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Supplemental Data

A Convergence of rRNA and mRNA Quality Control Pathways Revealed by Mechanistic Analysis of Nonfunctional rRNA Decay

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Supplemental Results

Because there are well-characterized large subunit mutations that affect sensitivity to cycloheximide (Kaufer and Warner, 1983; Liu and Liebman, 1996), it is possible that 25S:U2585A rRNA decay is resistant to cycloheximide inhibition because this mutation alters cycloheximide binding. Therefore, we repeated the transcriptional pulse-chase assay using hygromycin B. Hygromycin B is an aminoglycocide that inhibits translation elongation and causes misincorporation of amino acids. It is mechanistically distinct from cycloheximide, as it binds the decoding site in the small subunit and does not compete with cycloheximide binding (Poehlsgaard and Douthwaite, 2005; Stocklein and Piepersberg, 1980). Unlike cycloheximide, which diffuses easily across the cell membrane and acts immediately (Figure S1), aminoglycocides enter cells via both diffusion and active transport (Taber et al., 1987). Consequently, at a concentration selective for yeast growth (1.5 mg/mL), hygromycin B initially decreases but does not abolish protein synthesis (Figure S1). At 10 mg/mL we found hygromycin B rapidly inhibits translation, but total RNA was completely degraded (data not shown). Therefore we analyzed 18S:A1492C and 25S:U2585A rRNA stability in the presence of 1.5 mg/ml hygromycin B, which was sufficient to reduce the rate of radioactive amino acid incorporation by 50%. At this concentration, 18S:A1492C rRNA decay was completely blocked, but 25S:U2585A rRNA was still degraded (Figure S1).

Supplemental Experimental Procedures

Yeast strains and plasmids

The *xrn1*Δ strain, HFY1081, the temperature sensitive *rat1-1* strain, HFY1101, and their parental strains, HFY1200 and HFY1096, respectively, were a generous gift from A. Jacobson (University of Massachusetts, Worcester). Strains yRP1923 (*xrn1*Δ DCP2-GFP) and yRP1736 (*dcp1*Δ DHH1-GFP) and plasmids pRP469 (PGK1) and pRP1251 (PGK1-SL) were a kind gift from R. Parker (University of Arizona, Tucson). The temperature sensitive *rrp44-1* strain, D348, was a gift from D. Tollervey (University of Edinburgh). The *hbs1*Δ*dom34*Δ strain, ySC296, was created by crossing strain YNL001W (*MAT* **a** *dom34::KAN*) to ySC277 (*MAT* α *hbs1::KAN*). The *hbs1*Δ*ski7*Δ strain, ySC314, was created by crossing strain YOR076C (*MAT* **a** *ski7::KAN*) with ySC277 (*MAT* α *hbs1::KAN*), and the *dom34*Δ*xrn1*Δ strain, ySC318, was

created by crossing strain YNL001W (*MAT* **a** *dom34::KAN*) to ySC317 (*MAT* α *xrn1::KAN*). Tetrads from sporulated diploids were dissected; genotypes were confirmed by PCR. All rDNA reporter plasmids used in this study contain 18S and 25S sequence tags complementary to oligos FL125 and FL126, respectively (see Table S2). The pJV12 plasmids express tagged 18S and 25S rRNAs under control of the constitutive PGK1 promoter (Jeeninga et al., 1997; LaRiviere et al., 2006). Plasmids pSC39 and pSC40 express *GAL7* transcribed 35S rRNA. Their creation has been described elsewhere (Cole and LaRiviere, 2008). To create plasmid pSC39-U2585A, the 4.6 kb *Bgl*II to *Bgl*II fragment of pSC39 containing the portion of the 35S rDNA locus with U2585 was excised and replaced with the same fragment of pJV12-U2585A using T4 DNA ligase (NEB). Plasmids pSC40-A1492C and pSC40-U2585A were created similarly, except the *Bgl*II to *Bgl*II fragment was excised from pSC40 and replaced with the same fragment from pJV12-A1492C and pJV12-U2585A, respectively. All plasmids were confirmed by sequencing. Yeast transformations followed standard protocols (Gietz et al., 1992).

In Vivo [^{35}S] Methionine incorporation

[^{35}S] methionine incorporation was done as described previously (Carr-Schmid, 1999) using strain BY4742 with the following modifications. Ice-cold 100% TCA (0.1 mL) was added to 0.9 mL aliquots taken at indicated time points. In cultures containing translation inhibitors, cychoheximide or hygromycin B was added along with methionine to final concentrations of 0.1 mg/ml and 1.5 mg/mL, respectively.

Half-life analysis in the presence of hygromycin B

Transcriptional pulse-chases analyses of plasmid-derived rRNAs were done as previously described (Cole and LaRiviere, 2008) except hygromycin B was added at the time of transcriptional shut off to a final concentration of 1.5 mg/mL. RNA from aliquots taken at indicated time points was analyzed by northern blot as above. During transcriptional pulse-chase, cell division was monitored by OD_{600} to account for dilution of plasmid-derived rRNA during the time course. Corrected data were fit to single or to double rate equations using KaleidaGraph (Synergy Software); half-lives were calculated using these equations.

Table S1. Strains and plasmids used in this study

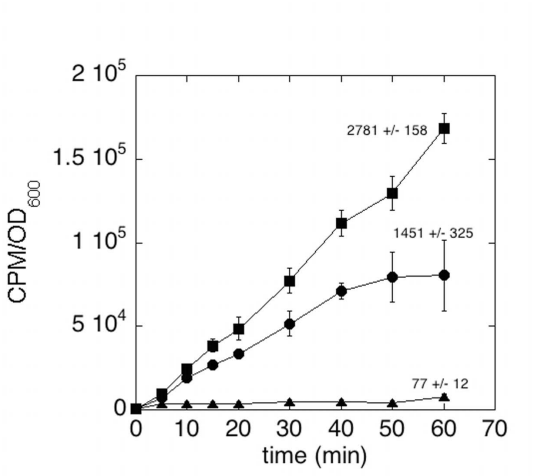
Strain or Plasmid	Genotype and notes	Source or reference
BY4741	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Invitrogen YKO
BY4742	*MAT* α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Invitrogen YKO
YOR001W	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 rrp6::KAN*	Invitrogen YKO
YOR076C	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ski7::KAN*	Invitrogen YKO
YGL173C	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 xrn1::KAN*	Invitrogen YKO
YMR080C	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 upf1::KAN*	Invitrogen YKO
YNL001W	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dom34::KAN*	Invitrogen YKO
YKR084C	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 hbs1::KAN*	Invitrogen YKO
ySC277	*MAT* α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 hbs1::KAN*	This Study
ySC296	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dom34::KAN hbs1::KAN*	This Study
ySC314	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 ski7::KAN hbs1::KAN*	This Study
ySC317	*MAT* α *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 xrn1::KAN*	This Study
ySC318	*MAT* **a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 dom34::KAN xrn1::KAN*	This Study
HFY1200	*MAT* **a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	(He and Jacobson, 2001)
HFY1081	*MAT* **a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100 xrn1::ADE2*	(He and Jacobson, 2001)
HFY1096	*MAT* α *ura3-52 trp1-Δ63 leu2-Δ1*	(He and Jacobson, 2001)
HFY1101	*MAT* α *ura3-52 trp1-Δ63 leu2-Δ1 rat1-1*	(He and Jacobson, 2001)
D348	*MAT* α *ura3-52 lys2 rrp44-1*	(Bousquet-Antonelli et al., 2000)
yRP1923	*MAT* **a** *leu2-3,112 trp1 ura3-52 his4-539 cup1::LEU2/PGK1pG/MFA2pG xrn1::URA3 DCP2-GFP (NEO)*	(Teixeira et al., 2005)
yRP1736	*MAT* **a** *leu2-3,112 trp1 ura3-52 his4 cup1::LEU2/PGK1pG/MFA2pG dcp1::URA3 DHH1-GFP(NEO)*	(Sheth and Parker, 2003)
pJV12-WT	*URA3, 2μ, rDNA (wild type)*	(Jeeninga et al., 1997)
pJV12-G530U	*URA3, 2μ, rDNA 18S:G530U*	(LaRiviere et al., 2006)
pJV12-A1492C	*URA3, 2μ, rDNA 18S:A1492C*	(LaRiviere et al., 2006)
pJV12-A2451G	*URA3, 2μ, rDNA 25S:A2451G*	(LaRiviere et al., 2006)
pJV12-U2585A	*URA3, 2μ, rDNA 25S:U2585A*	(LaRiviere et al., 2006)
pSC39	*TRP1, 2μ, GAL7-rDNA (wild type)*	(Cole and LaRiviere, 2008)
pWL160-A1492C	*TRP1, 2μ, GAL7-rDNA 18S:A1492C*	(LaRiviere et al., 2006)
pSC39-U2585A	*TRP1, 2μ, GAL7-rDNA 25S:U2585A*	This Study
pSC40	*URA3, 2μ, GAL7-rDNA (wild type)*	(Cole and LaRiviere, 2008)
pSC40-A1492C	*URA3, 2μ, GAL7-rDNA 18S:A1492C*	This Study
pSC40-U2585A	*URA3, 2μ, GAL7-rDNA 25S:U2585A*	This Study
pRP469	*URA3, CEN, GAL1-PGK1*	(Decker and Parker, 1993)
pRP1251	*URA3, CEN, GAL1-PGK1-SL*	(Doma and Parker, 2006)

Table S2. Oligonucleotides used in this study

Name	Sequence	Notes	Source or reference
FL125	5' - CGAGGATCCAGGCTTT	complementary to 18S tags	(Beltrame et al., 1994)
FL126	5' - ACTCGAGAGCTTCAGTACC	complementary to 25S tags	(Musters et al., 1989)
FL217	5' - ATCCCGGCCGCCTCCATCAC	complementary to SCR1	(Galani et al., 2004)
RP141	5' - AATTGATCTATCGAGGAATTCC	complementary to PGK1 tag	(Caponigro and Parker, 1995)

Figure S1. Effects of hygromycin B on translation and NRD. (A) Incorporation of [^{35}S] methionine in untreated cells (■), cells treated with 1.5 mg/mL hygromycin B (●) or cells treated with 100 µg/mL cycloheximide (▲). The average rate of [^{35}S] methionine incorporation in CPM/min is noted next to each curve. Data is an average of three independent trials (error: standard deviation). (B) Transcriptional pulse-chase and northern analysis of wild type cells (BY4741) carrying pSC40-A1492C (18S:A1492C) in the absence or presence of 1.5 mg/mL hygromycin B. (C) Same as in (B) except cells are carrying pSC40-U2585A (25S:U2585A). In both B and C, times indicated are relative to transcriptional shut off (i.e., glucose addition; t=0). Plasmid-derived rRNAs were detected with ^{32}P-labeled probe FL125 or FL126. Endogenous scR1 RNA, which served as a loading control, was also monitored by northern blotting (error: standard deviation).

A



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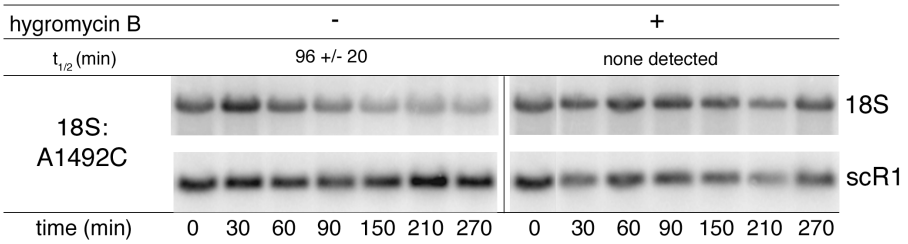

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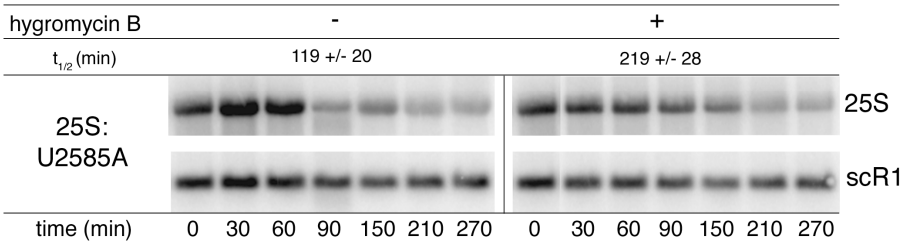


Figure S2. Analysis of 18S and 25S NRD in an alternate *xrn1*Δ strain. Transcriptional pulse-chase and northern analysis of 18S:A1492C rRNA expressed from pSC39-A1492C (left) and 25S:U2585A rRNA expressed from pSC39-U2585A (right) in wild type strain HFY1200 or the isogenic *xrn1*Δ strain HFY1081. Times indicated are relative to transcriptional induction (i.e., galactose addition; t=0). † indicates a likely 18S rRNA decay intermediate. Plasmid-derived 18S rRNA, 25S rRNA and endogenous scR1 RNA, which served as a loading control, were detected with ^{32}P-labeled oligo probes FL125, FL126 and FL217, respectively. Histogram reports average rRNA half-lives from at least three independent trials (error: standard deviation), and statistically significant differences (unpaired student t-test; $p<0.01$) in half-lives are indicated (*).

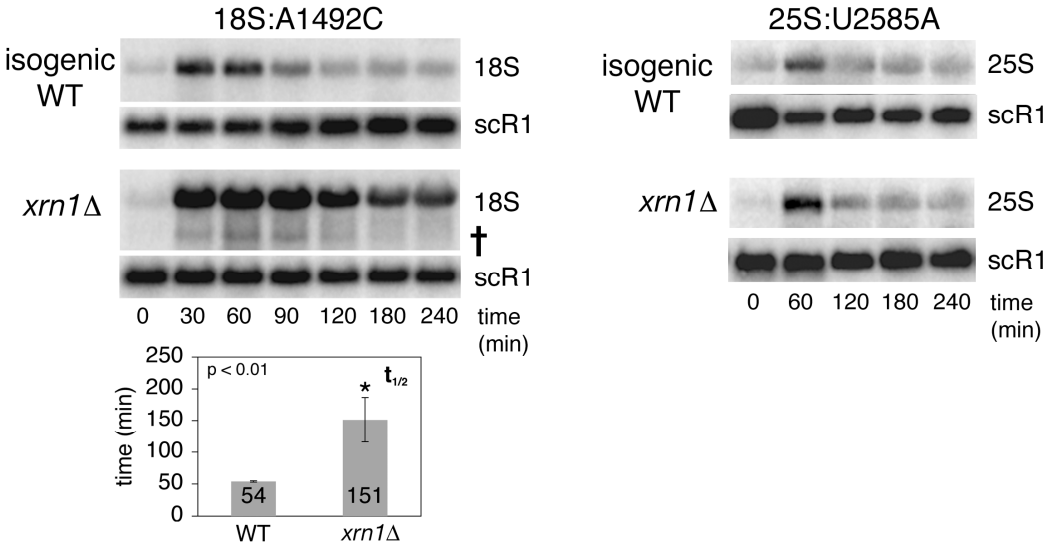


Figure S3. Transcriptional shut off and northern analysis of 25S:U2585A rRNA expressed from pSC39-U2585A in the temperature-sensitive *rat1-1* strain grown in galactose at the permissive temperature (23°C) or shifted to the non-permissive temperature (37°C) 3 hours prior to addition of glucose. Times indicated are relative to transcriptional shut off (i.e., glucose addition; t=0). Plasmid-derived rRNAs and endogenous scR1 RNA, which served as a loading control, were detected with ^{32}P-labeled probes (FL126 and FL217). Histogram reports average rRNA half-lives from at least three independent trials (error: standard deviation).

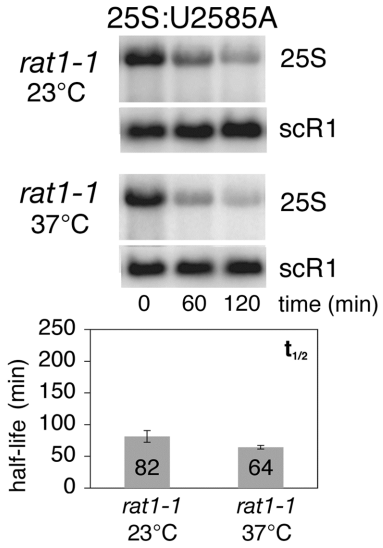


Figure S4. Analysis of 18S and 25S NRD in strain *rrp44-1*. (A) Northern analysis of total RNA from *rrp44-1* carrying plasmid pSC40 (25S:wild type) grown at 37°C. Top arrow indicates where the 25S:U2585A rRNA fragment runs. Bottom arrow points to the smaller 25S rRNA fragment present regardless of mutational status. (B) Northern blot of total RNA from *rrp44-1* carrying plasmid pSC40-U2585A (25S:U2585A) grown at 37°C or 23°C. The upper arrow points to the 25:U2585A rRNA fragment that is seen only at 37°C. This decay intermediate migrates separately from a smaller 25S rRNA band, which is also observed in northern analysis of wild type 25S rRNA (lower arrow). (C) Darker exposure of northern blots in Figure 4C showing the absence of smeared rRNA below full-length 18S rRNA at 37°C. (D) Darker exposure of northern blots in Figure 4D showing the presence of a fragment of 25S:U2585A rRNA and the absence of smeared rRNA below full-length 25S rRNA at 37°C.

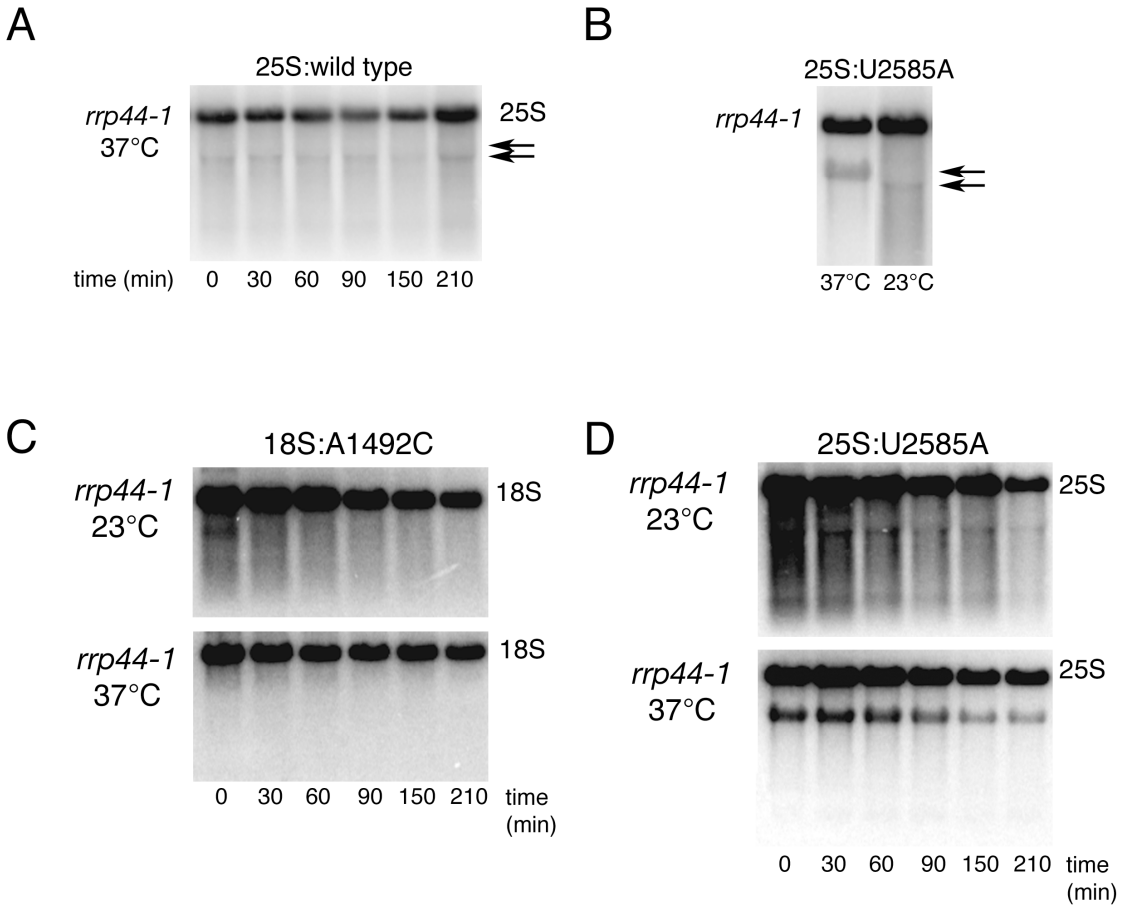


Figure S5. Analysis of 25S NRD in double mutant strains. Transcriptional pulse-chase analysis of 25S:U2585A rRNA expressed from pSC40-U2585A in wild type strain BY4741 or indicated isogenic mutant strains. Times indicated are relative to transcriptional shut off (i.e., glucose addition; t=0). Plasmid-derived 25S rRNA and endogenous scR1 RNA, which served as a loading control, were detected with ^{32}P-labeled probes (FL126 and FL217).

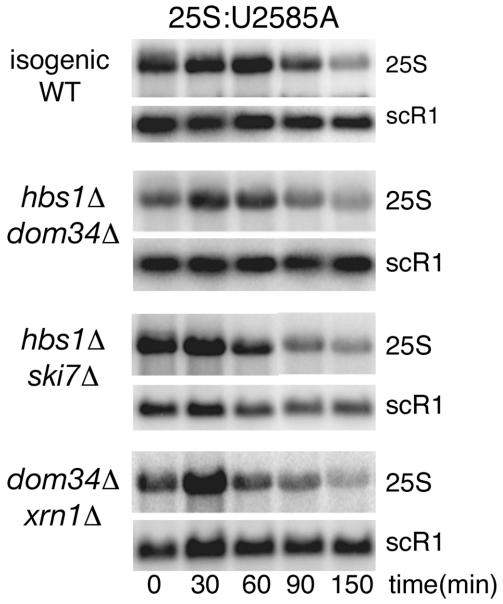


Figure S6. (A) *In situ* localization of 25S rRNAs in the absence of Xrn1p. FISH analysis of *xrn1*Δ cells (YGL173C) carrying no plasmid, pJV12-WT (25S:wild type) or pJV12-U2585A (25S:U2585A) using Alexa 594-labeled probe FL126. The nucleus is marked by DAPI staining. (B) Localization of P-bodies, as marked by GFP-tagged Dhh1p, in *dcp1*Δ cells. Cells were imaged live in YPD using an Olympus BX51 microscope, Qimaging Retiga Exi camera and Velocity Software (Improvision Inc.).

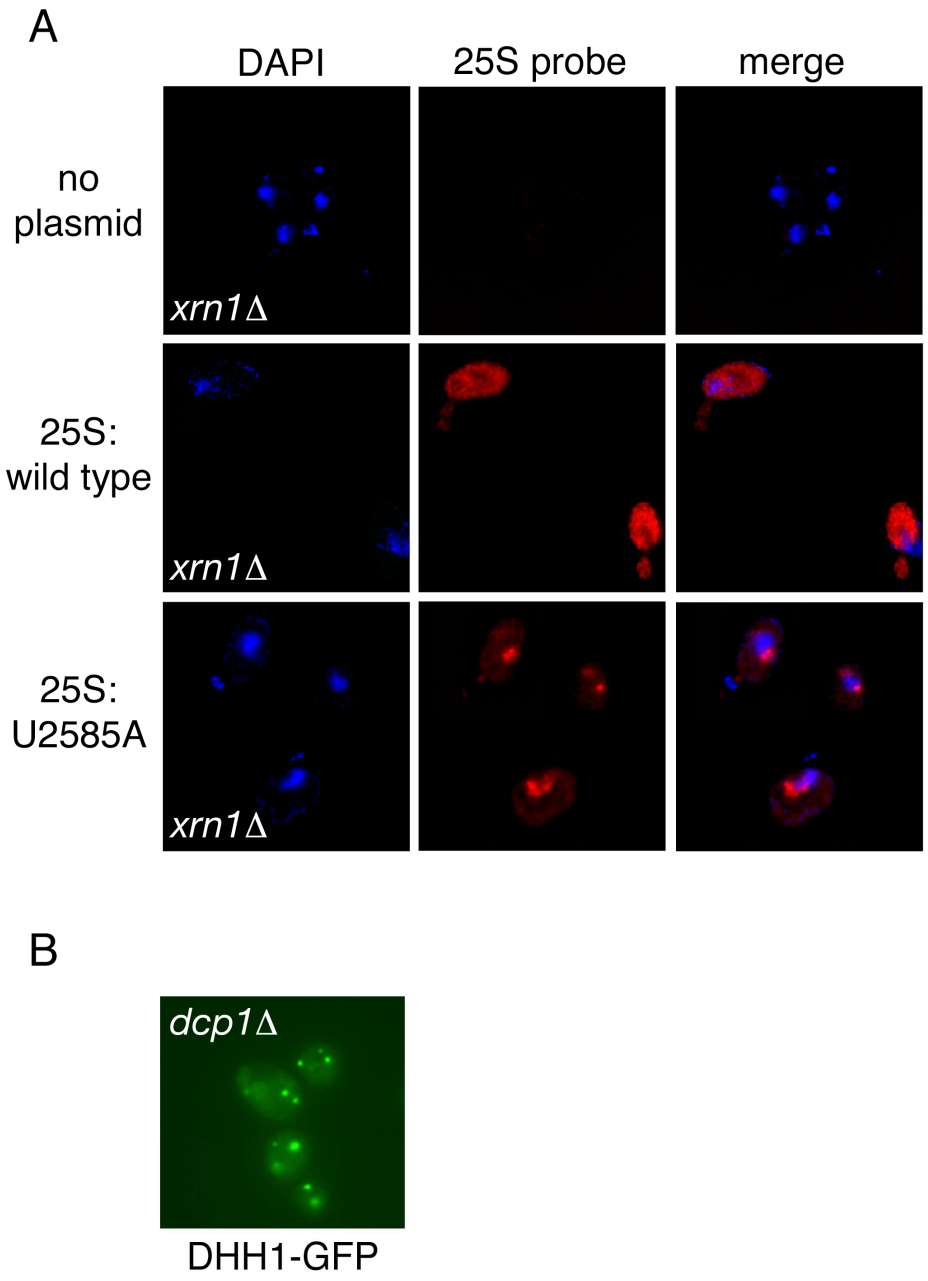
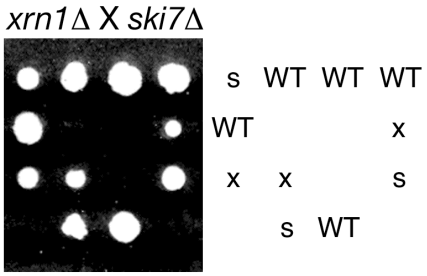


Figure S7. Deletion of *XRN1* and *SKI7* is synthetically lethal. Dissected tetrads from a cross between strains YOR076C (*MAT* **a** *ski7*Δ) and ySC317 (*MAT* α *xrn1*Δ). Spores were grown on YPD for 7 days at 23°C. Viable spores were wild type (WT), *ski7*Δ (s) or *xrn1*Δ (x). The same result was obtained when spores were grown at 30°C.



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