Supplemental Data

RIBOSOME PERFORMANCE IS ENHANCED BY A RICH CLUSTER OF PSEUDOURIDINES IN THE A-SITE FINGER REGION OF THE LARGE SUBUNIT

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Running title: A rich cluster of pseudouridines in rRNA

Supplemental data for experimental procedures

Yeast strains

The [PSI+] phenotype associated with prions was identified in several strains created in these studies. Routine curing of [PSI+] was done preventively for all strains by growing cells to colonies on solid medium containing 5 mM guanidine-hydrochloride (Gdn-HCl; Sigma) (1). The procedure was repeated two more times and cells were then restreaked onto fresh solid medium without Gdn-HCl.

The genotypes for 13 initial test strains of *Saccharomyces cerevisiae* are as follows. **Ys602** (WT, wild-type) - *MATα, ade2, his3, trp1, ura3, leu2*; **D1** - *MATα, ade2, his3, trp1, ura3, leu2, snr5::URA3*; **D2** - *MATα, ade2, his3, trp1, ura3, leu2, snr8::URA3*; **D3** - *MATα, ade2, his3, trp1, ura3, leu2, snr33::URA3*; **D4** (name YWD449, (2)) - *MATα, ade2, his3, trp1, ura3, leu2, snr81::TRP1*; **D5** (name YWD461, (2)) - *MATα, ade2, his3, trp1, ura3, leu2, rps22B::TRP1*; **(–)4Ψb** (name YS661, (3)) - *MATα, ade2, his3, his4, trp1, ura3, leu2, snr5::TRP1, snr8::HIS3*; **D7** - *MATα, ade2, his3, trp1, ura3, leu2, snr81::TRP1, snr33::URA3*; **D8** - *MATα, ade2, his3, trp1, ura3, leu2, rps22B::TRP1, snr81::URA3*; **D9** - *MATα, ade2, his3, trp1, ura3, leu2, rps22B::TRP1, snr5::ura3, snr33::HIS3*; **D10** - *MATα, ade2, his3, his4, trp1, ura3, leu2, snr5::TRP1, snr8::HIS3, snr81::ura3*; **D11** - *MATα, ade2, his3, his4, trp1, ura3, leu2, snr5::TRP1, snr8::HIS3, snr81::ura3, snr33::ura3*; **D12** - *MATα, ade2, his3, his4, trp1, ura3, leu2, snr5::TRP1, snr8::HIS3, snr81::ura3, rps22B::LEU2*; **D13** - *MATα, ade2, his3, his4, trp1, ura3, leu2, snr5::TRP1, snr8::HIS3, snr81::ura3, rps22B::LEU2, snr33::URA3*

The improved strain collection includes mutants with plasmids that refine the initial set of mutants, by: restoring certain Ψs in the test region, restoring Ψs outside of the test region, and restoring a protein (see Table S1 below). To maintain similar genetic backgrounds for the control and test strains, empty plasmids with suitable markers were introduced to cells where needed.

The genotypes of the strains in the improved collection are as follows. **Control cells (C)** – Ys602 with pRS316 [*URA3*], pDK141 [*ADE2*]; **(–)1Ψ –** D11 with pDK218 [*ADE2*], pDK257 [*URA3*]; **(–)2Ψa** – D13 with pDK219 [*ADE2*]; **(–)2Ψb** – D10 with pDK166 [*URA3*], pDK141 [*ADE2*]; **(–)2Ψc** – D9 with pDK159 [*URA3*], pDK157 [*ADE2*]; **(–)3Ψa** – D11 with pDK166 [*URA3*], pDK141 [*ADE2*]; **(–)3Ψb** – D10 with pRS316 [*URA3*], pDK164 [*ADE2*]; **(–)3Ψc –** D11 with pDK163 [*URA3*], pDK152 [*ADE2*]; **(–)3Ψd –** D11 with pDK163 [*URA3*], pDK154 [*ADE2*]; **(–)4Ψa** – D11 with pRS316 [*URA3*], pDK164 [*ADE2*]; **(–)5Ψa** – D13 with pDK164 [*ADE2*]; **(–)5Ψb** – D10 with pRS316 [*URA3*], pDK158 [*ADE2*]; **(–)6Ψ –** D11 with pRS316 [*URA3*], pDK158 [*ADE2*]; **(–)7Ψ –** D13 with pDK158 [*ADE2*].
The locations of the Ψs depleted in the test strains are summarized in Table 1.

Table S1
Plasmids used to restore protein and selected Ψs in the improved strain collection

Yeast strain	Parental strains with plasmids carrying expression cassettes for wild type or mutated snoRNA genes and for protein Rps22B. Only positions modified by mutant and wild-type snoRNAs are indicated.	Depleted but restored Ψs and protein	Depleted and not restored Ψs
(–)1Ψ	D11: pDK218 – snR5 (LSU: Ψ1004, Ψ1124), snR8 (LSU: Ψ960, Ψ986); pDK257 – snR81 (LSU: Ψ1052; U2: Ψ42)	LSU: Ψ960, Ψ986, Ψ1004, Ψ1052, Ψ1124; U2: Ψ42	LSU: Ψ1042
(–)2Ψa	D13: pDK219 – snR44 (SSU: Ψ106; LSU Ψ1056), snR5 (LSU: Ψ1004, Ψ1124), snR8 (LSU: Ψ960, Ψ986), Rps22B	SSU: Ψ106, Rps22B LSU: Ψ960, Ψ986, Ψ1004, Ψ1056, Ψ1124	LSU: Ψ1042, Ψ1052 U2: Ψ42
(–)2Ψb	D10: pDK166 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960, Ψ986), Rps22B	LSU: Ψ960, Ψ986, Ψ1124; U2: Ψ42	LSU Ψ1004, Ψ1052
(–)2Ψc	D9: pDK159 - snR5 (LSU: Ψ1124); pDK157- snR44 (SSU: Ψ106; LSU Ψ1056), Rps22B	SSU: Ψ106, Rps22B LSU: Ψ1056, Ψ1124	LSU: Ψ1004, Ψ1042
(–)3Ψa	D11: pDK166 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960, Ψ986), Rps22B	LSU: Ψ960, Ψ986, Ψ1124 U2: Ψ42	LSU: Ψ1004, Ψ1042, Ψ1052
(–)3Ψb	D10: pDK164 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960), Rps22B	LSU: Ψ960, Ψ1124 U2: Ψ42	LSU: Ψ986, Ψ1004, Ψ1052
(–)3Ψc	D11: pDK163 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960), Rps22B; pDK152 - snR81 (LSU: Ψ1052; U2: Ψ42)	LSU: Ψ960, Ψ1052, Ψ1124 U2: Ψ42	LSU: Ψ986, Ψ1004, Ψ1042
(–)3Ψd	D11: pDK163 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960), Rps22B; pDK154 - snR5 (LSU: Ψ1004, Ψ1124)	LSU: Ψ960, Ψ1004, Ψ1124 U2: Ψ42	LSU: Ψ986, Ψ1042, Ψ1052
(–)4Ψa	D11: pDK164 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960), Rps22B	LSU: Ψ960, Ψ1124 U2: Ψ42	LSU: Ψ986, Ψ1004, Ψ1042, Ψ1052
(–)5Ψa	D13: pDK164 - snR44/81 (SSU: Ψ106; U2: Ψ42), snR5 (LSU: Ψ1124), snR8 (LSU: Ψ960), Rps22B	SSU: Ψ106, Rps22B LSU: Ψ960, Ψ1124 U2: Ψ42	LSU: Ψ986, Ψ1004, Ψ1042, Ψ1052, Ψ1056,
(–)5Ψb	D10: pDK158 - snR44/81 (SSU: Ψ106; U2: Ψ42), Rps22B	U2: Ψ42	LSU: Ψ960, Ψ986, Ψ1004, Ψ1052, Ψ1124
(–)6Ψ	D11: pDK158 - snR44/81 (SSU: Ψ106; U2: Ψ42), Rps22B	U2: Ψ42	LSU: Ψ960, Ψ986, Ψ1004, Ψ1042, Ψ1052, Ψ1124
(–)7Ψ	D13: pDK158 - snR44/81 (SSU: Ψ106; U2: Ψ42), Rps22B	SSU: Ψ106, Rps22B U2: Ψ42	LSU: Ψ960, Ψ986, Ψ1004, Ψ1042, Ψ1052, Ψ1056, Ψ1124

Plasmid constructs

Plasmid constructs with snR5, snR8 and snR81 expression cassettes: To create an ADE2 plasmid with multiple cloning sites, a genomic fragment of the S. *cerevisiae ADE2* gene was amplified by PCR using oligos 1 and 2 (see below), and after digestion with NcoI/NsiI (New England Biolabs) was used to replace a NcoI/NsiI fragment in pRS316 (4), generating pDK141. To generate snR5 gene plasmids, a genomic DNA fragment of ~500 bp was amplified by PCR using oligos 3 and 4. After digestion with XhoI and SacII, the resulting DNA fragment was inserted into pRS415 (*LEU2*) and pDK141 (*ADE2*) to generate pDK146 and pDK154, respectively. To create snR5 with a mutation in the guide domain that is specific for the ASF, two PCR products were produced from pDK146 using pairs of oligos 3/5 and 6/4. These fragments were used together with flanking oligos 3 and 4 to generate a full expression cassette for snR5; following XhoI/SacII digestion, the product fragment was inserted into pDK141 and pRS316 (*URA3*), to yield pDK156 and pDK159, respectively. Two expression cassettes of snR8 were generated using oligos 7 and 8 from pNJ46 (one with a mutation in the guide domain of snR8 that targets the ASF helix) and pNJ44 (with wild type snR8) (5); after digestion with SacI/SacII fragments were inserted into SacI/SacII treated pDK156, pDK159, and pDK154 to generate pDK161 (*ADE2* plasmid with mutant snR5 and mutant snR8), pDK165 (*URA3* plasmid with mutant snR5 and wild type snR8), and pDK218 (*ADE2* plasmid with wild type snR5 and wild type snR8), respectively. The pDK160 construct is a *URA3*

plasmid and contains the same insert as pDK161. To generate a snR81 gene plasmid, a genomic DNA segment was amplified by PCR using oligos 34 and 35. After digestion with EcoRI and XhoI, the product was inserted into EcoRI/XhoI digested pDK141 and pRS316, generating pDK152 and pDK257, respectively.

Plasmids with Rps22B expression cassettes: A plasmid with an expression cassette that specifies wild type protein Rps22B was constructed in two steps. First, two fragments ~1000 bp, and ~1500nt were generated by PCR from genomic DNA using paired oligos 9/10 and 11/12 and VENT polymerase (New England Biolabs). The PCR products were used together with flanking oligos 9 and 12 to amplify ~2.5kb fragment using a mixture of VENT and TAQ polymerases (New England Biolabs). The PCR product was digested with SalI and KpnI and inserted into XhoI/KpnI digested pDK141, generating pDK157. A plasmid containing an expression cassette of Rps22B was created that includes a modified, intron-encoded snoRNA snR44, in which the domain A guide sequence of snR81 was substituted for domain B of snR44; this was constructed in two steps. First, two fragments ~780 bp, and ~750nt were generated by PCR from pDK157 using pairs of oligos 11/13 and 14/12. The PCR products were used together with flanking oligos 11 and 12 to amplify ~1.5kb fragment, which was digested with PflMI and KpnI and inserted into PflMI /KpnI digested pDK157, generating pDK158.

Plasmids with three expression cassettes for snoRNAs: To create plasmids with three expression cassettes of mutant and wild type snoRNAs, plasmids pDK161, pDK160, pDK165 and pDK218 were digested with XhoI/PstI; and plasmids pDK157 and pDK158 were digested with SalI/PstI. A fragment of ~5700nt from pDK158 was ligated with fragments of ~4500nt from pDK161, ~2700nt from pDK160 and ~2700nt from pDK165, to yield pDK164 (see Supplemental Fig. S2*B*), pDK163 and pDK166, respectively. A fragment of ~5700nt from pDK157 was ligated with a fragment ~4500nt from pDK218, to produce pDK219.

DNA oligonucleotides

DNA oligonucleotides for plasmid constructions:

1. CATGCCATGGAATGTGTCCATCTGACATTACTATTTTGC
2. CCAATGCATTAAGCGTTGATTTCTATGTATGAAGTCCAC
3. CCGCTCGAGGACGTTAATAGGAACTCATGGTG
4. TCCCCGCGGATAATTGAAGTATATGTACG
9. ACGCGTCGACCTTATATGCAATTCGGTTGCATAC
12. CGGGGTACCTTCTGGAGGTAAATGATCTATTG
11. TTTTCCTCAGTTGTGGGCCCATGTG
10. TTTCAATATATTCCTTCCCTCCAAC
13. AGTAGCTACACATGAGGTGTAATCCATAACCCTTCTTTTCTTTCCACATGGGATTAAATA
14. GGATTACACCTCATGTGTAGCTACTATATCCATTACCCGATACTTTTCCTCAAAATCTCA
5. TTGTTTTCTTAATTAAGAAAAACCATTAAAACTGGTGAATATTTCAATAATGAAAAGCTA
6. GTTTTTCTTAATTAAGAAAACAAATTATCATTGGGGGAAACTAGGTGTACATATCTTCAC
7. TCCCCGCGGATGCCATATTTCTCTGCCTTG
8. AAACGAGCTCGGCCGGGTAACAGAAAACTGTC
15. CGGGATCCACGATGGGCTCCTGAGCAAGCTTCGATCCCGTCGTTTTACAACG
16. CGGGATCCACGATGGGCTCTCAATAGCAAGCAAGCTTCGATCCCGTCGTTTTACAACG
17. CGGGATCCACGATGGGCTCTCAATAAGCAAGCTTCGATCCCGTCGTTTTACAACG
18. CCCGAGCTCTTATTATTATTTTTGACACCAGACCAACTGGT
34. CCGCTCGAGTGCCCAATTTATTGGATATTAC
35. CGGAATTCGACGTTCTTCGCACGCTCGGCA

Probes used for Northern analyses of snoRNAs:

snR5: TGTCTTAAGCATGGTAATCCGGAAGATCAG
snR8: ACGGATGTAAGATGGCACAGTGAATAGAAG
snR33: GATTGTCCACACACTTCTATATC
snR44: CACATGAGGTGTAATCCATAACC; CGATCACACCATCTAGTTATCAG
snR81: GGATTGCTCTTGGGACCGTAGTATCG

DNA oligonucleotides used in gene disruptions

snR33: 19.
ATTAAAAAAATAAGCTTAATGCCCTCTTTGTACGATGGTGTCACTCTAAAAGATTGTACTGAGAGTGCAC
20. TAAACAAGCTCAGTAGTAATACATAAAATAAAAAGTTTTGCAAATCCTGTGCGGTATTTCACACCG
snR5: 21. CAATTCTTTGAACTTACTTCATTTGCAGGATCCTTCAGGATAAGAAAACCAGATTGTACTGAGAGTGCAC
22. GAAGTATATGTACGATGTATACAATGTAAACAAGAGAGTAATGAAGTGAAGCTGTGCGGTATTTCACACCG
snR8: 23. CTCTTCTTTCATTTTTTAGTTATCTTTGATTTTCGGTTCCGAAGAAGGCAGATTGTACTGAGAGTGCAC

24. ATAAAATAGTAAAGGAAGCTATTGCTACAGATAAATATAAATGAATGAACTGTGCGGTATTTCACACCG
snR81: 25. GATGGAGTACCCTCATGCTTTGGCGCGTGCGCATCTATATAAGGTAGATTGTACTGAGAGTGCAC
26. GACTGACTGAATCATATTACTTAGTTACTACTACTCTTATCATGCAAGACTGTGCGGTATTTCACACCG
snR44: 27.
TTCAATGTTGCGCAACTCTAACGAAGAAATAATTATTGCCATTGTTTTTTAAGATTGTACTGAGAGTGCAC
28. ATGTTGTCTAAGTTGTGCGGAAGAATTTTGCCATTGAAAATTTTAAGAGTGCTGTGCGGTATTTCACACCG

DNA oligonucleotides used for pseudouridine mapping

U2: 29. TGCCAAAAAATGTGTATTGTAACAA
LSU: 30. TCCCGCATCGCCAGTTCTGCT; 31. CGTTCAATTAAGTAACAAGGACTTC; 32.
CGACCCCGGAACCTCTAATCATTCG
SSU: 33. GTAAAGGAACTATCAAATAAACG

References

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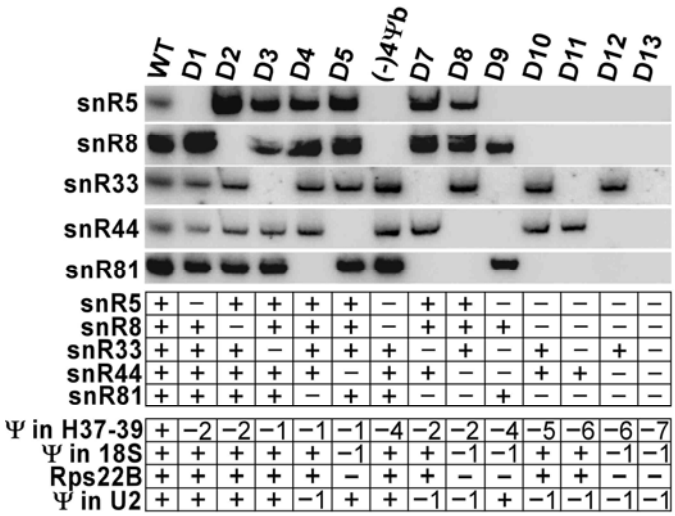
Supplemental Figures

Supplemental Figure S1



	WT	D1	D2	D3	D4	D5	(-)4Ψb	D7	D8	D9	D10	D11	D12	D13
snR5	+	−	+	+	+	+	−	+	+	−	−	−	−	−
snR8	+	+	−	+	+	+	−	+	+	+	−	−	−	−
snR33	+	+	+	−	+	+	+	−	+	−	+	−	+	−
snR44	+	+	+	+	+	−	+	+	−	−	+	+	−	−
snR81	+	+	+	+	−	+	+	−	−	+	−	−	−	−

	WT	D1	D2	D3	D4	D5	(-)4Ψb	D7	D8	D9	D10	D11	D12	D13
Ψ in H37-39	+	−2	−2	−1	−1	−1	−4	−2	−2	−4	−5	−6	−6	−7
Ψ in 18S	+	+	+	+	+	−1	+	+	−1	−1	+	+	−1	−1
Rps22B	+	+	+	+	+	−	+	+	−	−	+	+	−	−
Ψ in U2	+	+	+	+	−1	+	+	−1	−1	+	−1	−1	−1	−1

Fig. S1. Five snoRNAs were examined that together target the formation of 7 of the 10 Ψs in the H37-39 region (and other regions too). The initial set of 13 test strains was constructed by sequential disruption of snoRNA coding sequences for snR5, snR8, snR33, snR44 and snR81. The presence of the snoRNAs was examined by northern analysis and the patterns are summarized in a table under the hybridization data. The mutant strains are identified as D1, D2, etc. (top). A second table summarizes the effects on the Ψ patterns for: the H37-39 region, and other sites in 18S rRNA and U2 snRNA. Loss of small ribosomal protein Rps22B is also indicated. '-', deleted; '+', not deleted.

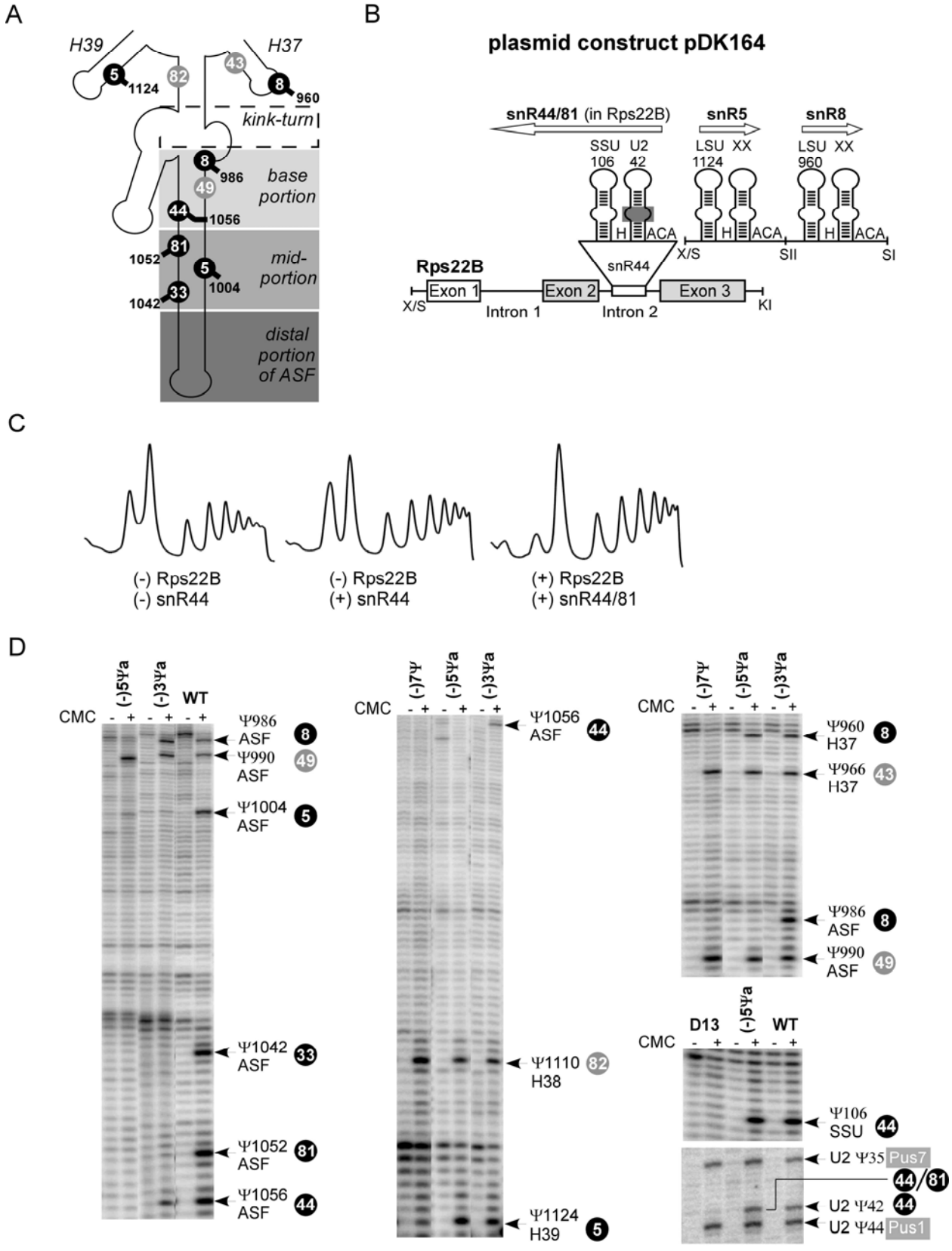

A
H39
H37
5
1124
82
43
8
960
kink-turn
8
986
49
base portion
44
1056
81
1052
5
1004
mid-portion
33
1042
distal portion of ASF
B
plasmid construct pDK164
snR44/81 (in Rps22B)
snR5
snR8
SSU U2
106 42
LSU XX
1124
LSU XX
960
H
ACA
X/S
SII
SI
snR44
Rps22B
Exon 1
Exon 2
Exon 3
X/S
Intron 1
Intron 2
KI
C
(-) Rps22B
(-) snR44
(-) Rps22B
(+) snR44
(+) Rps22B
(+) snR44/81
D
CMC
(-)5Ψa
(-)3Ψa
WT
Ψ986 ASF 8
Ψ990 ASF 49
Ψ1004 ASF 5
Ψ1042 ASF 33
Ψ1052 ASF 81
Ψ1056 ASF 44
(-)7Ψ
Ψ1056 ASF 44
Ψ1110 H38 82
Ψ1124 H39 5
Ψ960 H37 8
Ψ966 H37 43
Ψ986 ASF 8
Ψ990 ASF 49
D13
Ψ106 SSU 44
U2 Ψ35 Pus7
44/81
U2 Ψ42 44
U2 Ψ44 Pus1

Fig. S2. Re-engineering of snoRNAs and a protein gene for construction of strains missing 1-7 Ψs in three-helix ASF region.
(A) Schematic structure of the 3-helix H37-39 region with the locations of 10 Ψ modifications highlighted. Black circles, 7 Ψs removed for the depletion analysis; Gray circles, 3 Ψs not analyzed. The positions of the Ψs in the sequence are given in bold numbers (e.g., 1124 in H39). The numbers in the circles correspond to the snoRNAs that guide the Ψ modification (e.g., '5' refers to snoRNA species snR5).
(B) Structure of one of four plasmids (pDK164) with three snoRNA expression cassettes designed to restore: specific rRNA and U2 RNA Ψ modifications lost on depletion of natural snoRNAs with multiple Ψ guide motifs, and expression of protein Rps22B. The second intron of the Rps22 gene contains an artificial hybrid snoRNA snR44/81. A grey box in the hairpin-loop region of the snoRNA (upstream of box ACA) represents a replacement of an ASF-specific guide motif in snR44 with a guide motif from the snR81 snoRNA; the latter motif specifies a Ψ in U2 snRNA. The positions of the Ψ modifications restored are listed above the snoRNA. XX, designates a guide motif that has been inactivated by mutations; KI, SI and SII are restriction sites for *Kpn*I, *Sac*I, *SacII;* X/S, compatible ligated ends after digestion with *Xho*I and *Sal*I.
(C) Polysome analysis shows that small ribosomal protein Rps22B is produced from the expression plasmid. Although cells contain two alleles for the Rps22 protein, deletion of the Rps22B gene causes the level of free 40S subunits to be reduced and a large increase in free 60S subunits (left polysome pattern). Expression of snR44 from a cassette missing the third exon of Rps22B does not restore the level of 40S subunits (middle pattern). A normal polysome profile is observed with a plasmid containing a full expression cassette for Rps22B (right pattern). In addition, replacement of the second guide motif in snR44 with a guide motif from snR81 (snR44/81) does not interfere with pre-mRNA splicing.
(D) The Ψ modification status of yeast mutants (–)3Ψa, (–)5Ψa and (–)7Ψ containing expression plasmids for complementing guide snoRNAs. For example, (–)5Ψa is strain D13 carrying a plasmid with the three expression cassettes shown in B above. Total RNA was treated with (+) or without (-) CMC and subjected to primer extension analysis. The mutants are identified above the lanes of extension products. The labels at the right in each set of patterns identify: the individual Ψs by position, the relevant rRNA helix region, and the corresponding guide snoRNAs (circled numbers). Abbreviations include: SSU, small ribosomal subunit; U2, U2 snRNA; ASF, A-site finger; H38, helix 38; H37, helix 37; H39, helix 39; Pus7, Pus1, Ψ synthase enzymes that form Ψ in U2 snRNA.

Supplemental Figure S3

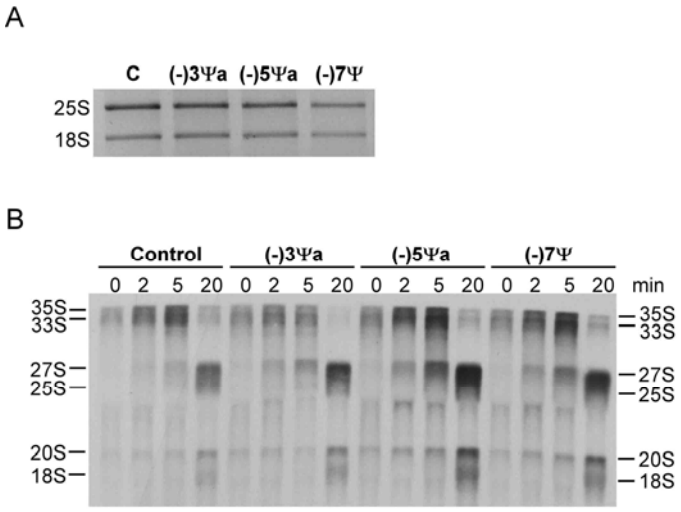


Fig. S3. A cluster of 7 Ψs in the three-helix region protects the large subunit at reduced temperature. Significantly slower growth of (–)7Ψ strain at 11°C is accompanied by changes in ribosomal subunit contents. (A) The steady state levels of both 18S and 25S rRNAs were substantially reduced when cells missing 7 Ψs were cultured in lower temperature to OD600= ~0.8. 18S and 25S rRNAs were examined by ethidium bromide staining of total RNA from equivalent numbers of cells. 'C', control cells; (–)3Ψa, (–)5Ψa, and (–)7Ψ, mutant strains. (B) The pre-rRNA processing is not impaired at lower temperature for strains missing Ψs in the three-helix region. Patterns of pre-rRNA processing were examined by pulse labeling. Cultures were grown at 30°C to OD600=0.8, transferred to 11°C for 1.5 hr., then pulse labeled with [3H] methionine for 3 min., followed by addition of an excess of cold methionine. Samples were collected at the times indicated, and RNAs were extracted, separated on a denaturing 1.2% agarose gel and transferred to a Zeta-probe membrane (Bio-Rad). The membrane was sprayed with En^{3}Hance (Dupont) and a radiograph prepared with X-ray film. Samples and chase times are identified above the gel lanes and the main precursor and product rRNA species are listed at the left.

Supplemental Figure S4

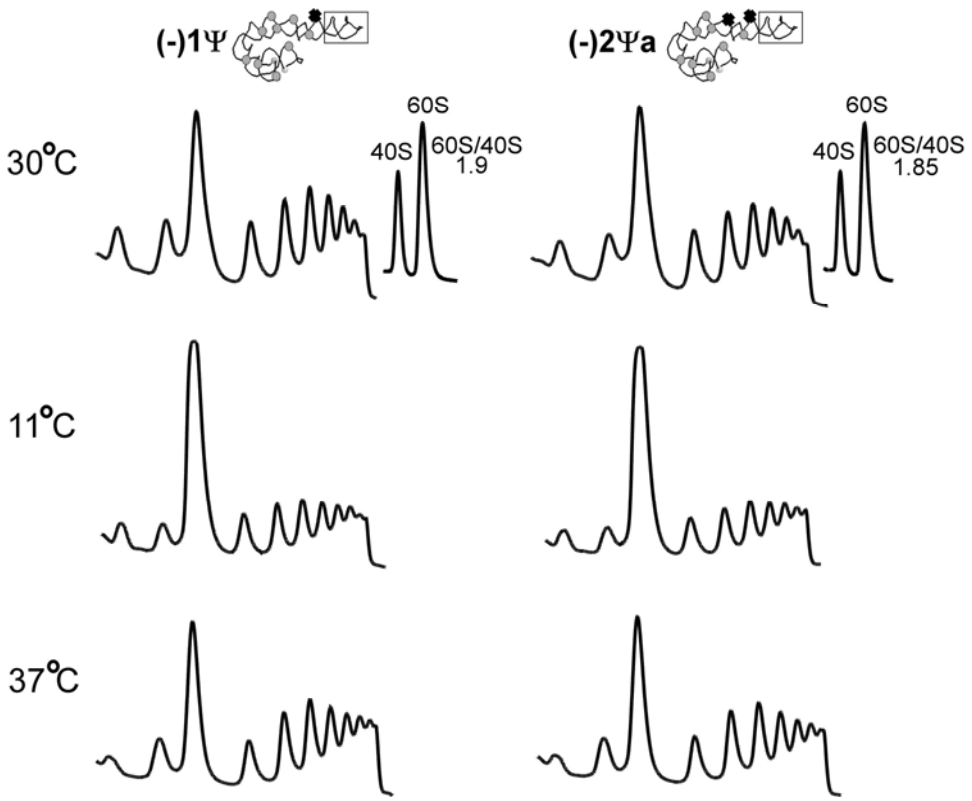


Fig. S4. Normal polysome profiles are obtained for mutants initially lacking 6-7 Ψs in the ASF region, when all but one or two Ψs are restored. Test cells were transformed with plasmids expressing wild type snoRNAs. Polysome analyses were carried out with cells grown at 30°C, and for a short time at 11°C (60 min.) and 37°C (45 min.).