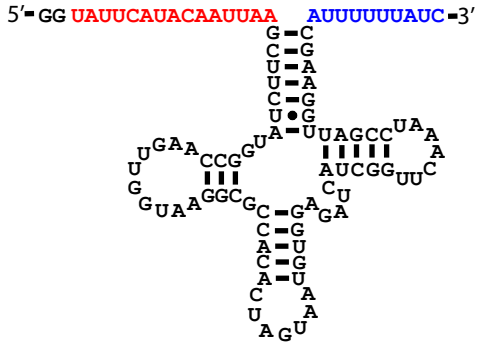
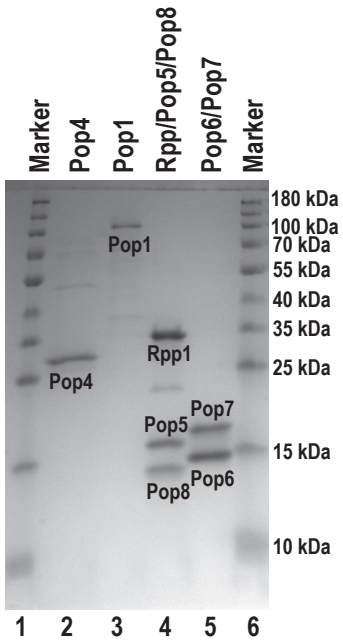
Supplementary Data

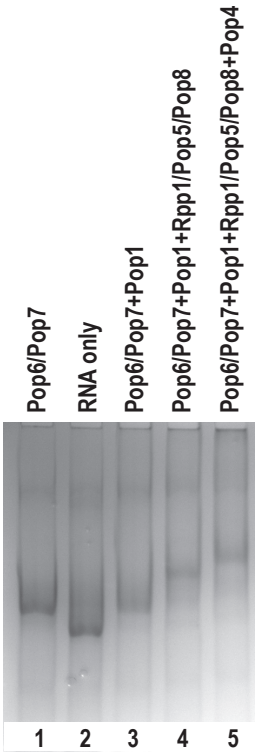
Supplementary Figures and Legends



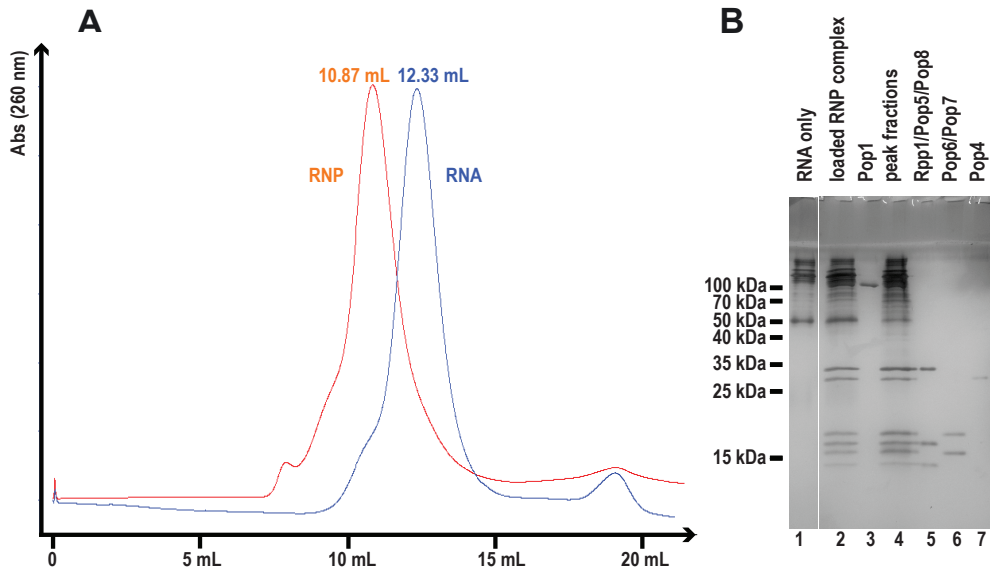
Supplementary Figure S1. *S. cerevisiae* tRNA^{Thr}(AGT) construct (chromosomal location chrIII:295469-295565, http://www.yeastgenome.org/cgi-bin/seqTools). 5'-leader is shown in red, 3'-trailer is shown in blue.



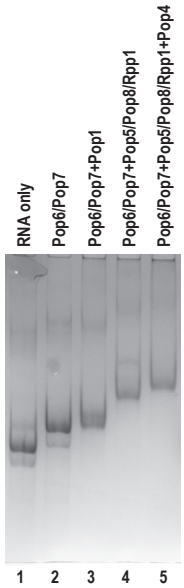
Supplementary Figure S2. RNase P proteins and protein subcomplexes used in the reconstitution of RNase P RNPs. Lanes 1, 6: protein size marker; lane 2: Pop4; lane 3: Pop1; lane 4: Rpp1/Pop5/Pop8 complex; lane 5: Pop6/Pop7 complex. Identities of the proteins have been confirmed using mass spectrometry. Coomassie Blue-stained SDS-PAAG. Molecular masses of the marker bands are shown on the right; protein identifiers are superposed on the gel next to the corresponding bands.



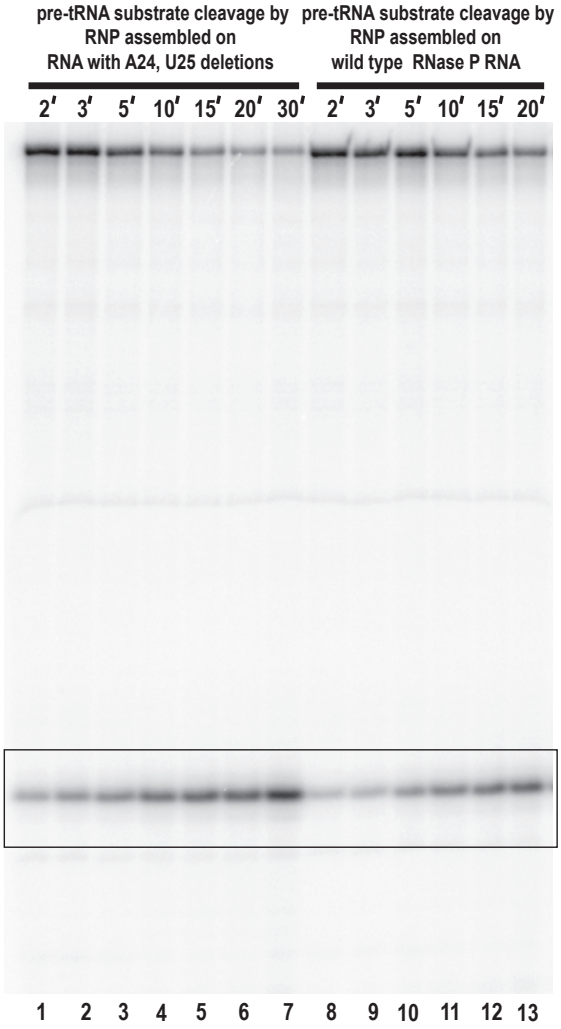
Supplementary Figure S3. EMSA analysis of the binding of RNase P proteins to the wild-type RNase P RNA. Proteins and RNA are taken at a 1:1 molar ratio; the resulting complexes are resolved on a native polyacrylamide gel. RNA is stained with Toluidine Blue. Lane 2: RNA only; lanes 1, 3, 4, 5: electrophoretic mobility shifts upon the addition of protein components as indicated above the gel.



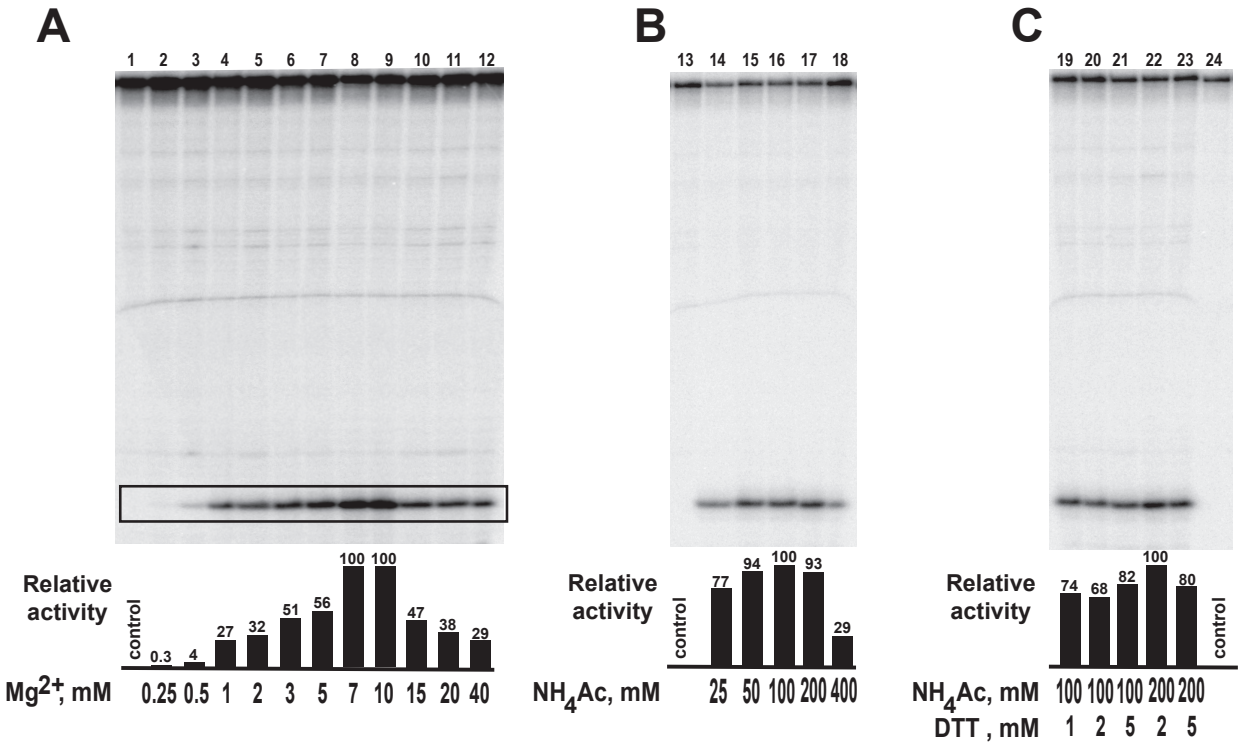
Supplementary Figure S4. Size-exclusion chromatography (SEC) analysis of the assembled 8-component RNase P RNP. (***A***) Comparison of gel filtration profiles for the refolded RNase P RNA (control, blue) and RNase P RNP containing proteins Pop1, Pop4, Pop5, Pop7, Pop7, Pop8, and Rpp1 (red). The samples were filtered through a Superdex 200 10/300GL size-exclusion column (GE Healthcare); the positions of the elution peaks are shown above the peaks. (***B***) SDS-PAAG analysis of the chromatographic peaks; silver-stained gel. Lane 1: refolded RNase P RNA was filtered through the size-exclusion column, concentrated, and loaded on the gel. Note that RNA is stained and runs as multiple bands as the conditions in the gel are not denaturing for RNA. Lane 2: the assembled 8-component RNase P RNP prior the SEC. Lane 3: purified Pop1 (control). Lane 4: the SEC fraction containing RNase P RNP. Lane 5: purified Rpp1/Pop5/Pop8 complex (control). Lane 6: purified Pop6/Pop7 complex (control). Lane 7: purified Pop4 (control).



Supplementary Figure S5. The deletion of bulged nucleotides A90, U93 from the catalytic core of RNase P RNA does not affect protein binding. The experiment is similar to the one shown in Fig. 2A, but the mutated RNase P RNA was used instead of the "wild-type" RNA. Protein components are added to the mutated RNase P RNA at a 1:1 molar ratio; the resulting complexes are resolved on a native polyacrylamide gel. RNA is stained with Toluidine Blue. Lane 1: RNase P RNA alone; lanes 2-5: electrophoretic mobility shifts upon the addition of protein components as indicated above the gel.



Supplementary Figure S6. The A24, U25 deletions do not negatively affect RNase P RNP activity. Comparison of the pre-tRNA substrate cleavage by RNP including RNase P RNA with the A24, U25 deletions and proteins Pop1, Pop4, Pop5, Pop6, Pop7, Pop8, Rpp1 (lanes 1-7) versus the RNP of the same protein composition assembled on the wild-type RNase P RNA (lanes 8-13). 2 pmol of RNPs (0.2 μM) were incubated with 20 pmol (2 μM) of 5'-end ^{32}P-labeled tRNA^{Thr} pre-tRNA. The incubation time varied as indicated above the gel. The assays were performed at 30^{0}C in a buffer containing 50 mM HEPES-NaOH pH 7.8, 100 mM ammonium acetate, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.5% glycerol, 0.5 μg/ml BSA. The lower level of the activity of the wild-type RNP is consistent with the reduced yield of the fully assembled RNP (compare Supplementary Fig. S3, lane 5 with Fig. 2, lane 5).

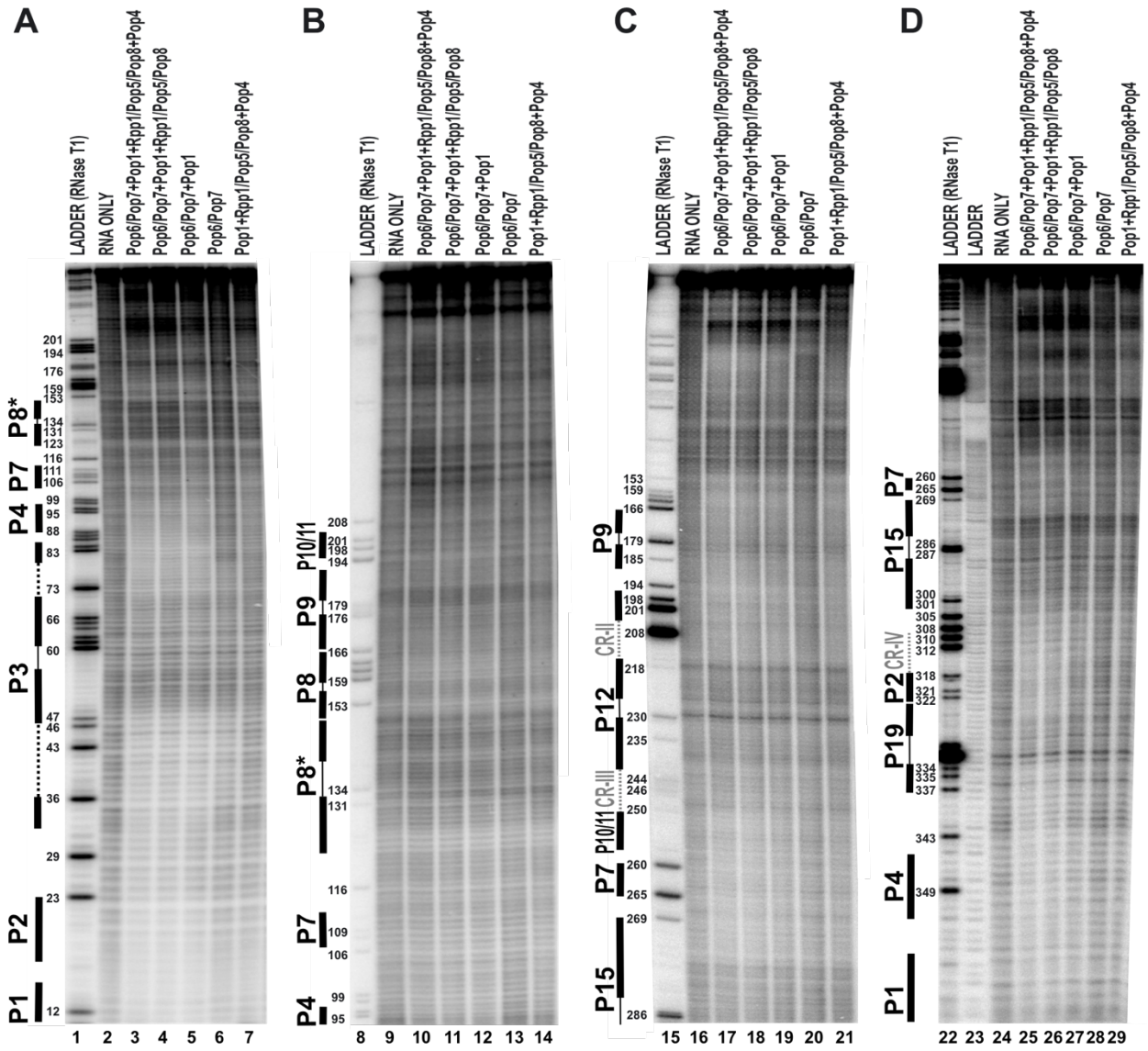


Supplementary Figure S7. *In vitro* assembled 8-component RNase P RNP (RNase P RNA plus proteins Pop1, Pop4, Pop5, Pop6, Pop7, Pop8, Rpp1) is catalytically active in a wide range of buffer conditions. 5'-end ^{32}P-labeled tRNA^{Thr} pre-tRNA (20 pmol, 1 μM) was digested by 2 pmol (0.1 μM) of RNase P RNP for 15 min at 30^{0}C in varying buffers. The gels were quantified; relative activities are plotted as vertical bars, the numerical values of the relative activities are shown above the bars.

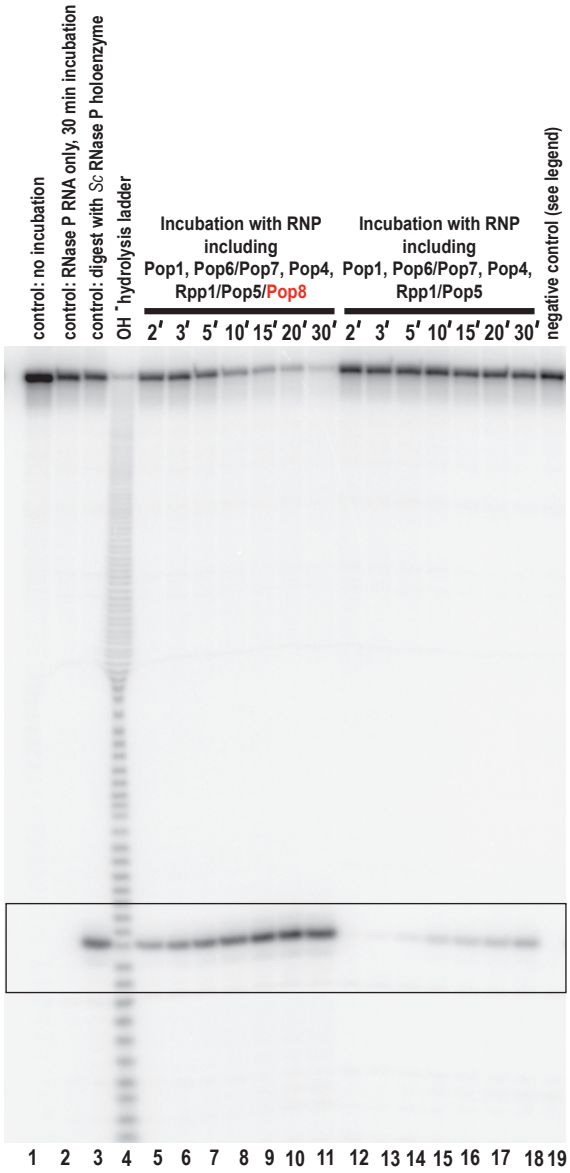
(***A***) buffers containing: 50 mM HEPES-NaOH pH 7.8, 100 mM ammonium acetate, 1 mM DTT, 0.5% glycerol, 0.5 μg/ml BSA and $MgCl_2$ at concentrations indicated below lanes (lanes 2-12). Lane 1: control (same buffer, 10 mM $MgCl_2$, no RNP added).

(***B)*** buffers containing: 50 mM HEPES-NaOH pH 7.8, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, 0.5% glycerol, 0.5 μg/ml BSA and varying concentrations of ammonium acetate as indicated below lanes (lanes 14-18). Lane 13: control (same buffer, 100 mM ammonium acetate, no RNP added).

(***C)*** buffers containing: 50 mM HEPES-NaOH pH 7.8, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.5% glycerol, 0.5 μg/ml BSA and varying concentrations of ammonium acetate and DTT as indicated below lanes (lanes 19-23). Lane 24: control (pre-tRNA).



Supplementary Figure S8. Hydroxyl radicals (Fe(II)-EDTA) footprinting assays for RNase P RNP assemblies. RNase P RNA was 5'-end (***A, B***) or 3'-end (***C, D***) ^{32}P-labeled. For the complete RNA coverage, the gels were run for the standard (***A, D***) and extended (***B, C***) duration. The sequence ladders (RNase T1 digest, identifies positions of guanines) are in lanes 1, 8, 15, 22. The secondary structure elements and nucleotide numbering (Fig. 1A) are marked on the left of the gels. Helical stems are shown by thick solid lines; terminal loops are shown by thin solid lines; large internal loops and conserved elements are shown by dotted lines. RNase P RNA assayed without proteins (reference) is in lanes 2, 9, 16, 24. Assays for RNP assemblies are in lanes 3-7, 10-14, 17-21, and 25-29. The protein compositions of RNPs are shown above corresponding lanes. RNase P RNA and proteins were taken at a 1:1 molar ratio.



Supplementary Figure S9. Comparison of the pre-tRNA substrate cleavage by the assembled 8-component RNP complex including Pop1, Pop6/Pop7, Pop4, Rpp1/Pop5/Pop8 and that of a complex having Rpp1/Pop5 instead of Rpp1/Pop5/Pop8. Lane 1: 5'-end ^{32}P-labeled tRNA^{Thr} pre-tRNA (control). Lane 2: pre-tRNA incubated with RNase P RNA for 30 min (control). Lane 3: pre-tRNA digested with *S. cerevisiae* RNase P isolated from yeast cells (control). Lane 4: pre-tRNA hydrolyzed in alkali (ladder). Lanes 5-11: 20 pmol (1.8 μM) of pre-tRNA was incubated with RNase P RNP assembled on 2 pmol (0.18 μM) of RNase P RNA and containing proteins Pop1, Pop6/Pop7, Pop4, and Rpp1/Pop5/Pop8. The incubation time varied from 2 to 30 min as indicated above the gel. Lanes 12-18: same as lanes 5-11, but for the RNP containing Rpp1/Pop5 instead of Rpp1/Pop5/Pop8. Lane 19: same as lane 18, but for RNP assembled on a ΔA90, ΔU93 mutant RNase P RNA (negative control). The assays were performed at 30^{0}C in a buffer containing 50 mM HEPES-NaOH pH 7.8, 100 mM ammonium acetate, 10 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.5% glycerol, 0.5 μg/ml BSA.

YEAST STRAINS

Yeast strain OE101

The *S. cerevisiae strain* OE101 (MAT**a** RPR2::TAPHIS8::TRP1 sep1::URA3 pep4::LEU2 nuc1::LEU2 ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112) was constructed using strain LSY389-34A [1] (MATa sep1::URA3 pep4::LEU2 nuc1::LEU2 ade2-1 trp1-1 his3-11,15 can1-100 ura3-1 leu2-3,112; a generous gift of Mark Schmitt) as the starting point. The affinity tag fused to the C-terminus of the Rpr2 gene was generated using PCR-based genomic tagging; the resultant tag TAPHIS8 was similar to the canonical TAP tag [2], but with eight histidine residues replacing the calmodulin-binding domain. The genomic sequence of the Rpr2 gene with the fused tag was confirmed by sequencing.

PLASMIDS AND OLIGONUCLEOTIDES

Plasmid yPop4MBP

Plasmid yPop4MBP was used for the expression and purification of protein Pop4. The plasmid was produced by inserting a product of PCR amplification of a codon-optimized Pop4 construct [3] into the *Xmn*I site of pMAL-c5X plasmid (New England Biolabs); one of the PCR primers contained a spacer with a TEV protease cleavage site.

The sequence of the relevant reading frame, including MBP, TEV cleavage site, and a codon-optimized Pop4 (color-coded) [3] is shown below:

ATG AAA ATC GAA GAA GGT AAA CTG GTA ATC TGG ATT AAC GGC GAT AAA GGC TAT AAC GGT CTC GCT
GAA GTC GGT AAG AAA TTC GAG AAA GAT ACC GGA ATT AAA GTC ACC GTT GAG CAT CCG GAT AAA CTG
GAA GAG AAA TTC CCA CAG GTT GCG GCA ACT GGC GAT GGC CCT GAC ATT ATC TTC TGG GCA CAC GAC
CGC TTT GGT GGC TAC GCT CAA TCT GGC CTG TTG GCT GAA ATC ACC CCG GAC AAA GCG TTC CAG GAC
AAG CTG TAT CCG TTT ACC TGG GAT GCC GTA CGT TAC AAC GGC AAG CTG ATT GCT TAC CCG ATC GCT
GTT GAA GCG TTA TCG CTG ATT TAT AAC AAA GAT CTG CTG CCG AAC CCG CCA AAA ACC TGG GAA GAG
ATC CCG GCG CTG GAT AAA GAA CTG AAA GCG AAA GGT AAG AGC GCG CTG ATG TTC AAC CTG CAA GAA
CCG TAC TTC ACC TGG CCG CTG ATT GCT GCT GAC GGG GGT TAT GCG TTC AAG TAT GAA AAC GGC AAG
TAC GAC ATT AAA GAC GTG GGC GTG GAT AAC GCT GGC GCG AAA GCG GGT CTG ACC TTC CTG GTT GAC
CTG ATT AAA AAC AAA CAC ATG AAT GCA GAC ACC GAT TAC TCC ATC GCA GAA GCT GCC TTT AAT AAA
GGC GAA ACA GCG ATG ACC ATC AAC GGC CCG TGG GCA TGG TCC AAC ATC GAC ACC AGC AAA GTG AAT
TAT GGT GTA ACG GTA CTG CCG ACC TTC AAG GGT CAA CCA TCC AAA CCG TTC GTT GGC GTG CTG AGC
GCA GGT ATT AAC GCC GCC AGT CCG AAC AAA GAG CTG GCA AAA GAG TTC CTC GAA AAC TAT CTG CTG
ACT GAT GAA GGT CTG GAA GCG GTT AAT AAA GAC AAA CCG CTG GGT GCC GTA GCG CTG AAG TCT TAC
GAG GAA GAG TTG GTG AAA GAT CCG CGT ATT GCC GCC ACT ATG GAA AAC GCC CAG AAA GGT GAA ATC
ATG CCG AAC ATC CCG CAG ATG TCC GCT TTC TGG TAT GCC GTG CGT ACT GCG GTG ATC AAC GCC GCC
AGC GGT CGT CAG ACT GTC GAT GAA GCC CTG AAA GAC GCG CAG ACT AAT TCG AGC TCG AAC AAC AAC
AAC AAT AAC AAT AAC AAC AAC CTC GGG ATC GAG GGA AGG GAT TAT GAT ATT CCA ACT ACT GCT AGC
GAG AAT CTG TAT TTT CAG GGT ATG GAC CGT ACC CAG ACC TTC ATC AAA GAT TGC CTG TTC ACT AAA
TGT CTG GAA GAC CCG GAA AAG CCG TTT AAC GAG AAC CGT TTC CAG GAT ACC CTG CTG CTC CTG CCG
ACT GAC GGT GGC CTG ACC TCC CGT CTG CAG CGC CAG CAA CGT AAA TCT AAG CTG AAC CTG GAT AAC

CTG CAG AAG GTT TCC CAG CTG GAA TCT GCG GAT AAA CAG CTG GAA AAG CGC GAC TAC CAG CGT ATC
AAC AAG AAC AGC AAG ATC GCT CTG CGT GAG TAC ATC AAT AAC TGC AAG AAA AAC ACC AAG AAA TGC
CTG AAG CTG GCG TAC GAA AAC AAG ATC ACC GAC AAA GAG GAC CTG CTG CAC TAC ATC GAA GAG AAA
CAC CCG ACT ATT TAT GAA AGC CTG CCG CAG TAT GTT GAT TTC GTG CCG ATG TAC AAA GAA CTG TGG
ATT AAC TAT ATC AAA GAG CTG CTG AAC ATC ACC AAG AAC CTG AAA ACC TTC AAC GGT AGC CTG GCT
CTG CTG AAA CTG TCT ATG GCG GAT TAC AAC GGC GCA CTG CTG CGT GTT ACT AAA TCC AAG AAC AAA
ACC CTG ATT GGT CTG CAG GGT ATC GTT ATT TGG GAC TCT CAG AAA TTC TTC ATC ATG ATT GTT AAA
GGT AAC ATC ATC GAC GAA ATT AAG TGC ATC CCG AAG AAG GGT ACT GTG TTC CAG TTC GAG ATT CCG
ATC TCC GAT GAC GAC GAT TCT GCT CTG CGT TAC TCC ATC CTG GGT GAT CGC TTC AAA TAC CGT AGC
GTT GAC CGC GCG GGT CGT AAA TTC AAG TCC CGT CGC TGT GAC GAT ATG CTG TAC TAT ATC CAG AAC
TAA

Plasmid 85Rpp1OP

Plasmid 85Rpp1OP was used to express the Rpp1/Pop5/Pop8 complex. This polycistronic expression construct was synthesized commercially and inserted into a pET21-based plasmid pET21R [3]. Codon-optimized reading frames for Pop5, Rpp1, Pop8 (color-coded) and linker sequences are shown below:

ATG GTT CGT CTG AAA AGC CGC TAT ATC CTG TTT GAA ATT ATC TTT CCG CCT ACC GAT ACC AAT GTT
GAA GAA AGC GTT AGC AAA GCA GAT ATT CTG CTG AGC CAT CAT CGT GCA AGT CCG GCA GAT GTT AGC
ATT AAA AGC ATT CTG CAA GAA ATT CGT CGT AGC CTG AGC CTG AAT CTG GGT GAT TAT GGT AGC GCA
AAA TGT AAT AGC CTG CTG CAG CTG AAA TAC TTC AGC AAT AAA ACC AGC ACC GGT ATT ATT CGT
TGT CAT CGT GAA GAT TGC GAT CTG GTT ATT ATG GCA CTG ATG CTG ATG AGC AAA ATT GGT GAT GTT
GAT GGC CTG ATT GTT AAT CCG GTT AAA GTT AGC GGC ACC ATC AAA AAA ATC GAA CAG TTT GCA ATG
CGT CGC AAC AGC AAA ATT CTG AAC ATT ATC AAA TGT AGC CAG AGC AGC CAT CTG AGC GAT AAC GAT
TTT ATC ATC AAC GAT TTC AAA AAA ATC GGT CGC GAA AAC GAA AAC GAG AAT GAG GAT GAT TAA
GGATCCCTGTAGAAATAATTTTGTTTAACTTTAATAAGGAGATATACC ATG CTG GTT GAT CTG AAT GTT CCG TGG
CCT CAG AAT AGC TAT GCA GAT AAA GTT ACC AGC CAG GCC GTT AAC AAT CTG ATT AAA ACC CTG AGC
ACC CTG CAT ATG CTG GGC TAT ACC CAT ATT GCA ATT AAC TTT ACC GTG AAC CAC AGC GAA AAA TTT
CCG AAT GAT GTG AAA CTG CTG AAC CCG ATT GAT ATT AAA CGT CGT TTT GGT GAA CTG ATG GAT CGT
ACC GGT CTG AAA CTG TAT AGC CGT ATT ACC CTG ATT ATT GAT GAT CCG AGC AAA GGT CAG AGC CTG
AGC AAA ATT AGC CAG GCA TTT GAT ATT GTT GCA GCA CTG CCG ATT AGC GAA AAA GGT CTG ACC CTG
AGT ACC ACC AAT CTG GAT ATT GAT CTG CTG ACC TTT CAG TAT GGT AGC CGT CTG CCG ACA TTT CTG
AAA CAC AAA AGC ATT TGT AGC TGC GTT AAT CGC GGT GTT AAA CTG GAA ATT GTT TAT GGT TAT GCC
CTG CGT GAT GTT CAG GCA CGT CGT CAG TTT GTT AGC AAT GTT CGT AGC GTT ATT CGT AGC AGC CGT
AGC CGT GGT ATT GTT ATT GGT AGC GGT GCA ATG AGT CCG CTG GAA TGT CGT AAT ATT CTG GGT GTG
ACC AGC CTG ATC AAA AAT CTG GGT CTG CCG AGC GAT CGT TGT AGC AAA GCA ATG GGT GAT CTG GCA
AGC CTG GTT CTG CTG AAT GGT CGT CTG CGT AAT AAA AGC CAT AAA CAG ACC ATT GTT ACC GGT GGT
GGT AGT GGT AAT GGT GAT GAT GTT GTT AAT GAT GTG CAG GGC ATT GAT GAC GTT CAG ACC ATT AAA
GTT GTT AAA CGT AGC ATG GAT GCA GAA CAG CTG GGT CAT GCA AGC AAA CGT CAT AAA CCG TAA
GAATTCATCTTAGTATATTAGTTAAGTATAAGAAGGAGATATACAT ATG GGC AAA AAA ACC TTT CGT GAG TGG CAG
TAT TTC AAA CTG AGC ATT ACC AGC TTT GAT CAG GAT GTT GAT GAT GCA CAT GCA ATT GAT CAG ATG
ACC TGG CGT CAG TGG CTG AAT AAT GCA CTG AAA CGT AGC TAT GGT ATT TTT GGT GAA GGT GTG GAA
TAT AGC TTT CTG CAC GTT GAT GAT AAA CTG GCC TAT ATT CGT GTT AAC CAC GCA GAT AAA GAT ACC
TTT AGC AGC AGC ATT AGC ACC TAT ATT AGC ACC GAT GAA CTG GTT GGT AGT CCG CTG ACC GTT AGC
ATT CTG CAA GAA AGC AGC AGC CTG CGT CTG CTG GAA GTT ACC GAT GAT GAT CGC CTG TGG CTG AAA
AAA GTT ATG GAA GAA GAG GAA CAG GAC TGC AAA TGC ATC TAA

Plasmid 5Rpp1

Plasmid 5Rpp1 was used for co-expression of Pop5 and Rpp1. 5Rpp1 is a polycistronic pET21-based expression construct containing *S. cerevisiae* Rpp1 and Pop5 genes. This plasmid was derived from the previously described plasmid 5Rpp1C [4] by removing the His_6 purification tag from the reading frame of Rpp1. The resulting reading frames of Rpp1 and Pop5 did not include any purification tags.

Plasmid pYRP2-HDV

Plasmid pYRP2-HDV was used to produce RNase P RNA for experiments were the homogeneity of the 3'-end of the *in vitro* transcribed RNA was critical. This plasmid (below) contained T7 promoter followed by the *S. cerevisiae* **RNase P RNA** construct with terminal m**u**tati**o**n**s**, and a self-cleaving HDV ribozyme construct (color-coded) [5]; the self-cleavage site is shown with an asterisk *. The construct was inserted into *EcoR*I and *Hind*III sites of pUC19; prior to *in vitro* transcription the plasmid was linearized with *Hind*III.

5′-
GAATTC**TAATACGACTCACTATA**G**G**GGAACAGTGGTAATTCCTACGTAAGAAACCTGTTTACAGAAGGATCCCCACCTATGGGCGGG
TTATCAGATATTATCAGGTGGGAAATTCGGTGGAACACAGTGGAGCCTTGTCCTCCGGGTTAATGTCGCTTTTGGCATTGGCCCCTG
CTCCTGAGAGAAGAAATATACTGGGGAACCAGTCTTTACCGACCGTTGTTATCAGAAATTCACGGAGTTCGGCCTAGGTCGGACTCC
GATGGGAACGGCAACGGTTGTTCCGTTTGACTTGTCGCCCGCTACGGCGTGAGCGTCAAGGTCTGTTGAGTGCAATCGTAGGACGTC
ATTAGTGGCGAACCCGATACCGATTACTGCTGCTGTTCC**C**G**T***GGGTCGGCTGCAGACCCAGCCTCCTCGCGGCCCGACCTGGGCAT
CTTCGGATGGCGAATGGGTCAAGCTT-3′

Plasmid pYRP2dead

Plasmid pYRP2dead was used to produce mutated RNase P RNA used in activity assays as negative controls. It was identical to pYRP2 [3], except for A90 and U93 (bulging P4 stem nucleotides essential for the catalytic activity of RNase P) that were deleted using site-directed mutagenesis.

***S. cerevisiae* tRNA^{Thr}(AGT) construct**

Transcribed RNA (below) included 5'-end **GG** added to facilitate transcription, 15-nucleotide-long leader, tRNA^{Thr}(AGT), followed by 10-nucleotide-long trailer (color-coded):

5′-**GG**UAUUCAUACAAUUAAGCUUCUAUGGCCAAGUUGGUAAGGCGCCACACUAGUAAUGUGGAG
AUCAUCGGUUCAAAUCCGAUUGGAAGCAUUUUUUAUC-3′

The leader, tRNA, and trailer sequences corresponded to *S. cerevisiae* genome (chromosomal location chrIII:295469-295565, http://www.yeastgenome.org/cgi-bin/seqTools).

RNA was produced using synthetic oligonucleotide preThr116 as a template.

preThr116 template oligonucleotide (including T7 promoter region):

5'-
GATAAAAAATGCTTCCAATCGGATTTGAACCGATGATCTCCACATTACTAGTGTGGCGCCTTACCAACTTGGCCATAGAAGCTTAAT
TGTATGAATACC TATAGTGAGTCGTATTA -3′

The expected secondary structure of the *S. cerevisiae* tRNA^{Thr}(AGT) construct is shown in Supplementary Fig. S1. This specific pre-tRNA substrate was selected to minimize potential RNA misfolding as judged by the partition function calculations in RNAfold [6].

DETAILED PROTOCOLS FOR EXPRESSION AND PURIFICATION OF PROTEINS

Expression and purification of protein Pop1

E. coli strain BL21 Star (DE3) (Invitrogen) carrying plasmid pLysSRARE (a plasmid encoding rare *E. coli* tRNAs and lysozyme, Novagen) was co-transformed with plasmids encoding *POP1* and codon-optimized *POP4* genes as previously described [3].

Cells were grown in 12 L of LB media supplemented with 0.2% (w/v) glucose, 50 mg/L ampicillin, 25 mg/L kanamycin, and 17 mg/L chloramphenicol at 32^{0}C to OD_{600}=0.3, at which point protein expression was

induced by the addition of IPTG to 1 mM. After 4 hrs of expression, the cells were cooled down on ice, pelleted by centrifugation at 4,000 g for 30 min, frozen in liquid nitrogen, and stored at -76^{0}C.

Frozen cells were thawed at room temperature and resuspended in Buffer A (50 mM Tris-HCl pH 7.5, 1 M NaCl, 50 mM KCl, 2 mM $MgCl_2$, 2 μM $ZnSO_4$, 100 mM DTT, 10 mM EDTA, 1 mM PMSF, and protease inhibitor (Pierce) as recommended) added to the total final volume of 120 ml. The suspension was incubated on ice for 30 min and divided into 4 equal parts. The cells in each aliquot were disrupted with 8 cycles of sonication (14 x 0.5 sec pulses each, with 3 min incubation on ice between cycles) using Sonic Dismembrator 500 (Fisher). The lysate was clarified by centrifugation at 16,000 g for 40 min, then Tween-20 (to 0.002% v/v) and polyethyleneimine (M_r 600,000-1,000,000, pH 7.5, to 0.5% v/v) were sequentially added. The mix was incubated on ice for 10 min, and centrifuged at 16,000 g for 10 min. Supernatant was collected, and 0.48 g of dry ammonium sulfate powder per 1 ml of supernatant were added. Following overnight incubation, the protein precipitate was pelleted by centrifugation at 16,000 g for 40 min.

The pellet was dissolved in 160 ml of Buffer SP (50 mM MES-NaOH pH 6.5, 50 mM KCl, 2 mM $MgCl_2$, 2 μM $ZnSO_4$, 10 mM DTT, 0.002% (v/v) Tween 20, 0.1 mM PMSF), and centrifuged at 16,000 g for 20 min. The supernatant was collected and its volume was adjusted with Buffer SP to reach the conductivity of 55 mS/cm. The solution was loaded on a 5 ml SP-Sepharose column (Amersham) that was pre-equilibrated with Buffer SP supplemented with 400 mM NaCl. The column was washed with 50 ml of Buffer SP supplemented with 400 mM NaCl, and Pop1 was eluted with a 400 mM-1 M gradient of NaCl in the SP buffer; Pop1 eluted at about 800 mM NaCl.

The fractions of interest were combined, diluted 2.7-fold with Buffer SP, and loaded on a 1 ml HiTrap Heparin HP column (Amersham) that was pre-equilibrated with Buffer SP supplemented with 400 mM NaCl. The column was washed with 10 ml of Buffer SP supplemented with 400 mM NaCl, and the proteins were eluted with a 400 mM-1.0 M gradient of NaCl in Buffer SP. The complex of interest eluted at ~800 mM NaCl.

The fractions of interest were pooled and concentrated to the final volume of 400 μl using an Amicon Ultra-4 concentrator (50,000 MWCO, Millipore), then loaded on a Superdex 200 gel-filtration column (Amersham) that was pre-equilibrated with Buffer SEC (50 mM MES-NaOH pH 6.5, 600 mM NaCl, 50 mM KCl, 2 mM $MgCl_2$, 2 μM $ZnSO_4$, 10 mM DTT, 0.002% (v/v) Tween 20). The fractions of interest were combined (care was taken to exclude truncated protein), then the protein was concentrated to 1.0 mg/ml using Amicon Ultra-0.5 concentrator (50,000 MWCO, Millipore), aliquoted, frozen in liquid nitrogen, and stored at -76^{0}C. The procedure yielded approximately 2 mg of Pop1.

Expression and purification of protein Pop4

E. coli strain BL21 Star (DE3) (Invitrogen) carrying plasmid pLysSRARE (a plasmid encoding rare *E. coli* tRNAs and lysozyme, Novagen) was transformed with plasmid yPop4MBP (above). Starter culture was grown overnight at 20^{0}C in LB media supplemented with 0.2% (w/v) glucose, 100 mg/L ampicillin, and 34 mg/L chloramphenicol (the cell culture was not allowed to reach the stationary phase), then diluted 50-fold into 3 L of LB media supplemented with 0.2% (w/v) glucose and 100 mg/L ampicillin, and grown at 20^{0}C until OD_{600} had reached ~0.3. At this point protein expression was induced by the addition of IPTG to 1 mM. After 26 hrs of growth at 20^{0}C, cells were cooled down on ice, pelleted by centrifugation at 4,000 g for 30 min, and stored at -76^{0}C.

Frozen cells were thawed at room temperature and resuspended in Buffer A (50 mM Tris-HCl pH 7.5, 1 M NaCl, 50 mM KCl, 2 μM $ZnSO_4$, 5 mM BME, 0.5 mM EDTA, 0.1 mM PMSF) added to the total final volume of 9 ml. The suspension was incubated on ice for 1 hr, then cells were disrupted with 4 cycles of sonication (14 x 0.5 sec pulses each, with 3 min incubation on ice between cycles) using Sonic Dismembrator 500 (Fisher). The lysate was clarified by centrifugation at 16,000 g for 20 min, then polyethyleneimine (M_r

600,000-1,000,000, pH 7.5) was added to 0.1% (v/v). The mix was incubated on ice for 10 min, and centrifuged at 16,000 g for 20 min. Supernatant was collected, and 0.48 g of dry ammonium sulfate powder per 1 ml of supernatant were added. Following overnight incubation, the protein precipitate was pelleted by centrifugation at 16,000 g for 20 min.

The pellet was dissolved in Buffer B (50 mM Tris-HCl pH 7.5, 400 mM NaCl, 100 mM KCl, 1% glycerol (v/v), 5 mM BME, 2 μM $ZnSO_4$, 0.5 mM EDTA, 0.1 mM PMSF); the total volume was adjusted to 32 ml. After centrifugation at 16,000 g for 20 min, the supernatant was loaded onto a gravity column filled with 2 ml of Amylose Resin (New England Biolabs) that was pre-equilibrated with Buffer B. The column was washed with 40 ml of Buffer B, and the protein was eluted with Buffer C (50 mM MES pH 6.0, 500 mM NaCl, 50 mM KCl, 1.5% glycerol, 5 mM BME, 0.5 mM EDTA, 0.1 mM PMSF, 2 μM $ZnSO_4$, and 10 mM maltose). Elution fractions of interest were combined and digested with TEV protease. TEV protease was added at a 1:1,200 ratio to the substrate as judged by UV absorption; the digestion was run at 4^0C for 24 hrs.

The product of digestion was diluted 2-fold with Buffer D (50 mM MES pH 6.0, 5 mM NaCl, 50 mM KCl, 1.5% glycerol, 5 mM BME, 0.5 mM EDTA, 0.1 mM PMSF, 2 μM $ZnSO_4$) and applied onto a 1 ml HiTrap SP Sepharose FF column (Amersham) that was pre-equilibrated with the Buffer D supplemented with NaCl to 225 mM. The column was washed with 10 ml of the buffer used in the column pre-equilibration and the protein was eluted with a 225 mM-1 M gradient of NaCl in Buffer D; Pop4 eluted at approximately 400 mM NaCl. The fractions of interest were combined, the protein was concentrated to 1.0 mg/ml using Amicon Ultra-0.5 concentrator (10,000 MWCO, Millipore), aliquoted, and stored at -76^0C. The procedure yielded approximately 2 mg of Pop4.

Expression and purification of proteins Pop3, Rpr2

Proteins Pop3 and Rpr2 were expressed in *E. coli* strain SHuffle T7 (New England Biolabs). Protein expression was induced by the addition of IPTG to 1 mM and continued at 14^0C for 24 hrs; for Rpr2 expression the growth media was supplemented with 3 μM Zn_2SO_4. Pop3 and Rpr2 purification followed the same protocol as used for Pop4 (above).

Expression and purification of the Rpp1/Pop5 complex

E. coli strain BL21 Star (DE3) (Invitrogen) carrying plasmid pLysSRARE (a plasmid encoding rare *E. coli* tRNAs and lysozyme, Novagen) was transformed with plasmid 5Rpp1, a polycistronic construct containing *S. cerevisiae* Rpp1 and Pop5 genes (above). Starter culture was grown at 20^0C in LB media supplemented with 0.2% (w/v) glucose, 100 mg/L ampicillin, 34 mg/L chloramphenicol until OD_{600} had reached ~0.6, then diluted 1000-fold into 2 L of autoinduction growth media [7] supplemented with 100 mg/L ampicillin. After 24 hrs of growth at 30^0C, cells were cooled down on ice, pelleted by centrifugation at 8,000 g for 10 min, and stored at -76^0C.

Frozen cells were thawed at room temperature and resuspended in Buffer A (50 mM Tris-HCl pH 8.4, 250 mM NaCl, 50 mM KCl, 5 mM BME, 1 mM EDTA, 1 mM PMSF), the total volume was adjusted to 30 ml. The cells were incubated on ice for 30 min and disrupted by 12 cycles of sonication (14 x 0.5 sec pulses each, with 3 min incubation on ice between cycles) using Sonic Dismembrator 500 (Fisher). The lysate was clarified by centrifugation at 16,000 g for 30 min, then polyethyleneimine (M_r 600,000-1,000,000, pH 7.5) was added to 0.5% (v/v). The mix was incubated on ice for 10 min, and centrifuged at 16,000 g for 20 min. Supernatant was collected, and 0.48 g of dry ammonium sulfate powder per 1 ml of supernatant were added.

Following overnight incubation, the protein precipitate was pelleted by centrifugation at 16,000 g for 45 min, resuspended in 80 ml of Buffer B (50 mM MES-NaOH pH 6.0, 50 mM KCl, 5 mM NaCl, 0.5 mM EDTA, 5 mM BME, 0.1 mM PMSF), and centrifuged at 16,000 g for 30 min. The supernatant was collected, and its conductivity was adjusted to 37 mS/cm with Buffer B (the final volume ~150 ml). The resulting solution was

loaded on a 5 ml SP-Sepharose column (Amersham) that was pre-equilibrated with Buffer B supplemented with 300 mM NaCl. The column was washed with 20 ml of Buffer B supplemented with 300 mM NaCl; the proteins were eluted with a 300 mM-1 M gradient of NaCl in Buffer B. The complexes of interest eluted at ~600 mM NaCl.

The fractions of interest were combined, diluted 2-fold with Buffer B, and loaded on a 1 ml HiTrap Heparin HP column (Amersham) that was pre-equilibrated with Buffer B supplemented with 300 mM NaCl. The column was washed with 10 ml of Buffer B supplemented with 300 mM NaCl, and the proteins were eluted with a 300 mM-1.2 M gradient of NaCl in Buffer B. The complex eluted at ~ 650 mM NaCl.

The fractions of interest were combined, concentrated to the final volume of 0.5 ml using an Amicon Ultra-4 concentrator (10,000 MWCO, Millipore), applied to a Superdex 75 10/300 GL column (Amersham) that was pre-equilibrated with SEC Buffer (10 mM Tris-HCl pH 7.0, 100 mM KCl, 400 mM NaCl, 5 mM DTT, 0.1 mM PMSF, 0.1 mM EDTA), and eluted with SEC Buffer. Fractions of interest were combined, supplemented with 1.5 mM TCEP, and concentrated to 10 mg/ml using an Amicon Ultra-4 concentrator (10,000 MWCO, Millipore). Glycerol (to 50% v/v) was added to the preparation, and the protein complex was stored at -20^{0}C; the protein remained stable for at least 6 months. This procedure yielded approximately 10 mg of the Rpp1/Pop5 complex.

Expression and purification of the Rpp1/Pop5/Pop8 complex

E. coli strain SHuffle T7 (New England Biolabs) was transformed with plasmid 85Rpp1OP (above). Starter culture was grown overnight at 20^{0}C in LB media supplemented with 0.2% (w/v) glucose, 100 mg/L ampicillin (the cell culture was not allowed to reach the stationary phase), then diluted 50-fold into 6 L of LB media supplemented with 0.2% (w/v) glucose and 100 mg/L ampicillin, and grown at 30^{0}C until OD_{600} had reached ~0.2. At this point protein expression was induced by the addition of IPTG to 1 mM. After 8 hrs of growth at 30^{0}C, cells were cooled down on ice, pelleted by centrifugation at 4,000 g for 30 min, and stored at -76^{0}C.

Frozen cells were thawed at room temperature and resuspended in Buffer A (50 mM Tris-HCl pH 8.4, 250 mM NaCl, 50 mM KCl, 5 mM BME, 1 mM EDTA, 1 mM PMSF, 0.5 mg/ml lysozyme), the total volume was adjusted to 15 ml. The cells were incubated on ice for 30 min, then frozen at -76^{0}C, thawed at room temperature, and incubated on ice for another 30 min. Cells were disrupted by 12 cycles of sonication (14 x 0.5 sec pulses each, with 3 min incubation on ice between cycles) using Sonic Dismembrator 500 (Fisher). The lysate was clarified by centrifugation at 16,000 g for 30 min, then polyethyleneimine (M_r 600,000-1,000,000, pH 7.5) was added to 0.5% (v/v). The mix was incubated on ice for 10 min, and centrifuged at 16,000 g for 20 min. Supernatant was collected, and 0.48 g of dry ammonium sulfate powder per 1 ml of supernatant were added.

Following overnight incubation, the protein precipitate was pelleted by centrifugation at 16,000 g for 45 min, resuspended in 80 ml of Buffer B (50 mM MES-NaOH pH 6.0, 50 mM KCl, 5 mM NaCl, 0.5 mM EDTA, 5 mM BME, 0.1 mM PMSF), and centrifuged at 16,000 g for 30 min. The supernatant was collected, and its conductivity was adjusted to 52 mS/cm with Buffer B (the final volume ~150 ml). The resulting solution was loaded on a 5 ml SP-Sepharose column (Amersham) that was pre-equilibrated with Buffer B supplemented with 300 mM NaCl. The column was washed with 20 ml of Buffer B supplemented with 300 mM NaCl; the proteins were eluted with a 300 mM-1 M gradient of NaCl in Buffer B. The complex of interest eluted at ~600 mM NaCl.

The fractions of interest were combined, diluted 1.5-fold with Buffer B, and loaded on a 1 ml HiTrap Heparin HP column (Amersham) that was pre-equilibrated with Buffer B supplemented with 450 mM NaCl. The column was washed with 10 ml of Buffer B supplemented with 450 mM NaCl, and the proteins were eluted with a 450 mM-1.0 M gradient of NaCl in Buffer B. The complex of interest eluted at ~750 mM NaCl.

The fractions of interest were combined, concentrated to the final volume of 0.5 ml using an Amicon Ultra-4 concentrator (30,000 MWCO, Millipore), applied to a Superdex 75 10/300 GL column (Amersham) that was pre-equilibrated with SEC Buffer (10 mM Tris-HCl pH 7.0, 100 mM KCl, 400 mM NaCl, 5 mM DTT, 0.1 mM PMSF, 0.1 mM EDTA, 1.5% (v/v) glycerol), and eluted with SEC Buffer. Fractions of interest were combined, supplemented with 1.5 mM TCEP, and concentrated to 9 mg/ml using an Amicon Ultra-4 concentrator (30,000 MWCO, Millipore). Glycerol (to 50% v/v) was added to the preparation, and the protein complex was stored at -20^{0}C; the protein remained stable for at least 6 months. This procedure yielded approximately 4 mg of the Rpp1/Pop5/Pop8 complex.

Supplementary Data References

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