Dhr1] ESF1 [Esf1] UTP14 [Utp14] WDR36 [Utp21] WDR43 [Utp5] |
| LSU-Ou | Outer layer | Outer layer | LSU knob formation | 38/55 | Abl1, Arx1, Bmt2, Bud20, Bud23, Cic1, Dbp7, Fpr3, Fpr4, Mrt4, Nop12, Nop13, Nop16, Puf6, Rae1/Gle2, Rcm1, Ssf1, Ssf2, Tri1 | DCAF13 [Sof1] DNAJC21 [Jjj1] |
| LSU-F | Outer layer | Inner layer | Initiator ? | 3/4 | Rsa3 | |
| snoRNP proteins | Inner layer | Inner layer | modify rRNA | 7/7 | none | DKC1 [Cbf5] <i>SNORD118 #</i> |
| Rnt1, nuclease | Inner layer | Inner layer | removal of SSU knobs | | | nuclease: LAS1L <i>RMRP #</i> |
| Rcl1, nuclease | Outer layer | Outer layer | release of transcripts | | | |
| Rrp5 | Inner layer | Outer layer | binds ITS1 | | | |
| rDNAPs | Axis | Axis | Packaging of rDNA ? | | Csm1, Fob1, Hmo1, Lrs4, Sir2, Tof2, Top1 | |
| Other (Pol A, Dbp3, Prp43) | Inner layer | Inner layer | transcription, early steps | | | TCOF1, POLR1A [Rpa190] POLR1C/D [RPA/C19; RPA/C40] |
| <p>* The naming conventions for these proteins and localizations are explained in Table 1 and in [1]. Additional human genes for which mutation impairs ribosome production are DDX11, DDX21, NOL11, PAK1IP1. There is little information concerning which subunits are affected. Orthologs have not been identified.</p> <p>** In the "Fraction Essential" column we indicate, for each category, the number of proteins that can be deleted, singly, without interrupting growth [2]. Each denominator indicates the total number of proteins in the category that have been examined.</p> <p>^x Not required for mitotic growth, although growth may slow [2].</p> <p>[°] [3, 4].</p> <p>⁺ Name of HUMAN GENE [Yeast name]. The homolog of Sdo1 (SBDS) is mutated in Schwachman-Diamond syndrome. Sdo1 is not concentrated in the nucleolus.</p> <p>[#] Non-coding RNAs.</p> | | | | | | |

Table S1 Subsets of Assembly Factors - related to Table 1

In addition to the information summarized in Table 1 and Table S2, this table specifies the suggested role of each subset of AFs and the fraction of the AFs in each group that are essential for mitotic growth [2]. We also list those for which homologs in man have been implicated in disease. The group of non-essential AFs in yeast includes proteins associated with the 5'-ETS (Bud21, Nan1/Utp17), other proteins required for making the SSU (Bud22, Cms1, Efg1, Fyv7, Nsr1, Rrp8, Rrt14, Slx9, Utp30) and proteins required for making the LSU (Bmt2, Bud20, Cgr1, Cic1/Nsa3, Dbp7, Fpr3, Fpr4, Jjj1, Loc1, Mrt4, Ngl2, Nop12, Nop13, Nop16, Puf6, Rrp6, Ssf1, Ssf2, Tri1).

Collection of data. The *Saccharomyces* Genome Database (SGD) was used to gather information on the abundance, molecular weight and amino acid sequence of assembly factors. For that database, BioPerl and CODONW were used to calculate isoelectric points and abundance data were averaged from multiple quantitative analyses of cells under standard growth conditions [5]. Predictor of Natural Disordered Regions (PONDR) was used to predict overall disorder based on the amino acid sequence (<http://www.pondr.com/>). FoldIndex was also used to make such estimates and the two algorithms gave similar estimates.

For human proteins, we used UniProt Knowledgebase (UniProtKB) and ExPASy to retrieve molecular weight and isoelectric point information. PONDR was again used to predict order.

Table S2. Master List of Assembly Factors and Their Physical Properties

| name | name | MW (kDa) | MW (kDa) | IEP | IEP | predicted disorder (%) | predicted disorder (%) | abundance mean | abundance STD | subset | tripartite domain |
|-----------------------|-----------|----------|----------|-------|-------|------------------------|------------------------|----------------|---------------|---------|-------------------|
| Nan1/Utp17 * | WDR75 | 101.22 | 94.49 | 6.31 | 5.65 | 17.58 | 17.31 | 6811 | 3486 | SSU-F | 5'-ETS |
| Utp5 * | WDR43 | 71.96 | 74.89 | 4.56 | 5.37 | 34.53 | 27.62 | 6874 | 7312 | SSU-F | 5'-ETS |
| Utp6 | UTP6 | 52.43 | 41.53 | 9.33 | 7.59 | 20.68 | 16.25 | 4712 | 2273 | SSU-F | 5'-ETS |
| Utp8 | | 80.16 | | 4.78 | | 18.93 | | 7512 | 3299 | SSU-F | 5'-ETS |
| Utp9 | | 65.23 | | 4.48 | | 24.7 | | 8516 | 7706 | SSU-F | 5'-ETS |
| Utp13 * | TBL3 | 91.00 | 89.03 | 5.06 | 6.44 | 21.05 | 21.41 | 6417 | 4007 | SSU-F | 5'-ETS |
| Utp15 * | UTP15 | 57.70 | 58.41 | 9.76 | 9.18 | 28.65 | 23.94 | 4549 | 3327 | SSU-F | 5'-ETS |
| Utp18 * | UTP18 | 66.39 | 62.00 | 4.61 | 8.93 | 31.99 | 33.63 | 6638 | 7492 | SSU-F | 5'-ETS |
| Utp21 * | WDR36 | 104.79 | 105.32 | 7.89 | 7.33 | 23 | 19.24 | 4129 | 2115 | SSU-F | 5'-ETS |
| Bud21/Utp16 | | 24.38 | | 6.55 | | 83.64 | | 3559 | 2746 | unknown | 5'-ETS |
| Dip2/Utp12 * | WDR3 | 106.32 | 106.09 | 5.89 | 6.2 | 21 | 24.18 | 5172 | 3868 | unknown | 5'-ETS |
| Fcf2 | DNTP2 | 25.64 | 84.46 | 10.17 | 5.86 | 71.43 | 60.58 | 3755 | 1318 | unknown | 5'-ETS |
| Imp3 # | IMP3 | 21.89 | 21.85 | 10.33 | 9.54 | 22.4 | 30.43 | 5499 | 5796 | unknown | 5'-ETS |
| Imp4 + | IMP4 | 33.48 | 21.85 | 9.5 | 9.54 | 31.38 | 30.43 | 4976 | 2363 | unknown | 5'-ETS |
| Mpp10 | MPHOSPH10 | 66.89 | 78.86 | 4.27 | 4.77 | 66.27 | 59.18 | 6676 | 3779 | unknown | 5'-ETS |
| Pwp2/Utp1 * | PWP2 | 103.94 | 102.45 | 4.71 | 5.76 | 17.44 | 17.3 | 5498 | 4011 | unknown | 5'-ETS |
| Rrp9 * | RRP9 | 65.04 | 51.84 | 5.43 | 7.97 | 34.73 | 38.53 | 4905 | 1950 | unknown | 5'-ETS |
| Sas10/Utp3 | UTP3 | 70.19 | 54.55 | 4.32 | 5.5 | 59.18 | 64.93 | 6159 | 3201 | unknown | 5'-ETS |
| Sof1 * | DCAF13 | 56.80 | 51.40 | 9.91 | 9.3 | 36.61 | 17.53 | 4982 | 2024 | unknown | 5'-ETS |
| Utp4 * | CIRH1A | 87.79 | 76.89 | 6.52 | 9.03 | 18.17 | 4.37 | 4299 | 2439 | unknown | 5'-ETS |
| Utp7/Kre31 * | WDR46 | 62.33 | 68.07 | 10.02 | 9.69 | 45.31 | 52.3 | 6067 | 2569 | unknown | 5'-ETS |
| Utp10 - | HEAR1 | 200.06 | 242.37 | 6.44 | 6.11 | 13.23 | 18.24 | 8060 | 3856 | unknown | 5'-ETS |
| Utp11 | UTP11L | 29.76 | 30.00 | 10.91 | 10.08 | 76.4 | 64.8 | 2954 | 1720 | unknown | 5'-ETS |
| Dbp8 † | DDX49 | 47.89 | 54.22 | 10.24 | 9.21 | 21.63 | 29.24 | 3904 | 3732 | SSU-F | pre-40S |
| Dhr2 † | | 82.73 | | 9.22 | | 33.47 | | 1734 | 1002 | SSU-F | pre-40S |
| Fyv7 | | 18.21 | | 10.99 | | 87.42 | | 2403 | 1324 | SSU-F | pre-40S |
| Noc4/Utp19 - | NOC4L | 63.62 | 58.46 | 6.01 | 7.08 | 14.31 | 26.16 | 3607 | 1572 | SSU-F | pre-40S |
| Rrp7 | RRP7A | 34.47 | 32.33 | 9.35 | 9.61 | 36.36 | 51.07 | 5813 | 2489 | SSU-F | pre-40S |
| Utp30 | RSL1D1 | 31.63 | 54.97 | 10.15 | 10.13 | 24.82 | 49.59 | 3195 | 1655 | SSU-F | pre-40S |
| Efg1 | | 27.13 | | 10.15 | | 66.09 | | 4774 | 2174 | SSU-In | pre-40S |
| Hca4/Dbp4/ Ecm24 † | DDX10 | 87.19 | 100.88 | 7.47 | 8.72 | 39.09 | 42.4 | 6606 | 7745 | SSU-In | pre-40S |
| Nop6 # | | 25.23 | | 10.87 | | 64.44 | | 5962 | 3007 | SSU-In | pre-40S |
| Nop9 #& | NOP9 | 77.72 | 69.43 | 8.38 | 6.86 | 22.07 | 46.23 | 4330 | 1859 | SSU-In | pre-40S |
| Nsr1/She5 # | NCL | 44.51 | 76.61 | 4.58 | 4.6 | 63.53 | 55.49 | 25614 | 21560 | SSU-In | pre-40S |
| Bfr2 | AATF | 61.16 | 63.13 | 4.41 | 4.82 | 37.88 | 55.89 | 7791 | 3997 | unknown | pre-40S |
| Bms1 ^ | BMS1 | 135.56 | 145.80 | 6.79 | 6.04 | 48.77 | 42.59 | 4889 | 3601 | unknown | pre-40S |
| Bud22 | SRFBP1 | 60.06 | 48.63 | 9.28 | 9.59 | 48.55 | 59.44 | 3797 | 1934 | unknown | pre-40S |
| Bud23 % | WBSCR22 | 30.74 | 31.88 | 9.48 | 8.95 | 34.18 | 44.13 | 4328 | 2802 | unknown | pre-40S |
| Cms1 | CMSS1 | 33.39 | 31.88 | 8.56 | 9.26 | 35.05 | 38.35 | 4906 | 3267 | unknown | pre-40S |
| Dim1 % | PNO1 | 35.96 | 27.92 | 10.16 | 9.75 | 28.3 | 48.02 | 5449 | 3874 | unknown | pre-40S |
| Dim2 # | PNO1 | 30.33 | 27.92 | 9.71 | 9.75 | 52.55 | 48.02 | 5172 | 2826 | unknown | pre-40S |
| Ecm16/Dhr1 † | DHX37 | 144.93 | 129.54 | 6.26 | 8.36 | 40.49 | 46.59 | 4312 | 1955 | unknown | pre-40S |
| Emg1/Nep1 % | EMG1 | 27.89 | 26.72 | 8.58 | 9.28 | 20.24 | 27.46 | 7148 | 5216 | unknown | pre-40S |
| Enp1/Meg1 | BYSL | 55.10 | 49.60 | 4.56 | 8.19 | 39.96 | 47.83 | 7222 | 4442 | unknown | pre-40S |
| Enp2 * | NOL10 | 81.72 | 80.30 | 6.54 | 8.64 | 48.09 | 31.54 | 5078 | 2812 | unknown | pre-40S |
| Esf2/Abt1 # | | 36.40 | | 8.37 | | 67.41 | | 4489 | 3189 | unknown | pre-40S |
| Faf1 | | 38.89 | | 10.02 | | 73.41 | | 2182 | 1168 | unknown | pre-40S |
| Fal1 † | EIF4A3 | 45.22 | 46.87 | 9.49 | 6.3 | 16.79 | 30.41 | 2165 | 629 | unknown | pre-40S |
| Kre33/Rra1 | NAT10 | 119.35 | 115.72 | 7.94 | 8.5 | 32.43 | 31.28 | 8767 | 5717 | unknown | pre-40S |
| Kri1 | KRI1 | 68.62 | 82.30 | 4.74 | 5.06 | 57.06 | 60.77 | 9342 | 12381 | unknown | pre-40S |
| Krr1 # | KRR1 | 37.17 | 43.66 | 10 | 9.78 | 49.05 | 43.31 | 6288 | 3601 | unknown | pre-40S |
| Lcp5 | NGDN | 40.79 | 35.89 | 6.91 | 9.57 | 62.59 | 61.13 | 2006 | 1325 | unknown | pre-40S |

Table S2 (contd.)

| Table S2. Master List of Assembly Factors and Their Physical Properties | | | | | | | | | | | |
|---|-----------|----------|----------|-------|-------|------------------------|------------------------|----------------|---------------|---------|-------------------|
| name | name | MW (kDa) | MW (kDa) | IEP | IEP | predicted disorder (%) | predicted disorder (%) | abundance mean | abundance STD | subset | tripartite domain |
| Mrd1 # | RBM19 | 101.11 | 107.33 | 6.5 | 6.13 | 46.34 | 35.34 | 4887 | 3492 | unknown | pre-40S |
| Nop14/Utp2 | NOP14 | 94.30 | 97.66 | 7.44 | 7.33 | 47.04 | 55.19 | 5441 | 3311 | unknown | pre-40S |
| Rio1/Rrp10 | RIOK1 | 56.11 | 65.45 | 6.53 | 5.84 | 36.57 | 44.27 | 2556 | 1383 | unknown | pre-40S |
| Rok1 † | DDX52 | 63.67 | 67.49 | 9.73 | 9.69 | 49.38 | 28.05 | 7571 | 8349 | unknown | pre-40S |
| Rrp3 † | DDX47 | 55.98 | 50.64 | 9.71 | 9.18 | 31.54 | 39.34 | 5173 | 2608 | unknown | pre-40S |
| Rrp12 · | RRP12 | 137.50 | 143.70 | 7.02 | 8.97 | 25.81 | 34.77 | 7240 | 3716 | unknown | pre-40S |
| Rrt14 | | 23.86 | | 10.56 | | 82.04 | | 3228 | 2867 | unknown | pre-40S |
| Sgd1 & | NOM1 | 102.84 | 96.25 | 5.74 | 8.28 | 36.93 | 53.72 | 2544 | 1238 | unknown | pre-40S |
| Slx9 | | 24.08 | | 10.73 | | 59.52 | | 4639 | 1530 | unknown | pre-40S |
| Utp14 | UTP14A | 102.97 | 87.97 | 4.68 | 7.67 | 73.08 | 71.47 | 5126 | 2989 | unknown | pre-40S |
| Utp14 | UTP14C | 102.97 | 87.18 | 4.68 | 6.7 | 71.87 | 63.17 | 5126 | 2989 | unknown | pre-40S |
| Utp20 & | UTP20 | 287.55 | 318.38 | 7.03 | 7.07 | 12.99 | 21.17 | 3802 | 2320 | unknown | pre-40S |
| Utp22 | NOL6 | 140.49 | 127.59 | 8.72 | 7.42 | 28.91 | 15.82 | 7828 | 4461 | unknown | pre-40S |
| Utp23 | UTP23 | 28.82 | 28.40 | 10.74 | 10.08 | 50 | 33.73 | 3903 | 1531 | unknown | pre-40S |
| Brx1 + | BRX1 | 33.54 | 44.40 | 9.81 | 9.92 | 28.52 | 47.88 | 10449 | 4015 | LSU-Ou | pre-LSU |
| Cic1/Nsa3 | RSL1D1 | 42.53 | 54.97 | 9.39 | 10.13 | 27.13 | 49.59 | 11772 | 9346 | LSU-Ou | pre-LSU |
| Fpr3/Npi46 | FPR3 | 46.49 | 39.96 | 4.09 | 8.12 | 79.08 | 11.9 | 10680 | 4750 | LSU-Ou | pre-LSU |
| Mak5 † | DDX24 | 87.05 | 96.33 | 8.76 | 9.14 | 42.69 | 44.7 | 4105 | 2344 | LSU-Ou | pre-LSU |
| Mak11 * | PAK1IP1 | 53.67 | 43.96 | 8.66 | 8.66 | 19.13 | 18.59 | 4910 | 2542 | LSU-Ou | pre-LSU |
| Mak21/Noc1 & | CEBPZ | 116.62 | 120.97 | 4.63 | 5.65 | 35.41 | 37.76 | 7700 | 3421 | LSU-Ou | pre-LSU |
| Mrt4 | MRT04 | 27.06 | 27.56 | 8.89 | 8.34 | 33.05 | 33.05 | 12109 | 5199 | LSU-Ou | pre-LSU |
| Noc2 · | NOC2L | 81.60 | 84.91 | 8.85 | 5.46 | 36.62 | 39.12 | 10327 | 7258 | LSU-Ou | pre-LSU |
| Noc3 · | NOC3L | 75.57 | 92.54 | 5.71 | 9.22 | 44.95 | 41.88 | 5027 | 2925 | LSU-Ou | pre-LSU |
| Nog1 ^ | | 74.42 | | 9.18 | | 41.58 | | 14566 | 7766 | LSU-Ou | pre-LSU |
| Nop2/Yna1 % | NOP2 | 69.78 | 89.30 | 4.68 | 9.27 | 52.91 | 60.84 | 11196 | 4039 | LSU-Ou | pre-LSU |
| Nop4/Nop77 # | RBM28 | 77.84 | 85.73 | 9.67 | 9.26 | 63.21 | 61.26 | 5443 | 2689 | LSU-Ou | pre-LSU |
| Nop7/Yph1 | PES1 | 69.86 | 68.00 | 5.26 | 6.93 | 44.46 | 41.84 | 10235 | 6330 | LSU-Ou | pre-LSU |
| Nop13 # | | 45.62 | | 9.94 | | 59.06 | | 6924 | 3734 | LSU-Ou | pre-LSU |
| Nop15 # | | 25.45 | | 9.91 | | 53.18 | | 7498 | 2435 | LSU-Ou | pre-LSU |
| Nop16 | NOP16 | 51.74 | 46.67 | 8.71 | 6.72 | 27.4 | 23.84 | 6417 | 2633 | LSU-Ou | pre-LSU |
| Puf6 # & | | 75.10 | | 6.88 | | 42.23 | | 9678 | 6137 | LSU-Ou | pre-LSU |
| Rix7 | NVL | 93.05 | 95.05 | 5 | 6.11 | 46.24 | 40.54 | 5682 | 4006 | LSU-Ou | pre-LSU |
| Rrp1 | RRP1 | 33.19 | 52.83 | 4.92 | 9.39 | 24.1 | 64.21 | 5406 | 2431 | LSU-Ou | pre-LSU |
| Rrp17 | NOL12 | 27.98 | 24.66 | 10.03 | 10.22 | 59.15 | 87.32 | 3792 | 2128 | LSU-Ou | pre-LSU |
| Ssf1 + | | 51.77 | | 9.96 | | 59.16 | | 5117 | 2126 | LSU-Ou | pre-LSU |
| Tif6/Cdc95 | EIF6 | 26.44 | 26.55 | 4.27 | 4.56 | 52.65 | 28.57 | 12902 | 7262 | LSU-Ou | pre-LSU |
| Ytm1/Cst14 | WDR12 | 51.35 | 47.70 | 6.96 | 5.57 | 35 | 15.82 | 6698 | 3329 | LSU-Ou | pre-LSU |
| Dbp6 † | DDX51 | 68.07 | 72.45 | 9.72 | 8.43 | 30.3 | 31.83 | 4861 | 2833 | LSU-F | pre-LSU |
| Rsa3 | | 24.64 | | 4.51 | | 40.91 | | 4878 | 1995 | LSU-F | pre-LSU |
| Urb1/Npa1 | URB1/NPA1 | 203.28 | 254.38 | 7.35 | 6.03 | 9.52 | 23.43 | 4666 | 1877 | LSU-F | pre-LSU |
| Urb2/Npa2 | URB2/NPA2 | 135.09 | 170.54 | 6.33 | 6.97 | 9.63 | 12.93 | 3932 | 2647 | LSU-F | pre-LSU |
| Bmt2 % | BMT2 | 38.57 | 46.32 | 10.28 | 5.75 | 26.71 | 27.65 | 803 | 687 | unknown | pre-LSU |
| Bud20 | ZNF93 | 18.52 | 15.19 | 9.3 | 9.9 | 50.6 | 79.85 | 5349 | 2142 | unknown | pre-LSU |
| Cgr1 | | 14.43 | | 11.2 | | 97.5 | | 4596 | 2094 | unknown | pre-LSU |
| Dbp7 † | DDX31 | 83.33 | 94.08 | 9.84 | 10.01 | 22.91 | 40.54 | 2715 | 1711 | unknown | pre-LSU |
| Dbp9 † | DDX56 | 68.07 | 61.58 | 9.72 | 9.34 | 33.52 | 32.8 | 7865 | 7383 | unknown | pre-LSU |
| Dbp10 † | DDX54 | 112.96 | 98.59 | 9.71 | 10.03 | 38.77 | 51.38 | 5311 | 3228 | unknown | pre-LSU |
| Drs1 † | DDX27 | 84.83 | 89.83 | 5.41 | 9.33 | 55.98 | 46.48 | 5785 | 2903 | unknown | pre-LSU |
| Ebp2 | EBNA1BP2 | 49.72 | 34.85 | 6.34 | 10.1 | 68.85 | 50 | 11139 | 10585 | unknown | pre-LSU |
| Erb1 * | | 91.66 | | 4.8 | | 37.79 | | 8395 | 4787 | unknown | pre-LSU |
| Fpr4 | | 43.86 | | 4.31 | | 66.07 | | 10074 | 6507 | unknown | pre-LSU |
| Ipi1 & | | 37.88 | | 10.14 | | 18.26 | | 3250 | 1614 | unknown | pre-LSU |
| Ipi3 * | | 61.76 | | 5.5 | | 15.5 | | 5697 | 2575 | unknown | pre-LSU |

Table S2 (contd.)

| Table S2. Master List of Assembly Factors and Their Physical Properties | | | | | | | | | | | |
|---|---------|----------|----------|-------|-------|------------------------|------------------------|----------------|---------------|----------|-------------------|
| name | name | MW (kDa) | MW (kDa) | IEP | IEP | predicted disorder (%) | predicted disorder (%) | abundance mean | abundance STD | subset | tripartite domain |
| Jjj1 | DNAJC21 | 68.77 | 62.02 | 6.11 | 5.34 | 47.29 | 56.69 | 2082 | 1449 | unknown | pre-LSU |
| Loc1 | | 23.62 | | 11.07 | | 79.9 | | 5272 | 4337 | unknown | pre-LSU |
| Mak16 | MAK16 | 35.68 | 35.36 | 4.9 | 5.27 | 48.37 | 60 | 4068 | 1908 | unknown | pre-LSU |
| Ng12 | PDE12 | 59.54 | 67.35 | 7.96 | 6.11 | 29.32 | 26.27 | 4050 | 2114 | unknown | pre-LSU |
| Nip7 | NIP7 | 20.38 | 20.64 | 9.75 | 8.66 | 12.15 | 11.1 | 8306 | 2906 | unknown | pre-LSU |
| Nop8 # | | 56.95 | | 5.75 | | 45.87 | | 7413 | 12383 | unknown | pre-LSU |
| Nop12 # | RBM34 | 51.96 | 48.56 | 10.13 | 10.11 | 45.11 | 51.32 | 7772 | 3542 | unknown | pre-LSU |
| Nop53 | NOP53 | 52.57 | 54.38 | 10.04 | 10.32 | 57.36 | 83.26 | 5690 | 3023 | unknown | pre-LSU |
| Nsa1 | WDR74 | 51.90 | 42.44 | 8.06 | 8.64 | 23.11 | 29.87 | 5501 | 2937 | unknown | pre-LSU |
| Nsa2 | NSA2 | 29.75 | 30.06 | 10.97 | 10.28 | 58.24 | 56.54 | 35483 | 35752 | unknown | pre-LSU |
| Nug1 ^ | NUGGC | 57.71 | 91.13 | 9.44 | 8.85 | 42.88 | 39.07 | 10176 | 5823 | unknown | pre-LSU |
| Rcm1 % | | 56.18 | | 9.02 | | 25.1 | | 2016 | 1355 | unknown | pre-LSU |
| Rea1/Mdn1 | MDN1 | 559.08 | 632.82 | 4.63 | 5.46 | 24.99 | 28.43 | 5887 | 5392 | unknown | pre-LSU |
| Rix1/Ipi2 | PELP1 | 86.71 | 119.69 | 5.34 | 4.29 | 18.35 | 61.33 | 4644 | 2074 | unknown | pre-LSU |
| Rlp7 | | 36.57 | | 10.14 | | 53.42 | | 8857 | 3068 | unknown | pre-LSU |
| Rlp24 | RSL24D1 | 23.98 | 19.62 | 10.21 | 9.99 | 70.85 | 47.24 | 6004 | 4065 | unknown | pre-LSU |
| Rpf1 + | RPF1 | 35.13 | 40.11 | 10.08 | 10.01 | 31.19 | 40.4 | 6121 | 3435 | unknown | pre-LSU |
| Rpf2 + | RPF2 | 39.60 | 35.58 | 9.57 | 10 | 39.83 | 33.99 | 9388 | 7843 | unknown | pre-LSU |
| Rrb1 * | GRWD1 | 52.21 | 49.41 | 4.23 | 4.82 | 40.12 | 30.94 | 9170 | 5803 | unknown | pre-LSU |
| Rrp6 | EXOSC10 | 84.03 | 100.83 | 7.15 | 8.68 | 30.42 | 30.73 | 4148 | 2743 | unknown | pre-LSU |
| Rrp8 % | | 46.00 | | 10.1 | | 39.03 | | 4016 | 1322 | unknown | pre-LSU |
| Rrp15 | RRP15 | 28.20 | 31.48 | 5.43 | 5.39 | 65.6 | 74.82 | 5969 | 1870 | unknown | pre-LSU |
| Rrs1 | RRS1 | 22.97 | 41.19 | 10.14 | 10.69 | 58.62 | 43.96 | 8448 | 3397 | unknown | pre-LSU |
| Rsa4 * | NLE1 | 57.03 | 53.32 | 9.18 | 6.92 | 16.89 | 15.88 | 4313 | 2097 | unknown | pre-LSU |
| Sda1 & | | 86.61 | | 6.93 | | 47.98 | | 4189 | 3101 | unknown | pre-LSU |
| Spb1 % | FTSJ3 | 96.48 | 96.55 | 8.29 | 8.53 | 50.89 | 61.98 | 5451 | 3109 | unknown | pre-LSU |
| Spb4 l | DDX55 | 69.43 | 68.54 | 9.54 | 9.32 | 28.22 | 26.17 | 3067 | 1157 | unknown | pre-LSU |
| Sqt1 * | | 47.12 | | 4.09 | | 22.74 | | 7749 | 3699 | unknown | pre-LSU |
| Ssf2 + | | 51.86 | | 9.69 | | 56.73 | | 2659 | 1604 | unknown | pre-LSU |
| Tri1 | | 26.48 | | 10.16 | | 50.44 | | 2300 | 1066 | unknown | pre-LSU |
| Fcf1/Utp24 | FCF1 | 21.63 | 23.36 | 10.48 | 9.74 | 37.04 | 49.49 | 2689 | 1920 | nuclease | N/A |
| Las1 | LAS1L | 59.35 | 83.06 | 8.05 | 4.64 | 25.3 | 35.69 | 1146 | 597 | nuclease | N/A |
| Nob1 | NOB1 | 51.74 | 46.67 | 8.71 | 6.72 | 43.14 | 29.13 | 5287 | 3149 | nuclease | N/A |
| Rcl1 | RCL1 | 40.17 | 40.84 | 9.23 | 9.36 | 34.33 | 23.32 | 6025 | 2731 | nuclease | N/A |
| Rnt1 | | 54.07 | | 9.06 | | 36.52 | | 2800 | 1291 | nuclease | N/A |
| Dbp3 | | 58.84 | | 9.73 | | 20.84 | | 13426 | 7719 | shared | N/A |
| Has1 | DDX18 | 56.73 | 75.40 | 9.88 | 9.52 | 23.98 | 31.04 | 12037 | 4084 | shared | N/A |
| Prp43/Ja1 | DHX15 | 87.54 | 90.93 | 6.38 | 7.12 | 39.11 | 40.13 | 8852 | 3735 | shared | N/A |
| Rrp5 | PDCD11 | 193.11 | 208.70 | 6.07 | 8.99 | 32.97 | 26.56 | 15799 | 9124 | shared | N/A |
| Cbf5 | DKC1 | 54.71 | 57.67 | 9.34 | 9.46 | 29.81 | 36.19 | 18964 | 8567 | snoRNP | N/A |
| Gar1 | GAR1 | 21.50 | 22.34 | 11.99 | 10.91 | 37.56 | 31.34 | 19463 | 10558 | snoRNP | N/A |
| Nhp2 | NHP2 | 17.13 | 17.20 | 10.45 | 8.48 | 35.26 | 50.33 | 16956 | 6672 | snoRNP | N/A |
| Nop1/Lot3 | FBL | 34.48 | 33.78 | 10.85 | 10.18 | 59.33 | 44.24 | 31821 | 28378 | snoRNP | N/A |
| Nop10 | DKCB1 | 6.64 | 7.70 | 10.78 | 10.01 | 63.79 | 42.19 | 14475 | 10128 | snoRNP | N/A |
| Nop56/Sik1 | NOP56 | 56.87 | 66.04 | 9.43 | 9.24 | 28.57 | 44.78 | 30862 | 19939 | snoRNP | N/A |
| Nop58/Nop5 | NOP58 | 56.96 | 59.57 | 9.55 | 9.03 | 44.23 | 36.86 | 27653 | 9644 | snoRNP | N/A |
| Snu13 | SNU13 | 13.57 | 14.17 | 8.04 | 8.72 | 18.25 | 20.31 | 50898 | 59087 | snoRNP | N/A |
| Csm1 | | 21.74 | | 4.82 | | 8.95 | | 2655 | 2061 | rDNAP | N/A |
| Fob1 | | 65.30 | | 6.1 | | 39.58 | | 1734 | 1079 | rDNAP | N/A |
| Hmo1 | | 27.54 | | 9.11 | | 47.56 | | 16564 | 14005 | rDNAP | N/A |
| Lrs4 | | 39.36 | | 10.21 | | 62.54 | | 1473 | 917 | rDNAP | N/A |
| Top1 | | 90.00 | | 9.22 | | 34.98 | | 5012 | 3280 | rDNAP | N/A |
| Top2 | | 164.21 | | 7.04 | | 27.94 | | 5856 | 4193 | rDNAP | N/A |

Table S2 (contd.)

| Table S2. Master List of Assembly Factors and Their Physical Properties | | | | | | | | | | | |
|---|------|----------|----------|------|-----|------------------------|------------------------|----------------|---------------|---------|-------------------|
| name | name | MW (kDa) | MW (kDa) | IEP | IEP | predicted disorder (%) | predicted disorder (%) | abundance mean | abundance STD | subset | tripartite domain |
| Rrn3 | | 72.34 | | 4.34 | | 27.43 | | 469 | 554 | RRN | N/A |
| Rrn5 | | 41.71 | | 6.85 | | 47.38 | | 811 | 568 | RRN | N/A |
| Rrn6 | | 102.01 | | 4.81 | | 34 | | 542 | 739 | RRN | N/A |
| Rrn7 | | 60.36 | | 8.25 | | 12.06 | | 1192 | 1122 | RRN | N/A |
| Rrn9 | | 42.79 | | 5.02 | | 41.92 | | 638 | 516 | RRN | N/A |
| Rrn10 | | 16.49 | | 4.85 | | 27.59 | | 1408 | 1842 | RRN | N/A |
| Rrn11 | | 59.24 | | 5.85 | | 36.29 | | 706 | 456 | RRN | N/A |
| Rpa12 | | | | | | | | 10655 | 5328 | RPA | N/A |
| Rpa14 | | | | | | | | 7820 | 10363 | RPA | N/A |
| Rpa34 | | | | | | | | 7722 | 3534 | RPA | N/A |
| Rpa43 | | | | | | | | 7471 | 3109 | RPA | N/A |
| Rpa49 | | | | | | | | 9948 | 3503 | RPA | N/A |
| Rpa135 | | | | | | | | 12623 | 6060 | RPA | N/A |
| Rpa190 | | | | | | | | 16112 | 8843 | RPA | N/A |
| Rpb1 | | | | | | | | 16726 | 11053 | RPB | N/A |
| Rpb2 | | | | | | | | 14925 | 6189 | RPB | N/A |
| Rpb3 | | | | | | | | 8911 | 4777 | RPB | N/A |
| Rpb7 | | | | | | | | 6897 | 3986 | RPB | N/A |
| Rpb8 | | | | | | | | 8439 | 4971 | RPB | N/A |
| Rpb9 | | | | | | | | 5395 | 2749 | RPB | N/A |
| Rpb11 | | | | | | | | 10860 | 6845 | RPB | N/A |
| Ret1 | | | | | | | | 4745 | 2562 | RPC | N/A |
| Rpc17 | | | | | | | | 3431 | 1714 | RPC | N/A |
| Rpc19 | | | | | | | | 15166 | 16514 | RPC | N/A |
| Rpc37 | | | | | | | | 4592 | 3392 | RPC | N/A |
| Rpc40 | | | | | | | | 15108 | 4005 | RPC | N/A |
| Rpc53 | | | | | | | | 3911 | 1354 | RPC | N/A |
| Rpc82 | | | | | | | | 4435 | 2123 | RPC | N/A |
| HHF1 | | | | | | | | 73439 | 141532 | histone | N/A |
| HHF2 | | | | | | | | 119590 | 176858 | histone | N/A |
| HHT1 | | | | | | | | 64338 | 101323 | histone | N/A |
| HHT2 | | | | | | | | 57624 | 99029 | histone | N/A |
| HTA1 | | | | | | | | 20958 | 21428 | histone | N/A |
| HTA2 | | | | | | | | 64804 | 39933 | histone | N/A |
| HTB1 | | | | | | | | 197857 | 267074 | histone | N/A |
| HTB2 | | | | | | | | 117817 | 122347 | histone | N/A |

[†] DExD/H-box proteins [#] RNA-binding proteins ⁺ brix domain [%] methyl transferases ^{*} WD repeats ⁻ HEAT repeats [&] ARM repeats [^] GTPases

Table S2 Master List of Nucleolar Assembly Factors and their Physical Properties

The listed AFs are strongly concentrated in the nucleolus and are essentially absent from the nucleoplasm and cytoplasm [1]. The shaded columns correspond to yeast

values while the non-shaded columns correspond to human values. The following AFs

were not included: Pfa1/Sqs1, Rrp36, Sen1/Cik3 and Utp25.

| Table S3. Physical Properties of Subgroups of Assembly Factors | | | | |
|---|---------------|----------------|------------|-------------|
| Motif Groupings | | Subunit | MW | IEP |
| ARM Motif | Ipi1 | LSU | 38 | 10.1 |
| | Nop9 | pre-40S | 78 | 8.4 |
| | Puf6 | LSU | 75 | 6.9 |
| | Sgd1 | LSU | 103 | 6.9 |
| | Utp20 | SSU | 287 | 7.0 |
| | mean +/- S.D. | | 116 +/- 88 | 7.8 +/- 1.2 |
| Brix Domain | Brx1 | LSU | 33 | 9.8 |
| | Imp4 | 5'-ETS | 33 | 9.5 |
| | Rpf1 | LSU | 35 | 10.0 |
| | Ssf1 | LSU | 52 | 10.0 |
| | Ssf2 | LSU | 52 | 9.7 |
| | mean +/- S.D. | | 41 +/- 9 | 9.8 +/- 0.2 |
| DEXD/H-box | Dbp3 | pre-40S & LSU | 59 | 9.7 |
| | Dbp4/Hca4 | pre-40S | 87 | 7.5 |
| | Dbp6 | LSU | 70 | 6.2 |
| | Dbp7 | LSU | 83 | 9.8 |
| | Dbp8 | SSU | 47 | 10.2 |
| | Dbp9 | LSU | 68 | 9.2 |
| | Dbp10 | LSU | 113 | 9.7 |
| | Dhr1 | SSU | 145 | 6.3 |
| | Dhr2 | SSU | 83 | 9.2 |
| | Drs1 | LSU | 84 | 5.4 |
| | Fal1 | SSU | 45 | 9.5 |
| | Has1 | pre-40S & LSU | 57 | 9.9 |
| | Mak5 | LSU | 87 | 8.8 |
| | Prp43 | pre-40S & LSU | 88 | 6.4 |
| | Rok1 | pre-40S | 63 | 9.7 |
| | Rrp3 | SSU | 55 | 9.7 |
| | Spb4 | LSU | 69 | 9.5 |
| | mean +/- S.D. | | 77 +/- 24 | 8.7 +/- 1.5 |
| GTPases or putative GTPases | Bms1 | SSU | 135 | 6.8 |
| | Efl1 | pre-LSU | 124 | 4.7 |
| | Lsg1 | pre-LSU | 73 | 9.2 |
| | Nog1 | pre-LSU | 74 | 9.2 |
| | Nog2 | pre-LSU | 55 | 10.2 |
| | Nug1 | pre-LSU | 58 | 9.4 |
| | mean +/- S.D. | | 80 +/- 32 | 8.9 +/- 1.2 |
| HEAT Repeat | Noc2 | LSU | 81 | 8.8 |
| | Noc3 | LSU | 75 | 5.7 |
| | Noc4/Utp19 | SSU | 63 | 6.0 |
| | Rrp12 | SSU | 137 | 7.0 |
| | Sda1 | LSU | 87 | 6.9 |
| | Utp10 | 5'-ETS | 200 | 6.4 |
| | mean +/- S.D. | | 107 +/- 47 | 6.8 +/- 1.0 |
| Methyl Transfer | Bmt2 | LSU | 39 | 10.3 |
| | Bud23 | SSU | 32 | 9.5 |
| | Dim1 | SSU | 36 | 10.2 |
| | Emg1 | pre-40S | 28 | 8.6 |

| Table S3. Physical Properties of Subgroups of Assembly Factors | | | | |
|---|------------------|----------------|-----------|-------------|
| Motif Groupings | | Subunit | MW | IEP |
| | Nop1 | snoRNP/5'-ETS | 34 | 10.8 |
| | Nop2 | LSU | 70 | 4.7 |
| | Rcm1 | LSU | 56 | 9.0 |
| | Rrp8 | LSU | 46 | 10.1 |
| | Spb1 | LSU | 96 | 8.3 |
| | mean +/- S.D. | | 49 +/- 20 | 9.0 +/- 1.7 |
| RNA-binding Proteins (Motifs) | Dim2/Pno1 (KH) | pre-40S | 30 | 9.7 |
| | Esf2 (RRM) | pre-40S | 36 | 8.4 |
| | Imp3 (S4 domain) | 5'-ETS | 21 | 10.3 |
| | Krr1 (KH) | pre-40S | 37 | 10.0 |
| | Mrd1 (RRM) | pre-40S | 101 | 6.5 |
| | Nop4 (RRM) | LSU | 77 | 9.4 |
| | Nop6 (RRM) | pre-40S | 25 | 10.9 |
| | Nop8 (RRM) | LSU | 57 | 5.7 |
| | Nop9 (pumilio) | pre40S | 77 | 8.3 |
| | Nop12 (RRM) | LSU | 51 | 12.1 |
| | Nop13 (RRM) | LSU | 46 | 9.9 |
| | Nop15 (RRM) | LSU | 25 | 9.9 |
| | Nsr1 (RRM) | pre-40S | 44 | 4.6 |
| | Puf6 (pumilio) | LSU | 75 | 6.9 |
| | mean +/- S.D. | | 50 +/- 23 | 8.7 +/- 2.0 |
| WD Repeat Proteins | Enp2 | pre-40S | 82 | 6.5 |
| | Erb1 | LSU | 92 | 4.8 |
| | Ipi3 | LSU | 62 | 5.5 |
| | Mak11 | LSU | 53 | 8.7 |
| | Rrb1 | LSU | 52 | 4.2 |
| | Rrp9 | 5'-ETS | 65 | 5.4 |
| | Rsa4 | LSU | 57 | 9.2 |
| | Sof1 | 5'-ETS | 57 | 9.9 |
| | Utp1 | 5'-ETS | 103 | 4.7 |
| | Utp4 | 5'-ETS | 88 | 6.5 |
| | Utp5 | 5'-ETS | 72 | 4.6 |
| | Utp7 | 5'-ETS | 62 | 10.0 |
| | Utp12 | 5'-ETS | 106 | 5.9 |
| | Utp13 | 5'-ETS | 91 | 5.0 |
| | Utp15 | 5'-ETS | 57 | 9.8 |
| | Utp17 | 5'-ETS | 101 | 6.3 |
| | Utp18 | 5'-ETS | 66 | 4.6 |
| | Utp21 | 5'-ETS | 104 | 7.9 |
| | Ytm1 | LSU | 51 | 7.0 |
| | mean +/- S.D. | | 75 +/- 19 | 6.6 +/-1.9 |
| snoRNP Proteins | Cbf5 | pre-40S | 54 | 9.3 |
| | Gar1 | pre-40S | 21 | 12.0 |
| | Nhp2 | pre-40S | 17 | 10.4 |
| | Nop1 | snoRNP/5'-ETS | 34 | 10.8 |
| | Nop10 | snoRNP/pre-40S | 6 | 10.8 |
| | Nop56/Sik1 | snoRNP/5'-ETS | 56 | 9.4 |
| | Nop58 | snoRNP/5'-ETS | 57 | 9.5 |

| Table S3. Physical Properties of Subgroups of Assembly Factors | | | |
|---|----------------|-----------|--------------|
| Motif Groupings | Subunit | MW | IEP |
| Snu13 (U3) | snoRNP/5'-ETS | 13 | 8.0 |
| mean +/- S.D. | | 32 +/- 19 | 10.0 +/- 1.1 |

Table S3 Physical Properties of Subgroups of Assembly Factors

The quadrants illustrated in Figure 3B/C are differentially enriched in subsets of AFs.

This table lists the physical properties of the relevant groups.

| Table S4A. Isoelectric Point and Predicted Disorder Extremes | | | | | | |
|---|---|------------------|--|------------------|-----------------------------------|------------------|
| | most acidic (<25th percentile) | | most basic (>75th percentile) | | most disordered (> 60%) | |
| | yeast AFs | human AFs | yeast AFs | human AFs | yeast AFs | human AFs |
| 5'-ETS | (< 4.72) | (< 5.81) | (> 9.69) | (> 9.24) | (> 60%) | (> 60%) |
| | <i>Pwp2</i> | <i>PWP2</i> | <i>Utp15</i> | <i>DCAF13</i> | <i>Mpp10</i> | <i>DNTTIP2</i> |
| | <i>Utp18</i> | <i>WDR75</i> | <i>Sof1</i> | <i>IMP4</i> | <i>Fcf2</i> | <i>UTP11L</i> |
| | <i>Utp5</i> | <i>UTP3</i> | <i>Utp7</i> | <i>IMP3</i> | <i>Utp11</i> | <i>UTP3</i> |
| | <i>Utp9</i> | <i>WDR43</i> | <i>Fcf2</i> | <i>WDR46</i> | <i>Bud21</i> | |
| | <i>Sas10</i> | <i>MPHOSPH10</i> | <i>Imp3</i> | <i>UTP11L</i> | | |
| | <i>Mpp10</i> | | <i>Utp11</i> | | | |
| pre-40S | (< 6.53) | (< 6.91) | (> 9.93) | (> 9.49) | (> 60%) | (> 60%) |
| | <i>Rio1</i> | <i>NOP9</i> | <i>Krr1</i> | <i>NGDN</i> | <i>Lcp5</i> | <i>KRI1</i> |
| | <i>Mrd1</i> | <i>UTP14C</i> | <i>Faf1</i> | <i>SRFBP1</i> | <i>Nsr1</i> | <i>NGDN</i> |
| | <i>Ecm16</i> | <i>EIF4A3</i> | <i>Utp30</i> | <i>RRP7A</i> | <i>Nop6</i> | <i>UTP14C</i> |
| | <i>Noc4</i> | <i>RBM19</i> | <i>Efg1</i> | <i>DDX52</i> | <i>Efg1</i> | <i>UTP14A</i> |
| | <i>Sgd1</i> | <i>BMS1</i> | <i>Dim1</i> | <i>PNO1</i> | <i>Esf2</i> | |
| | <i>Kri1</i> | <i>RIOK1</i> | <i>Dbp8</i> | <i>KRR1</i> | <i>Utp14</i> | |
| | <i>Utp14</i> | <i>KRI1</i> | <i>Rrt14</i> | <i>UTP23</i> | <i>Faf1</i> | |
| | <i>Nsr1</i> | <i>AATF</i> | <i>Slx9</i> | <i>RSL1D1</i> | <i>Rrt14</i> | |
| | <i>Enp1</i> | <i>NCL</i> | <i>Utp23</i> | | <i>Fyv7</i> | |
| | <i>Bfr2</i> | | <i>Nop6</i> | | | |
| | | | <i>Fyv7</i> | | | |
| pre-LSU | (< 5.48) | (< 6.09) | (> 9.85) | (> 9.93) | (> 60%) | (> 60%) |
| | <i>Rrp15</i> | <i>URB1</i> | <i>Nop15</i> | <i>RSL24D1</i> | <i>Nop4</i> | <i>NOP2</i> |
| | <i>Drs1</i> | <i>BMT2</i> | <i>Nop13</i> | <i>RPF2</i> | <i>Rrp15</i> | <i>RBM28</i> |
| | <i>Rix1</i> | <i>CEBPZ</i> | <i>Ssf1</i> | <i>DDX31</i> | <i>Fpr4</i> | <i>PELP1</i> |
| | <i>Nop7</i> | <i>WDR12</i> | <i>Rrp17</i> | <i>EBNA1BP2</i> | <i>Ebp2</i> | <i>FTSJ3</i> |
| | <i>Rix7</i> | <i>NOC2L</i> | <i>Nop53</i> | <i>RBM34</i> | <i>Rlp24</i> | <i>RRP1</i> |
| | <i>Rrp1</i> | <i>MDN1</i> | <i>Rpf1</i> | <i>RSL1D1</i> | <i>Fpr3</i> | <i>RRP15</i> |
| | <i>Mak16</i> | <i>RRP15</i> | <i>Rrp8</i> | <i>NOL12</i> | <i>Loc1</i> | <i>ZNF93</i> |
| | <i>Erb1</i> | <i>DNAJC21</i> | <i>Nop12</i> | <i>NSA2</i> | <i>Cgr1</i> | <i>NOP53</i> |
| | <i>Nop2</i> | <i>MAK16</i> | <i>Ipi1</i> | <i>NOP53</i> | | <i>NOL12</i> |
| | <i>Rea1</i> | <i>GRWD1</i> | <i>Rlp7</i> | <i>RRS1</i> | | |
| | <i>Mak21</i> | <i>EIF6</i> | <i>Rrs1</i> | | | |
| | <i>Rsa3</i> | <i>PELP1</i> | <i>Tri1</i> | | | |
| | <i>Fpr4</i> | | <i>Rlp24</i> | | | |
| | <i>Tif6</i> | | <i>Bmt2</i> | | | |
| | <i>Rrb1</i> | | <i>Nsa2</i> | | | |
| | <i>Sqt1</i> | | <i>Loc1</i> | | | |
| <i>Fpr3</i> | | <i>Cgr1</i> | | | | |

| Table S4B Outliers from the Comparison of Yeast vs Human | | | | | | |
|---|---------------------------|---------------|----------------------------|---------------|--------------------------|---------------|
| | isoelectric point outlier | | predicted disorder outlier | | molecular weight outlier | |
| | yeast homolog | human homolog | yeast homolog | human homolog | yeast homolog | human homolog |
| 5'-ETS | Fcf2 | DNTTIP2 | Sof1 | DCAF13 | Utp10 | HEAR1 |
| | Utp18 | UTP18 | | | Fcf2 | DNTTIP2 |
| pre-40S | Utp14 | UTP14A | Nop9 | NOP9 | Utp30 | RSL1D1 |
| | Fal1 | EIF4A3 | Utp30 | RSL1D1 | Utp20 | UTP20 |
| | Enp1 | BYSL | | | Nsr1 | NCL |
| pre-LSU | Ebp2 | EBNA1BP2 | Rrp1 | RRP1 | Rix1 | PELP1 |
| | Drs1 | DDX27 | Rix1 | PELP1 | Nug1 | NUGGC |
| | Fpr3 | FPR3 | Fpr3 | FPR3 | Urb2 | URB2 |
| | Rrp1 | RRP1 | | | Urb1 | URB1 |
| | Bmt2 | BMT2 | | | Rea1 | MDN1 |
| | Nop2 | NOP2 | | | | |

Table S4 Extreme Values of Physical Parameters of Assembly Factors - related to Figure 2

(A) Isoelectric point and predicted disorder extremes for principal subsets (5'-ETS, pre-40S, pre-LSU) in both yeast and man. AFs are listed from top-down in order of increasing acidity for the most acidic and increasing basicity for the most basic. The entries for predicted disorder are organized in comparable fashion. The italicized AFs are those for which both yeast and human homologs have extreme values. The AFs that appear in multiple lists of extremes are highlighted.

(B) Outliers from the comparison of yeast vs human principal groups of AFs (5'-ETS, pre-40S, pre-LSU). Homolog pairs are listed from top-down in order of increasing difference between yeast and human values. The AFs that appeared in multiple lists of outliers are highlighted.

IQR Rule

The interquartile range (IQR) was calculated by subtracting the first quartile (Q_1) from the third quartile (Q_3) in each set of AF parameters. The boundaries for low and high outliers were then determined using the IQR rule (Equation 1, Equation 2).

$$\text{low outliers} < Q_1 - 1.5 * IQR \quad \text{Eq. 1}$$

$$\text{high outliers} > Q_3 + 1.5 * IQR \quad \text{Eq. 2}$$




















| Table S5. Assembly Factors with Shared Motif Signatures | | | | | | |
|--|---------------------------------|---|---|--------------------------------------|---|------------------------|
| | Signature Name, Activity | Color code | Prototypes | Number of Motifs in Prototype | Proteins with Variant Motif Signatures | Larger Grouping |
| 1 | DExD/H-box |  | Dbp7, [Dbp8], Dbp9 | 10 | Dbp3, Dbp4, Dpb6, Dbp10, [Dhr1], Dhr2, Drs1, Fal1, [Has1], Mak5, [Prp43], Rok1, [Rrp3], Spb4 | NTPases |
| 2 | GTPase/AAA ATPase |  | Rea1 | 3 | Bms1, [Fun12c], Lsg1c, Nog1, Nog2/Nug2, Nug1, [Sen1] | |
| 3 | ATPase |  | Drg1c, Rix7 | 8 | | |
| 4 | snoRNP |  | Nop56, Nop58 | 9 | | snoRNP proteins |
| 5 | HMG-like |  | Nhp2, Snu13 | 7 | (Cic1/Nsa3) | |
| 6 | Gar1 |  | Gar1 | 1 | | |
| 7 | RRM |  | Mrd1, Nop4, Nop12, Nop13, Nop15, [Nsr1] | 5 | Esf2, Nop6, Nop8, (Rrp7) | RNA-binding |
| 8 | Brix domain |  | Ssf1, Ssf2 | 4 | Brx1, Imp4, Rpf1, Rpf2 | |
| 9 | Methyl Transferases |  | Nop1 | 3 | Bmt2, Bud23, [Dim1], Emg1, Nop2, Rcm1, Rrp8, Spb1, [YGR283C] | |
| 10 | KH domain |  | Dim2, Krr1 | 3 | | Structural |
| 11 | ARM/HEAT |  | Ipi1, Nop9, Puf6, Rrp12, Sda1, Sgd1, Utp10, Utp20 | 1 | Mak21/Noc1, Noc3, (Noc4/Utp19) | |
| 12 | WD repeat and WD-like |  | Nan1/Utp17, Rrp9, Utp4, Utp18 | 6 | Enp2, Erb1, (Esf1), Ipi3, Mak11, (Nsa1), Rrb1, (Rrp5), Rsa4, Sof1, Utp1, (Utp5), Utp6, Utp7, (Utp9), Utp12, Utp13, Utp15, Utp21, Ytm1 | |
| 13 | Prolyl isomerase |  | Fpr3, Fpr4 | 8 | | Other |
| 14 | Zn-finger |  | Bud20, Jjj1 | 5 | Rei1 | |
| 15 | Kinases |  | Rio2c | 5 | Rio1c | |
| 16 | PUA domain |  | Cbf5*, Nip7 | 3 | | Various |
| 17 | GTP-binding |  | Efl1c | 10 | | |
| 18 | Unknown |  | Utp3 | 2 | Lcp5 | Various |
| 19 | |  | Sdo1 | 1 | | |
| Assembly Factors without Shared Motif Signatures | | | | | | |
| Alb1, Arx1, Bfr2, Bud21, Bud22, Cgr1, Ebp2, Efg1, Enp1, [Fcf2], [Fyv7], Imp3, Kre33, Kri1, Las1, Loc1, Ltv1c, Mak16, Mpp10, Mrt4, Ngl2, [Nob1], Noc2, Nop7, Nop16, Npa1/Urb1, Npa2, Nsa2, Pfa1/Sqs1c, Rcl1, Rix1, Rlp7, Rlp24, Rnt1, Rrp1, Rrp6, Rrp15, Rrs1, Rrt14, Rsa3, [Slx9], Snm1, Tif6, Tri1, Utp2, Utp8, Utp11, Utp14, Utp22, [Utp24/Fcf1], Yvh1. | | | | | | |
| <p>Color scheme: Green for 5'-ETS, Blue for pre-40S, Red for pre-LSU.</p> <p>Motif signatures under consideration are listed by the <i>Saccharomyces</i> Genome Database. If a motif is found in only a single AF, that AF is considered to lack shared motifs.</p> <p>Variant motif signatures can have more or fewer of the motifs by comparison to the prototypes.</p> <p>Entries in parentheses are marginally-related to the groups in which they are included.</p> <p>Domain assignments for AFs in brackets are provisional, judging from differences between two publications [6, 7].</p> <p>* Cbf5 is the pseudouridine synthase of H/ACA snoRNPs. These proteins have been recovered in association with the pre-40S segment of rRNA, unlike Nop1 and other box C/D snoRNP proteins, that are recovered with the 5'-ETS [6, 7].</p> <p>The suffix, c, signifies that the AF localizes largely to the cytoplasm.</p> <p>@ Low-stringency BLAST searches detect the following relations: Mrt4 with Rpp0, Tri1 with Uaf30 and Enp1, Fcf1/Utp24 with Utp23, and Tif6 with eIF6. Rlp7 and Rlp24 are considered placeholders for Rpl7 and Rpl24 [8] and Rrs1 binds Rpl11.</p> <p>The colors indicate the segments of rRNA with which the AFs are associated: Green: 5'-ETS, Blue: pre-40S, Red: pre-LSU. Purple: snoRNP.</p> | | | | | | |

Table S5 Hierarchic Clustering Motif Signatures - relevant to Figure 3D

AFs with motif(s) that are shared with at least one other AF were tabulated and assigned to 19 groups ("motif signatures"). The lower part of the table lists the AFs that do not share motif signatures with others.

Table S6. RNA-Binding Proteins Associated with rRNA Domains

| | 5'-ETS | pre-40S domains | | | | ITS 1 | pre-LSU domains | | | | | | | |
|------------------------|---|---------------------------|---|--|---------------------------------|------------|---|---|-------------|------------|------------|------------|-------------------------------|-------------|
| RNA domain | ETS | 5' Domain | Central | 3' Major | 3' Minor | ITS1 | ITS2-Ssf1 | D1 | D2 | D3 | D4 | D5 | D6 | late |
| RRM, KH, S4 or pumilio | Imp3 (10.3) | <u>Esf2</u> (8.4) | Kri1 (4.7) <u>Krr1</u> (10.1) Nsr1 ^{In} (4.6) | <u>Mrd1</u> (6.5) Nop6 (10.9) Nop9 ^{In} (8.4) | Pno1 (9.7) | Rrp5 (6.1) | | Nop12 (10.1) Nop15 (9.9) Rrp5 (6.1) | Nop4 (9.7) | | Puf6 (6.9) | | <u>Nop8^F</u> (5.7) | Nop13 (9.9) |
| DExD/H | | Dbp4 (7.5) Dbp8 (10.2) | Rok1 (9.7) | | Dhr1 (6.3) <u>Dhr2</u> (9.2) | | Dbp7* (9.8) Dbp9* (9.7) Dbp10 (9.7) | Has1 (9.9) | | Drs1 (5.4) | | | | |
| Brix domain | Imp4 (9.5) | | | | | | <u>Ssf1</u> (10.0) Ssf2 (9.7) | Brx1 (9.8) | Rpf1 (10.1) | | | Rpf2 (9.6) | | |
| MeTransferase | | | | Emg1 (8.6) | Rrp8 (10.1) | | | | | | | | <u>Nop2</u> (4.7) | |
| snoRNP proteins | Nop1 (10.8) <u>Nop5</u> ₆ (9.4) <u>Nop5</u> ₈ (9.5) <u>Snu1</u> ₃ (8.0) | | Cbf5 (9.3) Gar1 (12.0) <u>Nhp2</u> (10.4) Nop10 (10.8) | | | | | | | | | | | |

Derived from experiments in which AFs have been recovered along with 3'-truncated segments of rRNA [6, 7, 9, 10].
 The superscripts (In, F) designate AFs that belong to the SSU-In or LSU-F subsets.
 IEP values from the *Saccharomyces* Genome Database are indicated in parentheses.
 The underlined AFs interact with Nop1 on the basis of biochemical and genetic experiments, as is summarized in the *Saccharomyces* Genome Database.

Table S6 Assembly Factors Associated with Successive rRNA Domains - related to Table 3, Figure 4A.

The RNA-binding proteins listed were identified in experiments using 3'-truncated rRNAs that were expressed in yeast, retrieved from lysates and analyzed, as described in the text. Their IEPs are indicated in parentheses. Many additional AFs are also known to contact rRNA judging from cross-linking studies and cryo-EM studies. The column entitled ITS2-Ssf1 corresponds to a very early LSU precursor.

Table S7. Subsets of Assembly Factors in More Primitive Cell Types

| Subset of AFs | Orthologs in LECA * | Orthologs in <i>Archaea</i> | Candidates in <i>E. coli</i> |
|---|--|-------------------------------|---|
| SSU-F | Dbp8, Dhr2, Fyv7, #Nan1/Utp17, Noc4, Rrp7, #Utp5, #Utp6, #Utp13, #Utp15, Utp25 | | ARB1, BUD23, CDC14, CFD1, DBP5, DBP8, DED1, DIM1, DIS3 DRS1, FPR3, Kre33, MDN1, MTR4, NOP2, RCL1, REX2, RIX7, RLI1, RNT1, ROK1, RRP5, RRP6, SKI6 |
| SSU-In | Efg1, Nsr1 | | |
| LSU-F | Dbp6, Npa1/Urb1, Npa2/Urb2 | | |
| LSU-Ou | Brx1, Cic1, Mak11, Mrt4, Noc2, Noc3, Nop4, Nop7, Nop13, Nop15, Nop16, Puf6, Rrp1, Ytm1, Ssf1 | Nog1, Rix7, Tif6 | |
| snoRNP | Gar1, Nhp2, Nop56/Sik1, Nop58 | Gar1, Nop1, Nop10, Nop56/Sik1 | |
| shared | Dbp3, Prp43 | | |
| Derived from [11]. * We have included only those AFs that we have localized. # These AFs associate with the 5-ETS in yeast: Utp5, Utp6, Utp13, Utp15, Nan1/Utp17. | | | |

Table S7 Assembly Factors in Evolutionary Context - related to Table 1

Comparison of each subunit-specific subset with the likely repertoire of AFs in more primitive organisms. Based on [11].

Supplementary Figures and Legends

Figure S1

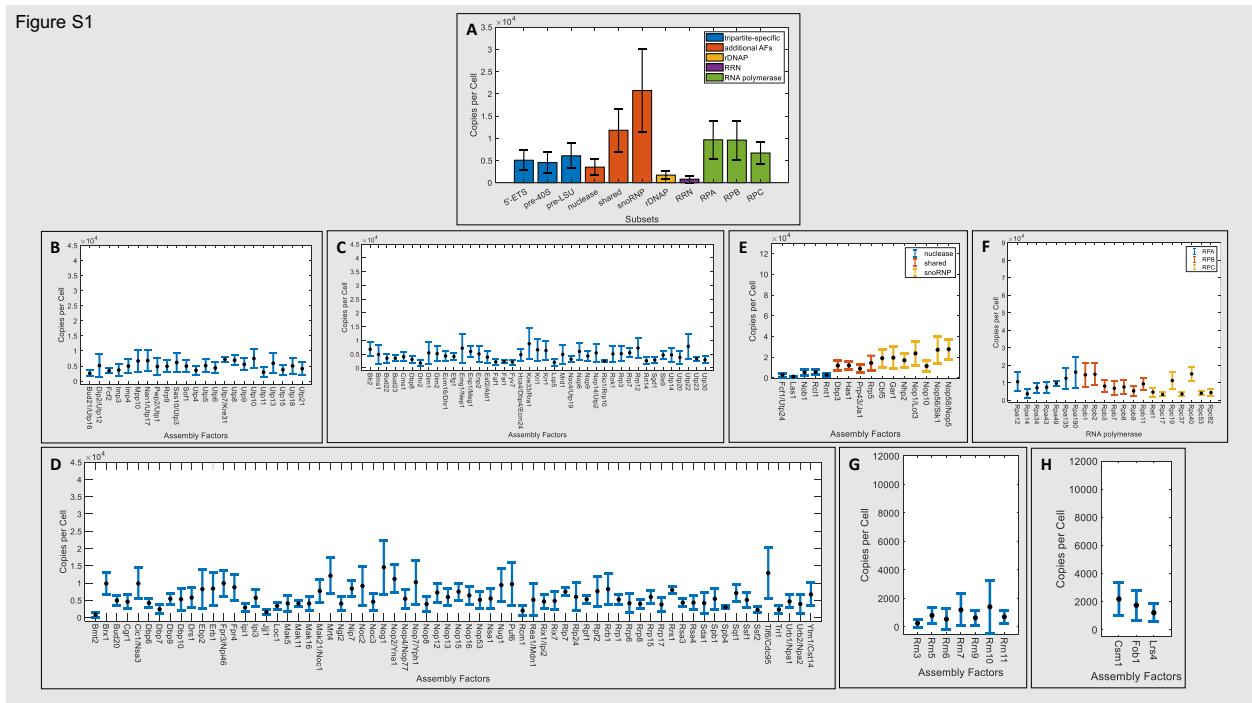


Figure S1 Copy Number per Cell of Single Assembly Factors - related to Figure 2

(A) Copy number estimate bar graphs. Mean values and standard deviations for single AFs as well as proteins associated with rDNA, and RNA polymerases. See Figure S1 and Table S4 for detail. Outlier values for each AF (based on the IQR rule - see Methods) and outlier AFs for each subset (due to unreasonably high standard deviations) were removed before these calculations were made. The following AFs were removed: Nsr1/She5, Nsa2, Hmo1, Top1 and Top2.

The representation is as in Figure 2 but concerns single proteins of **(B) 5'-ETS**, **(C) pre-40S**, **(D) pre-LSU**, **(E) additional AFs**, **(F) RNA polymerase**, **(G) Rrn proteins**, **(H) rDNAPs**. Outlier values for each AF were removed from consideration (Nsr1/She5, Nsa2, Hmo1, Snu13, Top1 and Top2). Nuclease: nucleases that cleave rRNA, Shared:

proteins needed to make both SSU and LSU, RPA/B/C: subunits of RNA polymerase A/B/C, Rrn: regulators of RNA polymerase A, rDNAPs: proteins that colocalize with the rDNA filament. For identification of the subsets of AFs, see Table S1.

Figure S2

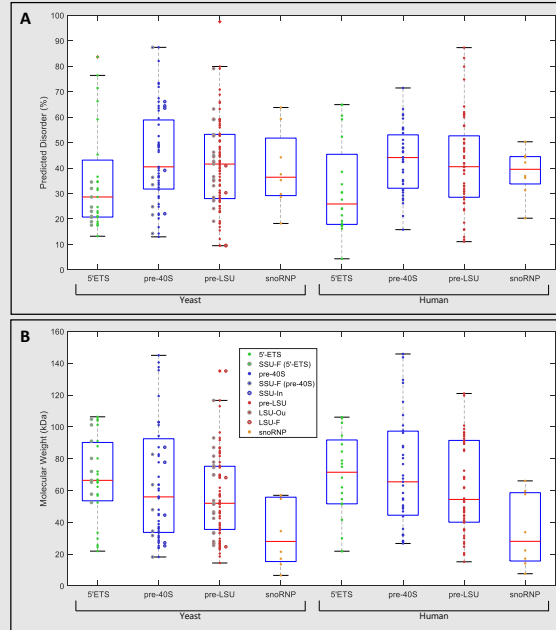


Figure S2 Predicted Disorder and Molecular Weight of Yeast and Human Assembly Factors

Assembly factors were grouped - as in Figure 3A - to illustrate the characteristics of those that associate with the 5'-ETS, the pre-40S segment, or the pre-LSU segment. In each case, the central column of values includes all relevant AFs listed in Table S2. For the 5'-ETS, the points to the left are SSU-F. For the pre-40S, the points to the left are SSU-F and the points to the right are SSU-In. For the pre-LSU, the points to the left are LSU-Ou and the points to the right are LSU-F. Human AFs are subgrouped according to the information for their yeast homologs.

Figure S3

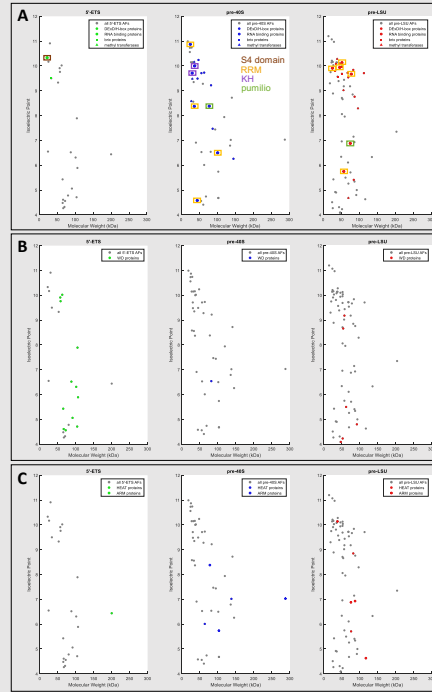


Figure S3 Overlay Representation of Physical Properties for Subsets of Assembly Factors - related to the 2-dimensional plots of Figure 3B/C

All AFs present in the each of the tripartite domains are shown in gray.

(A) RNA-associated proteins. The AFs in the upper quadrants include those with RNA-binding motifs (RRMs, KH and pumilio domains). They have a mean IEP of pH 8.5 and mean size of 64 kDa (pH 8.5, 64 kDa), DExD/H-box proteins (pH 8.7, 77 kDa), the methyl transferases (pH 9.0, 49 kDa), and Brix domain proteins (pH 9.8, 41 kDa). snoRNP proteins, although not illustrated, have the following mean values (pH 10.0, 32 kDa).

(B) WD-repeat proteins. The AFs with WD repeats are found mostly in the lower two quadrants (pH 6.6, 54 kDa).

(C) HEAT/ARM-repeat proteins. The AFs with HEAT repeats (pH 6.8, 107 kDa) and ARM repeats (pH 7.1, 116 kDa) have broadly distributed IEPs.

Figure S4

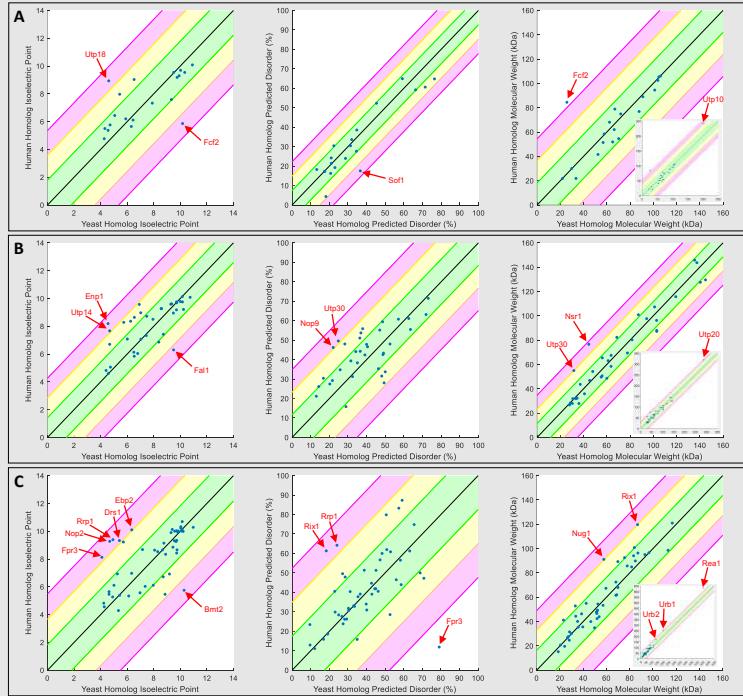


Figure S4 Yeast vs Human Comparison of Physical Properties of Assembly Factors - related to Figure 3

The comparative diagonal plots show isoelectric points, predicted disorder and molecular weights of **(A)** 5'-ETS, **(B)** pre-40S and **(C)** pre-LSU AFs. Colored margins indicate how far away yeast and human values are from each other, with green being 1 standard deviation, yellow being 2 standard deviations, and pink being 3 standard deviations. AF pairs were considered outliers if the difference between their yeast and human values (when compared to all AFs in their subset) was more than 2 standard deviations away from 0. These are indicated with red arrows using yeast names.

Figure S5

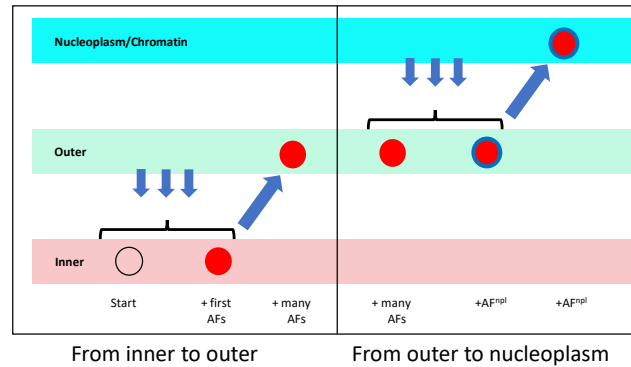


Figure S5 Vectorial 2-Phase Partitioning

On the left, in a typical T-diagram, we illustrate the progressive loading of AFs onto nascent rRNA within the inner compartment. At "start," no AFs have been added so the circle is empty. Due to arrival of latent AFs from the outer layer (vertical blue arrows) the nascent rRNPs progressively acquires AFs (red filling) and becomes phase compatible with the outer layer/volume. The nascent particles therefore shift in an energetically downhill fashion to the outer layer/volume. At the right, the further maturation of precursor particles that reside in the outer layer. Since multiple AFs that are conspicuous in the nucleoplasm are also found in the outer compartment, we propose that when they arrive in the outer layer (blue arrows) they bind to the surface of immature particles (represented by the blue surface on the red particles). Those particles therefore can diffuse in energetically neutral fashion into the nucleoplasmic volume. Our previous article [1] introduces the use of T-diagrams.

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