The Paf1 Complex Subunit Rtf1 Buffers Cells Against the Toxic Effects of [*PSI*$^+$] and Defects in Rkr1-Dependent Protein Quality Control in *Saccharomyces cerevisiae*

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ABSTRACT The Rtf1 subunit of the Paf1 complex is required for specific histone modifications, including histone H2B lysine 123 monoubiquitylation. In *Saccharomyces cerevisiae*, deletion of *RTF1* is lethal in the absence of Rkr1, a ubiquitin-protein ligase involved in the destruction of nonstop proteins, which arise from mRNAs lacking stop codons or translational readthrough into the poly(A) tail. We performed a transposon-based mutagenesis screen to identify suppressors of *rtf1Δ rkr1Δ* lethality and found that a mutation in the gene encoding the protein chaperone Hsp104 rescued viability. Hsp104 plays a role in prion propagation, including the maintenance of [*PSI*$^+$], which contributes to the synthesis of nonstop proteins. We demonstrate that *rtf1Δ* and *rkr1Δ* are synthetically lethal only in the presence of [*PSI*$^+$]. The deletion, inactivation, and overexpression of *HSP104* or the overexpression of prion-encoding genes *URE2* and *LSM4* clear [*PSI*$^+$] and rescue *rtf1Δ rkr1Δ* lethality. In addition, the presence of [*PSI*$^+$] decreases the fitness of *rkr1Δ* strains. We investigated whether the loss of *RTF1* exacerbates an overload in nonstop proteins in *rkr1Δ* [*PSI*$^+$] strains but, using reporter plasmids, found that *rtf1Δ* decreases nonstop protein levels, indicating that excess nonstop proteins may not be the cause of synthetic lethality. Instead, our data suggest that the loss of Rtf1-dependent histone modifications increases the burden on quality control pathways in cells lacking Rkr1 and containing [*PSI*$^+$].

DURING transcription elongation, various proteins modify chromatin in coordination with RNA polymerase II (Pol II) to ensure accurate and efficient transcription of nucleosomal templates (Li *et al.* 2007). Changes in chromatin include nucleosome remodeling, the exchange of histone variants for canonical histones, and histone modifications such as the methylation, ubiquitylation, and acetylation of lysine (K) residues. The conserved Paf1 complex (Paf1C), which consists of Paf1, Ctr9, Leo1, Cdc73, and Rtf1, associates with Pol II on all actively transcribed genes (Mayer *et al.* 2010) and couples the modification of histones to transcription elongation (reviewed in Crisucci and Arndt 2011; Jaehning 2010). Paf1C is required for multiple histone modifications associated with active genes, including the monoubiquitylation of H2B K123, a modification for which the Rtf1 subunit of Paf1C plays a prominent role (Ng *et al.* 2003; Wood *et al.* 2003; Warner *et al.* 2007; Tomson *et al.* 2011). Rad6 is the ubiquitin-conjugating enzyme (E2) for H2B K123 ubiquitylation, while Bre1 is the ubiquitin-protein ligase (E3) (Hwang *et al.* 2003). This modification is a prerequisite for downstream histone H3 methylation (Dover *et al.* 2002; Sun and Allis 2002; Ng *et al.* 2003; Wood *et al.* 2003). In yeast, loss of H2B K123 ubiquitylation broadly impacts gene expression and chromatin structure (Mutiu *et al.* 2007; Batta *et al.* 2011). In humans, errors in Paf1C-dependent histone modifications can lead to aberrant gene expression and tumorigenesis (reviewed in Crisucci and Arndt 2011).

Paf1C has several functions in addition to promoting specific histone modifications, including directing the proper 3′-end formation of transcripts (Mueller *et al.* 2004; Penheiter *et al.* 2005; Sheldon *et al.* 2005; Nordick *et al.* 2008; Nagaike *et al.* 2011). Depletion of human Paf1C (hPaf1C) subunits impairs mRNA cleavage, polyadenylation, and

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export to the cytoplasm (Nagaike *et al.* 2011). Additionally, loss of hCdc73 results in aberrantly processed and polyadenylated histone mRNAs (Farber *et al.* 2010). In yeast, deletion of Paf1C subunits leads to decreased poly(A) tail length and alternative poly(A) site usage (Mueller *et al.* 2004; Strawn *et al.* 2009). These observations indicate that Paf1C is essential for both the proper expression and the processing of a subset of RNAs and that loss of Paf1C can result in aberrant transcripts, which are inefficiently exported or translated. In support of this idea, erroneous transcripts resulting from loss of Paf1C are substrates for mRNA quality control pathways, including nonsense mRNA decay (Penheiter *et al.* 2005; reviewed in Jaehning 2010 and Crisucci and Arndt 2011).

RKR1, which is required for viability in *Saccharomyces cerevisiae* strains lacking the Paf1C subunit Rtf1, encodes a conserved RING finger-containing ubiquitin-protein ligase (Braun *et al.* 2007). Deletion of *RKR1* also causes severe growth defects in strains lacking *PAF1* or *CTR9* (Braun *et al.* 2007). Given that *rkr1Δ* causes severe synthetic growth defects in strains with an *htb1-K123R* mutation, Rkr1 most likely functions in a pathway parallel to the histone modification functions of Paf1C to promote an important cellular process (Braun *et al.* 2007). Interestingly, Rkr1 is required for the proper ubiquitylation and degradation of nonstop proteins in yeast and physically associates with ribosomes (Fleischer *et al.* 2006; Wilson *et al.* 2007; Bengtson and Joazeiro 2010). Nonstop proteins can result from mRNAs lacking stop codons (nonstop mRNAs), and both the mRNAs that encode these nonstop proteins and the resulting nonstop proteins themselves are targeted for degradation (van Hoof *et al.* 2002; Wilson *et al.* 2007; Bengtson and Joazeiro 2010), thus implicating Rkr1 in a protein quality control pathway (Bengtson and Joazeiro 2010). Importantly, mutations in *RKR1* homologs in higher eukaryotes are associated with neurodegeneration and colon cancer (Ivanov *et al.* 2007; Chu *et al.* 2009).

To investigate the relationship between the transcription factor Rtf1 and the protein quality control factor Rkr1, we performed a transposon-based mutagenesis screen to identify suppressors of *rtf1Δ rkr1Δ* synthetic lethality in *S. cerevisiae*. We found that mutations in the gene encoding the Hsp104 chaperone rescue lethality of an *rtf1Δ rkr1Δ* strain. Enhanced or depleted levels of Hsp104 alter [*PSI*$^+$] prion propagation (Chernoff *et al.* 1995), suggesting a role for this prion in *rtf1Δ rkr1Δ* synthetic lethality. Consistent with this idea, we found that conditions for curing [*PSI*$^+$] restore viability of *rtf1Δ rkr1Δ* strains and that transfer of [*PSI*$^+$] to *rtf1Δ rkr1Δ* [*psi*$^-$] cells causes lethality. Additionally, we found that the presence of [*PSI*$^+$], presumably through increased nonstop proteins, negatively influences the fitness of *rkr1Δ* strains even in the presence of Rtf1. Unexpectedly, the absence of *RTF1* in a *rkr1Δ* background causes a decrease in the levels of nonstop reporter proteins. Our results suggest that Rtf1 and its H2B ubiquitylation function protect cells against the combined deleterious effects of [*PSI*$^+$] and defects in Rkr1-mediated protein quality control.

Materials and Methods

Yeast strains and standard growth conditions

KY *S. cerevisiae* strains are isogenic with FY2, a *GAL2*$^+$ derivative of S288C and are listed in the Supporting Information, Table S1 (Winston *et al.* 1995). Yeast deletion mutants, crosses, and transformants were created using standard protocols (Ausubel *et al*. 1988; Rose *et al.* 1991). Yeast were grown on rich (YPD), synthetic complete (SC), synthetic minimal (SD), 5-fluoroorotic acid (5-FOA), or sporulation media as specified and prepared as previously described (Rose *et al.* 1991). Strains were typically cured of prions by streaking for single colonies onto YPD supplemented with 5 mM guanidine hydrochloride. For creating [prion−] strains from diploids, tetrad dissections were performed on YPD containing 2.5 mM guanidine hydrochloride.

Plasmids

The *his3* nonstop plasmid, pAV240 (*LEU2*-marked), and protein A nonstop plasmid, pAV184 (*URA3*-marked), were gifts from Ambro van Hoof (Wilson *et al.* 2007). The *sup35NM*-GFP (*URA3*- or *LEU2*-marked) and *RNQ1*-GFP plasmids were gifts from Susan Liebman (Zhou *et al.* 2001). The *URA3*-marked plasmid carrying *RTF1*, pKA69, was used to maintain *rtf1Δ rkr1Δ* viability (Stolinski *et al.* 1997). *HSP104* was driven by a GPD promoter on a 2μ pRS424 (*TRP1*) plasmid (Mumberg *et al.* 1995; Rubel *et al.* 2008). This plasmid was used to derive plasmids for overexpression of *URE2*, *LSM4*, *LSM2*, and *RNQ1*. The open reading frames and 3′ UTR sequences of *URE2*, *LSM4*, *LSM2*, and *RNQ1* were amplified by PCR from a plasmid source and inserted in place of *HSP104* using the *Sac*I and *Bam*HI sites (Nagalakshmi *et al.* 2008). The *ade1-14* and *sup35-Y351C* alleles (Bradley *et al.* 2003) were amplified from strains provided by Susan Liebman and cloned using *Xma*I and *Sac*I sites into pRS306 for two-step gene replacement of the *ADE1* or *SUP35* gene, respectively (Scherer and Davis 1979).

Mutagenesis and confirmation of genetic suppressors

Transposon (Tn) mutagenesis to identify suppressors of *rtf1Δ rkr1Δ* synthetic lethality was performed by transforming a *LEU2*-marked set of integrating plasmids (described in Kumar *et al.* 2000) into an *rtf1Δ rkr1Δ* strain (KY1663) carrying an *RTF1/URA3/CEN/ARS* plasmid (pKA69) and selecting on SC-leucine (L) medium. Transformants were replica-plated onto SC-L containing 5-FOA to select for colonies that had lost the *RTF1* plasmid. Fifteen thousand colonies were screened and 55 candidates were purified and analyzed further. Stable integration of the transposon was verified by streaking strains onto YPD and replica-plating onto SC-L and 5-FOA. Thirty-nine candidates passing this test were selected from 5-FOA plates. These strains, *rtf1Δ rkr1Δ TnSup*::*LEU2*, were then used in backcrosses with an

rtf1Δ strain containing pKA69 because a functional copy of *RTF1* is required for sporulation (data not shown). Tetrad analysis of these crosses confirmed that only one Tn was present per candidate by 2:2 sorting of *LEU2*. This cross also confirmed that every *rtf1Δ rkr1Δ* Leu$^-$ spore was 5-FOA^S while every *rtf1Δ rkr1Δ* Leu$^+$ spore was 5-FOA^R, verifying linkage of the Tn to suppression of lethality. Fifteen *rkr1Δ TnSup*::*LEU2* strains from these crosses were taken through a second backcross with an *rtf1Δ* strain to verify that the transposon rescued *rtf1Δ rkr1Δ* lethality independently of pKA69 and 5-FOA. In this case, we expected suppression of lethality only in *rtf1Δ rkr1Δ TnSup*::*LEU2* strains. Three Tn mutants passed these genetic criteria, one of which is described in this article. The Tn insertion was recovered as previously described by rescuing the insertion in yeast with linearized pRSQ2-*URA3*, and the plasmid insert was sequenced with an M13 oligonucleotide primer (Burns *et al.* 1994). In the described candidate, the Tn mapped within *HSP104*, 68 bp from the 3′-end of the open reading frame. A mutation in *HSP104* was verified to suppress *rtf1Δ rkr1Δ* lethality by creating a precise *KanMX* replacement of *HSP104* and performing tetrad analysis of a triple heterozygous diploid strain (*rtf1Δ/RTF1 rkr1Δ/RKR1 hsp104Δ/HSP104*).

High-copy-number suppressor screen

To obtain high-copy-number suppressors of *rtf1Δ rkr1Δ* synthetic lethality, a 2μ *LEU2*-marked plasmid library of genomic fragments (Yoshihisa and Anraku 1989; Rubel *et al.* 2008) was transformed into an *rtf1Δ rkr1Δ* strain (KY2205) carrying pKA69. Approximately 13,500 Leu$^+$ colonies were screened for the ability to grow on 5-FOA medium, indicating loss of pKA69. One hundred and thirty-one candidates were verified by testing on 5-FOA medium, and plasmids were isolated by standard extraction methods (Hoffman and Winston 1987). Plasmids were then retransformed into KY2205, and 48 exhibited the suppression phenotype. Of these, 21 contained either *RTF1* or *RKR1*. Of the remaining candidates, six unique plasmids remained. One plasmid, which was isolated seven times, contained the gene *LSM4*. Another plasmid, which was isolated three times, contained multiple open reading frames, including *URE2*. A third plasmid, obtained twice, also contained multiple open reading frames, including *HSP104*.

Yeast dilution growth assays

Unless stated otherwise, yeast strains were grown to saturation at 30° in rich or selective media, washed with sterile water, and diluted into 1×10^8 cells/ml stocks from which 10-fold dilutions were made. Two microliters of cell suspension were plated on appropriate control and selective media, and plates were incubated at 30° for the specified number of days. Media for testing *rtf1Δ* and *rkr1Δ* phenotypes contained 0.8 μg/ml cycloheximide, 50 μM cadmium chloride, 10% ethanol, or 15 mM caffeine in YPD or SC as indicated.

Cytoduction

An *rtf1Δ rkr1Δ ade1-14* [*psi*$^-$] strain (KY2286), created by dissection onto YPD containing guanidine hydrochloride (GuHCl), was transformed with *RTF1/URA3* (pKA69) and depleted of mitochondrial DNA (ρ^0 conditions) by growth in liquid culture with ethidium bromide. Using previously described methods (Wickner *et al.* 2006), this recipient strain was used for cytoduction with two *kar1* donor strains, L2261 ([*PIN*$^+$] [*psi*$^-$]) and L2265 ([*PSI*$^+$] [*pin*$^-$]) (Mathur *et al.* 2009). Transfer of cytoplasm to the recipient strain was confirmed by growth on YP medium containing 3% glycerol (YPG), and transfer of the donor prion was confirmed by live-cell confocal microscopy of plasmid-encoded GFP-tagged prion domains. The *kar1* donor strains and GFP plasmids were gifts from Susan Liebman.

Live-cell confocal microscopy

Strains were transformed with *sup35NM*-GFP plasmid (Zhou *et al.* 2001) to test for the presence of [*PSI*$^+$] or a *RNQ1*-GFP plasmid to test for the presence of [*PIN*$^+$] and patched onto selective media containing 100 μM $CuSO_4$. Plates were protected from light and incubated at 30° for several days. Live-cell imaging was performed on wet mounts using a Leica TCS SP5 confocal microscope (Leica Microsystems, Buffalo Grove, IL).

Immunofluorescence

A *rkr1Δ* strain (KY2289) was transformed with either an *HA-RKR1* (pMB11) or an untagged *RKR1* plasmid (pPC65) (Braun *et al.* 2007). These strains were grown to midlog phase and prepared as previously described (Amberg *et al.* 2006). Briefly, cells were fixed with formaldehyde, treated with zymolyase 20T, and adhered to a polylysine slide before overnight incubation with 1:500 anti-HA (Roche) and a 1-hr incubation with 1:250 Alexa 647 (Molecular Probes). Slides were mounted with ProLong GOLD Antifade DAPI reagent (Invitrogen) and imaged using a Leica TCS SP5 confocal microscope (Leica Microsystems, Buffalo Grove, IL).

Western analysis of nonstop protein levels

Strains transformed with pAV184 (Wilson *et al.* 2007) were grown at 30° in SC-uracil (U) liquid culture containing 2% galactose to an OD_{600} of 0.7–0.9. Cells were normalized to 10.8 OD_{600} units, and extracts were made using glass-bead lysis in 20% trichloroacetic acid as previously described (Cox *et al.* 1997; Zheng *et al.* 2010). An equal amount of each extract (5 μl) was run on a 15% SDS-polyacrylamide gel and transferred to nitrocellulose membrane for Western analysis using standard methods (Harlow and Lane 1988). The membrane was probed with peroxidase–anti-peroxidase (1:2000 dilution; Sigma) to assay levels of protein A and anti-G6PDH antibody (1:50,000 dilution; Sigma) as a loading control. Immunoreactivity was measured using chemiluminescence (Perkin-Elmer) and a 440 CF digital imaging station (Kodak).

Results

Genetic suppressors of rtf1Δ rkr1Δ synthetic lethality

To investigate the basis for the lethality of strains lacking Rtf1 and the ubiquitin-protein ligase Rkr1, we performed a Tn-based mutagenesis screen for suppressors of the *rtf1Δ rkr1Δ* synthetic lethal interaction. A library of plasmids containing yeast genomic DNA and *LEU2*-marked transposon insertions (Kumar *et al.* 2000) was transformed into an *rtf1*Δ *rkr1*Δ strain, which carried a *URA3*-marked *RTF1* plasmid for viability. Approximately 15,000 transformants were screened for loss of the *URA3*-marked *RTF1* plasmid on medium containing 5-FOA. Following phenotypic confirmation, *rtf1*Δ *rkr1*Δ *TnSup*::*LEU2* candidates were crossed to an *rtf1Δ* strain containing a *URA3*-marked *RTF1* plasmid to verify the 2:2 sorting of the *LEU2*-marked transposon, indicating only one insertion site, as well as linkage of the 5-FOA resistance to the *LEU2* marker in *rtf1*Δ *rkr1*Δ colonies. Fifteen strains met these requirements, and *rkr1Δ TnSup*::*LEU2* strains from those crosses were backcrossed to an *rtf1Δ* strain to verify that the Tn insertion rescued lethality of the *rtf1*Δ *rkr1*Δ double mutants in the absence of the *RTF1*-containing plasmid. One candidate that met these criteria is described here.

Following the plasmid rescue of the transposon insertion (Burns *et al.* 1994), DNA sequencing revealed a transposon insertion in the 3′-coding region of *HSP104*. Hsp104 encodes a heat-shock protein that can disrupt aggregated proteins (Parsell *et al.* 1994) and is involved in the maintenance and propagation of yeast prions (reviewed in Grimminger-Marquardt and Lashuel 2010). To confirm that the Tn mutation rescued *rtf1*Δ *rkr1*Δ synthetic lethality by disrupting Hsp104 function, we generated an *hsp104Δ* strain and crossed it to a *rkr1Δ* strain. Double mutants from this cross were then mated with an *rtf1Δ* strain. The diploids, which are heterozygous for three genes (*rtf1*Δ, *rkr1*Δ, and *hsp104*Δ), were subjected to tetrad analysis. Surprisingly, the *rtf1*Δ *rkr1*Δ double mutant segregants were alive and healthy, indicating dominant suppression by *hsp104*Δ (Figure 1A). In addition, the *hsp104Δ rtf1Δ rkr1Δ* triple mutants were viable, independently confirming the identification of an *hsp104* mutation in our suppressor screen (Figure 1A).

Transposon insertion in HSP104 cures cells of [PSI⁺]

Deletions in the C-terminal domain of Hsp104 have been shown to weaken its ATPase activity and ability to propagate prions, particularly [*PSI*⁺], an aggregate of the translation termination factor Sup35 (Chernoff *et al.* 1995; Mackay *et al.* 2008). Because the transposon insertion disrupted the C-terminal domain of Hsp104, we investigated if the *rtf1Δ rkr1Δ* strain used in the suppressor screen was [*PSI*⁺] and whether the *hsp104Tn*::*LEU2* suppressor mutation cleared [*PSI*⁺] from this strain. A plasmid expressing the GFP-tagged prion domain of Sup35 was transformed into the original *rtf1Δ rkr1Δ [RTF1*, *URA3*, *CEN/ARS]* strain used in our transposon mutagenesis screen, and transformants were visualized by live-cell imaging using a confocal microscope (Zhou *et al.* 2001). Previous studies have shown that this GFP-tagged Sup35 protein appears as small fluorescent puncta in [*PSI*⁺] cells and as diffuse fluorescence in [*psi*⁻] cells (Zhou *et al.* 2001). Using this method, we found that the original strain used in our screen was indeed [*PSI*⁺] and that a transposon insertion within *HSP104* resulted in [*psi*⁻] conditions in all cells examined (Figure 1B). Given the importance of Hsp104 in prion propagation, we next investigated the role of prions in the genetic interaction between *RTF1* and *RKR1*.

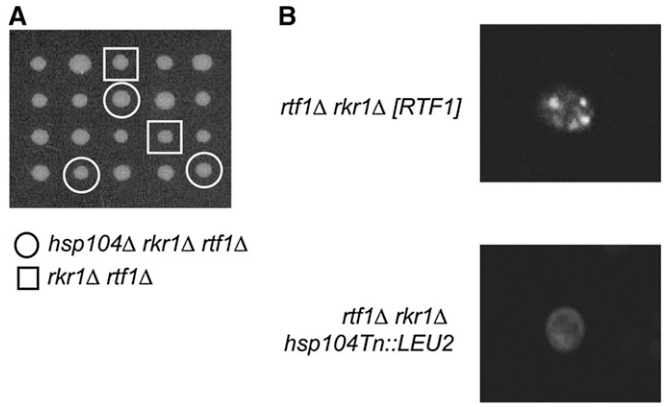

Figure 1 Mutation of *HSP104* suppresses *rtf1Δ rkr1Δ* synthetic lethality and cures [*PSI*⁺]. (A) Tetrad dissections of crosses between an *rtf1Δ* strain (KY958) and a *rkr1Δ hsp104Δ* strain. Dissections were done on YPD and incubated at 30° for 3 days. Double mutant *rtf1Δ rkr1Δ* segregants are highlighted by boxes and triple mutants are highlighted by circles. Note that suppression of *rtf1Δ rkr1Δ* synthetic lethality occurs with or without *hsp104Δ* cosegregation, indicating that *hsp104Δ* acts as a dominant suppressor in the diploid. (B) The strain originally used for transposon-mediated mutagenesis (KY1663) and the *hsp104Tn*::*LEU2* mutant recovered from the transposon-based suppressor screen were transformed with a *LEU2*-marked or a *URA3*-marked *pCUP1-SUP35NM-GFP* plasmid. Strains were patched onto SC-L or SC-U plates containing 100 μM $CuSO_4$ and incubated in the dark at 30° before live-cell imaging was performed by confocal microscopy. Observations were made of at least three transformants per strain and 100 cells per transformant. Representative images are shown. No variability was seen among cells with respect to the GFP pattern.

RKR1 genetic interactions are rescued by curing strains of [PSI⁺]

As noted above, *HSP104* is required for the propagation of yeast prions (Chernoff *et al.* 1995; Shorter and Lindquist 2006). Therefore, the loss of prions in an *hsp104Δ/HSP104* heterozygous diploid strain likely rescued lethality between *rtf1Δ* and *rkr1Δ*, independently of whether the *hsp104*Δ mutation actually segregated with the *rtf1*Δ and *rkr1*Δ mutations (Figure 1A). Growth on media containing GuHCl has been shown to cure yeast of prions by inactivating the ATPase domain of Hsp104 (Ferreira *et al.* 2001; Jung and Masison 2001). To determine if clearing prions through this method could also rescue *rtf1Δ rkr1Δ* lethality, an *rtf1Δ/RTF1 rkr1Δ/RKR1* heterozygous diploid was sporulated and tetrads were dissected onto YPD or YPD containing 2.5 mM

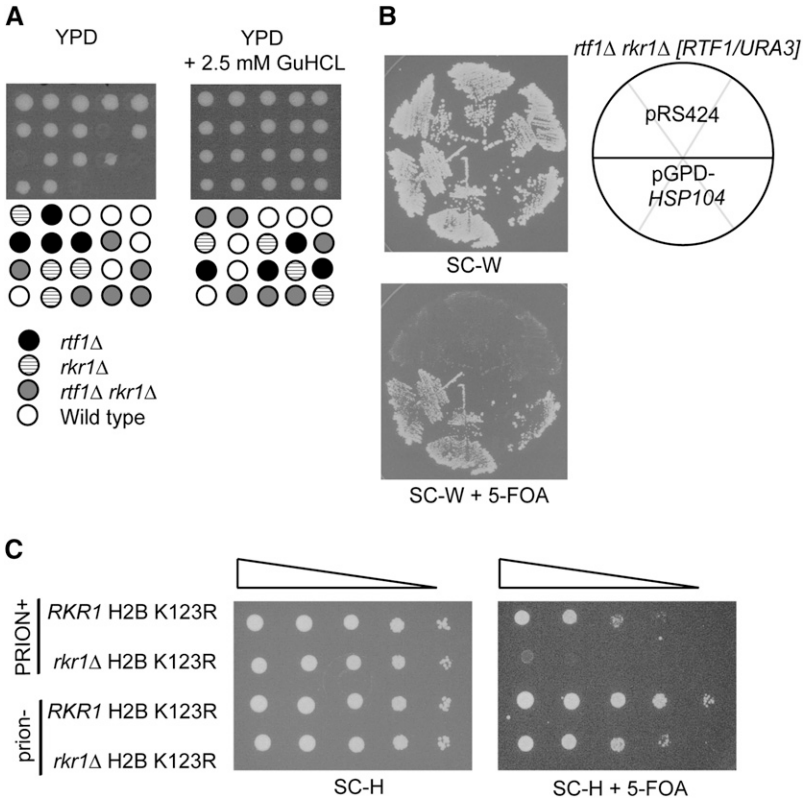

Figure 2 Inactivation or overexpression of *HSP104* rescues *rkr1Δ* synthetic genetic interactions. (A) Heterozygous *rtf1Δ/RTF1 rkr1Δ/RKR1* diploids (KY2202 mated by KY453) were dissected onto YPD or YPD containing 2.5 mM GuHCl and incubated at 30° for 3 days. (B) An *rtf1Δ rkr1Δ [RTF1/URA3]* strain (KY1663) was transformed with either a 2μ *TRP1*-marked *pGPD-HSP104* plasmid or a *TRP1*-marked empty vector (pRS424), and transformants were purified on SC-W and replica-plated to SC-W + 5-FOA (W = tryptophan). Plates were incubated at 30° for 3 days. (C) Wild-type (KY2203) or *rkr1Δ* (KY2204) strains, lacking both endogenous histone H2A and H2B gene copies and containing a *URA3*-marked wild-type copy of *HTA1-HTB1*, were transformed with a *HIS3*-marked *HTA1-htb1 K123R* plasmid. Strains were cured by streaking onto YPD + 5 mM GuHCl. Dilution growth assays were performed on SC-H or SC-H + 5-FOA, and cells were incubated at 30° for 2 days (H = histidine). "PRION+" indicates uncured cells; "prion−" indicates cells passaged on medium containing GuHCl.

GuHCl. Strikingly, tetrad analysis revealed that *rtf1Δ rkr1Δ* double mutants grew as well as wild-type strains on YPD containing GuHCl but were inviable on YPD alone (Figure 2A). Therefore, inactivation of *HSP104* by mutation or treatment with GuHCl rescues *rtf1Δ rkr1Δ* synthetic lethality.

HSP104 is required for the propagation of several yeast prions, including [*PSI*$^+$], [*URE3*], and [*PIN*$^+$] (reviewed in Haslberger *et al.* 2010). Similar to a loss of *HSP104* function, overexpression of *HSP104* can also alleviate yeast of [*PSI*$^+$], but it has not been shown to affect the propagation of other yeast prions (Chernoff *et al.* 1995; Shorter and Lindquist 2006). Therefore, to investigate if *rtf1Δ rkr1Δ* lethality might be rescued by loss of [*PSI*$^+$] or of yeast prions in general, we overexpressed *HSP104* to test for rescue of synthetic lethality. An *rtf1Δ rkr1Δ* strain, which carried an *RTF1 URA3*-marked plasmid to allow growth, was transformed with a *TRP1*-marked *HSP104* overexpression plasmid. Transformants were grown under selective conditions and replica-plated to medium containing 5-FOA to select for loss of the *RTF1* plasmid. Interestingly, the *HSP104* high-copy plasmid allowed for growth of the *rtf1Δ rkr1Δ* double mutants (Figure 2B). Taken together, our results indicate that deletion, inactivation, or overexpression of *HSP104* suppresses *rtf1Δ rkr1Δ* synthetic lethality by clearing [*PSI*$^+$].

A defect in H2B K123 ubiquitylation can phenocopy an *rtf1Δ* mutation with respect to *rkr1Δ* synthetic growth defects (Braun *et al.* 2007). We therefore investigated if the inactivation of Hsp104 could also rescue the genetic interaction between *rkr1Δ* and *htb1-K123R*, a derivative of H2B that lacks the ubiquitylation site for Rad6-Bre1. To answer this question, we performed a plasmid shuffle experiment with *RKR1* and *rkr1Δ* strains, which carried a *URA3*-marked *HTA1-HTB1* plasmid and a *HIS3*-marked *HTA1-htb1K123R* plasmid and were deleted for the chromosomal H2A and H2B genes. Serial dilution analysis was conducted on 5-FOA medium to select for cells that had lost the *URA3*-marked wild-type *HTA1-HTB1* plasmid and retained the *HIS3*-marked *HTA1-htb1K123R* plasmid. As previously shown, the *rkr1Δ* mutation causes a strong synthetic growth defect in combination with the H2B K123R substitution by this assay (Figure 2C) (Braun *et al.* 2007). However, if these strains were first cured of prions by passaging on medium containing 5 mM GuHCl prior to plating on 5-FOA medium, *rkr1Δ htb1K123R* strains were viable (Figure 2C).

Overexpression of the prion-coding genes URE2 and LSM4 rescues rtf1Δ rkr1Δ lethality

Over 20 potential or verified prions have been identified in budding yeast, with [*PSI*$^+$] being one of the best characterized (Alberti *et al.* 2009). Interactions between different prions can be both positive and negative. The *de novo* formation and propagation of some prions require the presence of other prions (Derkatch *et al.* 2001), while the maintenance of some prions may be negatively impacted by the presence of other prions, possibly by affecting chaperone activity (reviewed in Crow and Li 2011).

In a second, unbiased suppression screen (see *Materials and Methods*), we identified high-copy-number plasmids that rescued *rtf1Δ rkr1Δ* synthetic lethality. Confirming

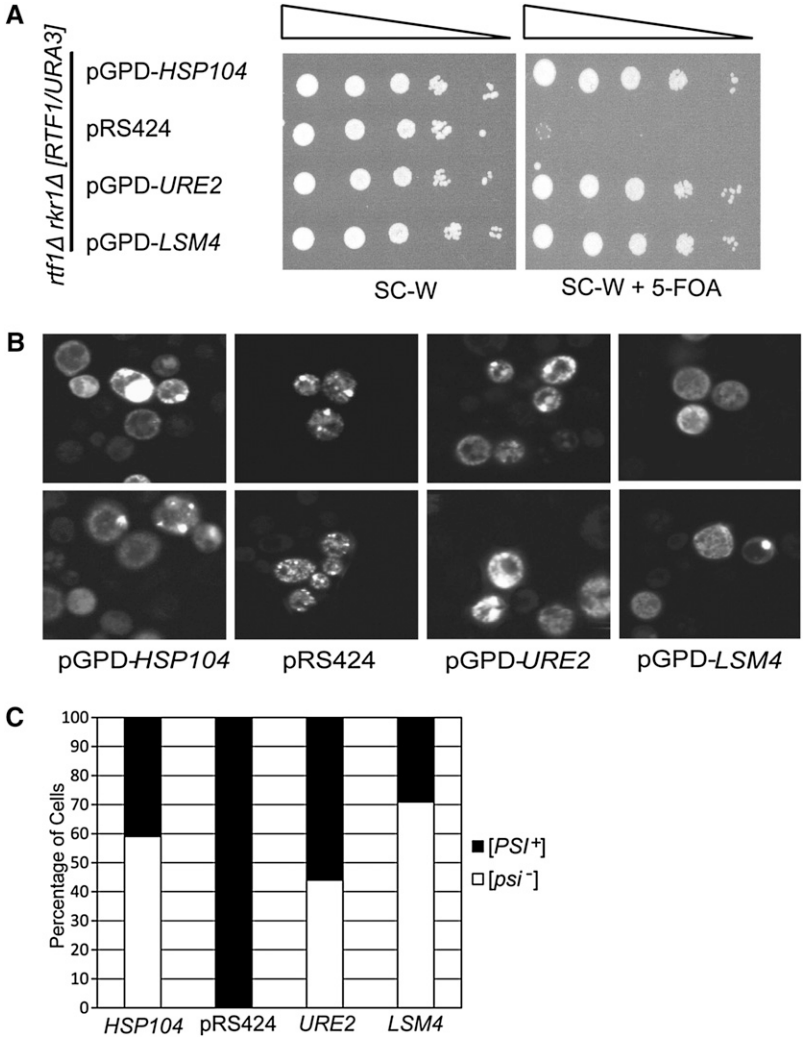

Figure 3 Overexpression of *HSP104*, *URE2*, or *LSM4* rescues *rtf1Δ rkr1Δ* lethality and clears [*PSI*$^+$]. (A) An *rtf1Δ rkr1Δ* strain (KY2205) carrying a *URA3*-marked *RTF1* plasmid was transformed with 2μ *TRP1*-marked p*GPD-HSP104*, p*GPD-URE2*, p*GPD-LSM4*, or empty vector. Dilution assays were performed on SC-W or SC-W + 5-FOA, and plates were incubated at 30° for 2 days. (B) Strains from A were transformed with *LEU2*-marked p*CUP1-SUP35NM-GFP*. Transformants were patched onto SC-L-W containing 100 μM $CuSO_4$ and incubated in the dark at 30° before live-cell imaging was performed using confocal microscopy. Two representative images are shown for each strain. (C) The percentage of [*PSI*$^+$] and [*psi*$^-$] cells from among 50–100 cells from three separate transformants.

earlier results, one plasmid suppressor contained *HSP104*. Interestingly, several other suppressor plasmids contained *URE2*, which encodes the prion [*URE3*] (reviewed in Masison *et al.* 2000), or *LSM4*, which codes for a protein with a prion-forming domain (Alberti *et al.* 2009). The overexpression of [*URE3*] has been shown to antagonize the propagation of [*PSI*$^+$] (Schwimmer and Masison 2002). Therefore, to further test the hypothesis that the clearance of [*PSI*$^+$] suppresses *rtf1Δ rkr1Δ* lethality, we transformed an *rtf1Δ rkr1Δ* double mutant strain carrying an *RTF1 URA3*-marked plasmid with overexpression plasmids for the prions [*URE3*] or [*LSM4*] and assessed the growth of these strains on 5-FOA medium (Derkatch *et al.* 2001; Alberti *et al.* 2009). *HSP104* overexpression served as a positive control. As expected, the *rtf1Δ rkr1Δ* strain was unable to grow without a plasmid source of *RTF1*. However, the overexpression of the prion-encoding genes *URE2* and *LSM4* suppressed *rtf1Δ rkr1Δ* synthetic lethality to the same degree as overexpression of *HSP104* (Figure 3A). We also investigated the effects of overexpressing the gene *RNQ1*, which encodes the prion [*PIN*$^+$] (Derkatch *et al.* 2001), on *rtf1Δ rkr1Δ* synthetic lethality. Although some transformants revealed suppression of *rtf1Δ rkr1Δ* synthetic lethality by *RNQ1* overexpression, others did not, possibly because [*PSI*$^+$] and [*PIN*$^+$] variants can differentially affect each other's propagation (Figure S1) (Bradley and Liebman 2003).

Collectively, our results indicate that [*PSI*$^+$] is negatively impacted by overexpression of [*URE3*] and [*LSM4*] prions and that [*PSI*$^+$] causes *rtf1Δ rkr1Δ* mutants to be inviable. To investigate if the aggregation propensity of [*PSI*$^+$] is negatively affected under each of these conditions, we used live-cell confocal microscopy to image the presence of [*PSI*$^+$] in strains overexpressing *HSP104*, *URE2*, or *LSM4*. As expected, *rtf1Δ rkr1Δ [RTF1*, *URA3*, *CEN/ARS]* cells expressing the Sup35NM-GFP 2μ plasmid contained fluorescent puncta, confirming the presence of [*PSI*$^+$] (Figure 3B). In strains that overexpressed Sup35NM-GFP and also *HSP104*, *URE2*, or *LSM4*, we observed a large number of cells that exhibited diffuse fluorescence and were apparently cured of [*PSI*$^+$] (Figure 3, B and C).

The recovery of *URE2* and *LSM4* from an independent genetic screen as suppressors of *rtf1Δ rkr1Δ* lethality further indicated the importance of [*PSI*$^+$] to this genetic interaction.

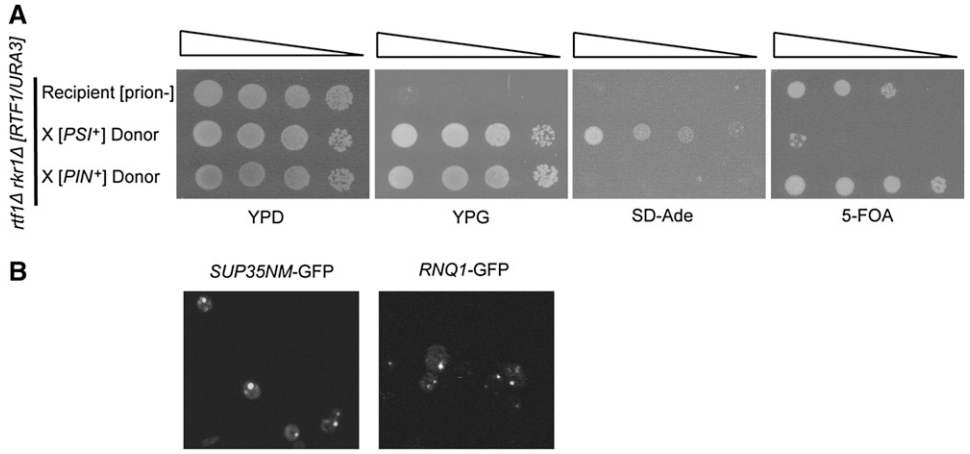

Figure 4 Cytoduction of [*PSI*$^+$] into cured *rtf1Δ rkr1Δ* strains causes synthetic lethality. (A) An *rtf1Δ rkr1Δ ade1-14* strain (KY2286) cured of prions by dissection on medium containing GuHCl was transformed with a *URA3*-marked *RTF1* plasmid and made ρ^0 by growth in ethidium bromide-containing medium. This strain served as a recipient for cytoduction with donor *kar1* strains containing only [*PSI*$^+$] (L2265) or [*PIN*$^+$] (L2261). A dilution growth assay is shown with the recipient and cytoductants on YPD and YPG to show successful cytoduction of recipients, on SD-Ade to show transfer of [*PSI*$^+$], and on 5-FOA to score for loss of the *RTF1/URA3* plasmid. Plates were incubated at 30° for 2–6 days. (B) Cytoductants from A were transformed with a *HIS3*-marked *pCUP1-SUP35NM-GFP* or *pCUP1-RNQ1-GFP* plasmid, patched on selective plates containing 100 μM $CuSO_4$, and incubated in the dark at 30° before live-cell imaging was performed by confocal microscopy. Observations were made of at least three transformants per strain and 100 cells per transformant. Representative images are shown. No variability was seen among cells with respect to the GFP pattern.

However, due to the known roles of Lsm4 in regulating RNA processing and degradation as part of the Lsm complex (reviewed in Beggs 2005), we asked whether the suppression of *rtf1Δ rkr1Δ* lethality by *LSM4* overexpression could be explained by a disruption of Lsm complex function. To this end, we constructed a plasmid to overexpress another member of the Lsm complex, Lsm2, which is not known to have a prion-forming domain. Unlike the case for *LSM4*, the *LSM2* overexpression plasmid did not rescue *rtf1Δ rkr1Δ* synthetic lethality (Figure S1). Collectively, our data indicate a positive correlation between overexpression of certain prions, clearance of [*PSI*$^+$], and suppression of *rtf1Δ rkr1Δ* inviability.

[PSI$^+$] causes rtf1Δ rkr1Δ synthetic lethality

To confirm that the combined loss of *RTF1* and *RKR1* causes synthetic lethality only in [*PSI*$^+$] conditions, we performed cytoduction experiments using a viable, cured *rtf1Δ rkr1Δ* strain transformed with a *URA3*-marked *RTF1* plasmid as a recipient strain and specific prion-containing donor strains. The recipient also carried the *ade1-14* nonsense allele to monitor the transfer of [*PSI*$^+$], which causes translational read-through of *ade1-14* and production of functional Ade1 protein (Chernoff *et al.* 1995). Cytoductions were performed with two donor strains (Mathur *et al.* 2009), one carrying [*PSI*$^+$] and the other carrying [*PIN*$^+$]. Only cytoduction of [*PSI*$^+$] resulted in the inability to lose the *RTF1* plasmid, as indicated by growth on SD-Ade and death on 5-FOA media (Figure 4A). We confirmed transfer of [*PSI*$^+$] or [*PIN*$^+$] by live-cell confocal microscopy with GFP tagged-prion domains (Figure 4B). These results confirm that the prion [*PSI*$^+$] causes *rtf1Δ rkr1Δ* lethality.

Synthetic lethality is due in part to [PSI$^+$]-mediated nonsense suppression

To investigate if the lethal effect of [*PSI*$^+$] on *rtf1Δ rkr1Δ* double mutants is due to the presence of a prion or to a reduction in Sup35 function, we introduced the *sup35-Y351C* mutation into our genetic background and asked whether this mutation could inhibit the growth of *rtf1Δ rkr1Δ* [*psi*$^-$] double mutants. The *sup35-Y351C* mutation was previously shown to increase readthrough of stop codons (Bradley *et al.* 2003) and impair the growth of a *rkr1Δ* strain (Bengtson and Joazeiro 2010). Interestingly, *rtf1Δ rkr1Δ sup35-Y351C* [*psi*$^-$] strains grew more slowly than *rtf1Δ rkr1Δ SUP35* [*psi*$^-$] cells (Figure 5). The increased nonsense suppression due to *sup35-Y351C* did not fully recapitulate the effects of [*PSI*$^+$] in *rtf1Δ rkr1Δ* strains; however, this may be due to the effect of the *sup35-Y351C* mutation in our strain background, as [*PSI*$^+$] and *sup35* alleles can cause different phenotypes in different strain backgrounds (True *et al.* 2004). Additionally, our results may indicate that Sup35 aggregates or that additional consequences of [*PSI*$^+$], not duplicated by the *sup35-Y351C* allele, contribute to the *rtf1Δ rkr1Δ* genetic interaction. Regardless, the slow growth of *rtf1Δ rkr1Δ sup35-Y351C* cells suggests that an increase in nonsense suppression plays at least a partial role in *rtf1Δ rkr1Δ* [*PSI*$^+$] lethality.

[PSI$^+$] impacts rkr1Δ phenotypes

Our results demonstrate that the presence of [*PSI*$^+$] greatly affects *rkr1Δ* genetic interactions. To further assess the physiological impact of [*PSI*$^+$] on these strains, we assayed the growth of *rtf1Δ* and *rkr1Δ* strains in the presence or absence of prions under conditions of cell stress. Both *rtf1Δ* and *rkr1Δ* strains are sensitive to caffeine and cadmium chloride (Figure 6A). Interestingly, the $CdCl_2$ sensitivity of *rkr1Δ* strains was strongly alleviated by curing prions through GuHCl treatment (Figure 6A). In addition, a slight ethanol sensitivity of *rkr1Δ* strains was detected only in uncured conditions. Together, these results suggest that prions influence the fitness of strains lacking *RKR1*, particularly under conditions of cell stress. We verified that [*PSI*$^+$] influenced *rkr1Δ* phenotypes by transforming a *rkr1Δ* strain with the *HSP104*, *URE2*, and *LSM4* overexpression plasmids and

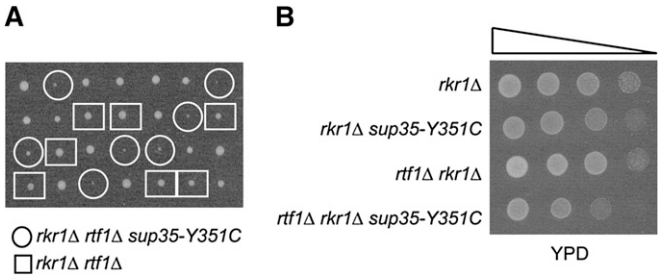

Figure 5 Nonsense suppression impairs growth of *rtf1Δ rkr1Δ* cells. (A) Tetrad dissections of crosses between a [*psi*$^-$] *rkr1Δ sup35-Y351C* strain (KY2292) and a [*psi*$^-$] *rtf1Δ rkr1Δ SUP35* strain (KY2286). Dissections were done on YPD and incubated at 30° for 2 days. Double-mutant *rtf1Δ rkr1Δ SUP35* segregants are highlighted by boxes while *rtf1Δ rkr1Δ sup35-Y351C* triple mutants are highlighted by circles. (B) A tetrad from A was further analyzed by fivefold serial dilution analysis on YPD and incubated at 30° for 1 day. Growth differences are less apparent on YPD on later days.

by measuring cadmium chloride sensitivity. Overexpressing these genes suppressed the sensitivity of *rkr1Δ* cells to cadmium chloride, similarly to the effects seen in a cured strain obtained by passage on GuHCl (Figure 6, A and B). Additionally, we tested the effect of the *sup35-Y351C* mutation on the phenotype of a *rkr1Δ* [*psi*$^-$] strain and found that it causes sensitivity to cadmium chloride, as seen for *rkr1Δ* [*PSI*$^+$] cells (Figure 6, A and C). This result demonstrates that [*PSI*$^+$]-mediated nonsense suppression causes this *rkr1Δ* phenotype and affects the fitness of strains lacking *RKR1*.

While the curing of prions did not suppress the caffeine sensitivity of *rkr1Δ* strains, it did partially suppress the caffeine sensitivity of an *rtf1Δ* strain (Figure 6A). Interestingly, double-mutant *rtf1Δ rkr1Δ* strains obtained by dissection onto medium containing GuHCl were more sensitive to caffeine than either cured single mutant strain (Figure 6A). These data demonstrate that both *RTF1* and *RKR1* are required for cell viability under certain growth conditions even in the absence of prions. These results also correlate with our observation that a residual growth defect is apparent for GuHCl-treated *rkr1Δ htb1-K123R* double mutants compared to GuHCl-treated *RKR1 htb1-K123R* strains (Figure 2C).

rtf1Δ suppresses the elevated levels of nonstop proteins in rkr1Δ cells

In our phenotypic analyses, we found that *rkr1Δ* mutants are sensitive to cycloheximide (CHX) (Figure 6A). Furthermore, the *RKR1* genetic interactors *rtf1Δ* and *htb1-K123R* also confer sensitivity to CHX (Figure 6A and Figure S2A), and this phenotype is not rescued by clearing cells of prions (Figure 6C). CHX inhibits ribosome translocation during protein synthesis (Schneider-Poetsch *et al.* 2010) as well as the decay of nonsense and nonstop mRNA (Frischmeyer *et al.* 2002; Wagner and Lykke-Andersen 2002). Therefore, the CHX sensitivity of cells lacking Rkr1 or Rtf1 may indicate a requirement for these proteins under conditions of impaired translation and/or mRNA quality control.

In previous studies, cells lacking Rkr1 exhibited an increase in nonstop protein levels without affecting the levels of nonstop mRNA (Wilson *et al.* 2007; Bengtson and Joazeiro 2010). As the prion form of Sup35, [*PSI*$^+$] results in suppression of nonsense codons in nonsense mRNAs as well as readthrough of normal stop codons (Paushkin *et al.* 1996; Wilson *et al.* 2005). Rkr1 has been reported to interact with ribosomes and be localized in the cytoplasm, where it is then necessary for nonstop protein degradation (Fleischer *et al.* 2006; Bengtson and Joazeiro 2010). Because our previous studies indicated nuclear localization of Rkr1 (Braun *et al.* 2007), we decided to re-examine the localization of Rkr1 using different strains, an N-terminally tagged HA-Rkr1 construct, and better visualization using confocal microscopy. Here, we found that Rkr1 is predominantly, although not exclusively, cytoplasmic, thus supporting its role in nonstop protein degradation in our strains (Figure S3).

Given the role of Rkr1 in degrading nonstop proteins and the role of [*PSI*$^+$] in generating nonstop proteins, we hypothesized that the lethality of *rtf1Δ rkr1Δ* [*PSI*$^+$] cells might be due to an overabundance of these proteins. To test this idea and examine if *RTF1* plays a role in regulating nonstop protein levels, we used a nonstop reporter plasmid containing the *HIS3* gene without a stop codon (Wilson *et al.* 2007). Because wild-type cells carrying the reporter efficiently degrade the *his3* nonstop transcript and protein, they fail to grow on media lacking histidine (Wilson *et al.* 2007). However, cells lacking *RKR1* are unable to degrade the His3 nonstop protein and therefore grow on media lacking histidine (Figure 7A) (Wilson *et al.* 2007). Cells lacking *RTF1* alone did not exhibit a *his3* nonstop phenotype in the presence or absence of prions (Figure 7A). Interestingly, however, absence of the histone H2A variant Htz1, which was previously shown to increase nonstop transcript levels (Wilson *et al.* 2007), exhibited only a *his3* nonstop phenotype in [*PSI*$^+$] conditions (Figure 7A). This result indicates that Sup35 aggregation exacerbates the nonstop phenotype in some strains, although not detectably in *rkr1Δ* strains by this assay. As for *rtf1Δ* cells, we did not observe a *his3* nonstop phenotype for H2B K123R strains, suggesting that loss of H2B ubiquitylation does not cause an increase in nonstop RNA or protein levels as measured by this reporter (Figure S2B).

To test if deletion of *RTF1* and *RKR1* additively elevate nonstop protein levels, we measured expression of the *his3* nonstop reporter in viable, cured *rtf1Δ rkr1Δ* strains. Surprisingly, deletion of *RTF1* suppressed the *his3* nonstop phenotype of a *rkr1Δ* mutant, as indicated by reduced growth on –His medium (Figure 7B). We confirmed this result using a second nonstop reporter in which the protein A gene lacks a stop codon (Wilson *et al.* 2007). Total protein A levels were measured by Western analysis (Figure 7C). Using this assay, we observed that the curing of prions caused a decrease in nonstop protein A levels in the *rkr1Δ* strain (Figure 7C). Also, in agreement with results obtained with the *his3*

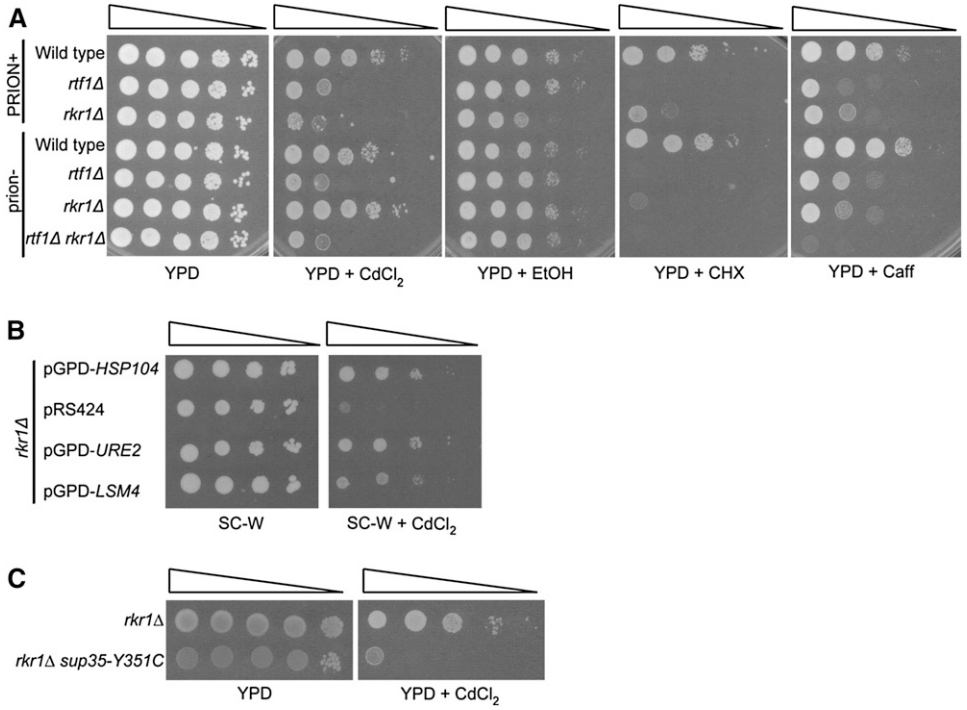

Figure 6 The presence of [*PSI*$^+$] affects *rkr1Δ* phenotypes. (A) Wild-type (KY761), *rtf1Δ* (KY2211), and *rkr1Δ* (KY2236) strains, or these strains first passaged onto YPD + 5 mM GuHCl, as well as an *rtf1Δ rkr1Δ* [*psi*$^-$] strain (KY2209), were used for dilution growth assays on YPD or YPD containing 50 μM $CdCl_2$, 10% EtOH, 15 mM caffeine, or 0.8 μg/ml cycloheximide and incubated at 30° for 2–6 days. "PRION+" indicates uncured cells; "prion−" indicates cells passaged on medium containing GuHCl. (B) A *rkr1Δ* strain (KY2236) was transformed with 2μ *TRP1*-marked p*GPD-HSP104*, p*GPD-URE2*, p*GPD-LSM4*, or empty vector (pRS424). Fivefold serial dilution analysis of these transformants was performed on SC-W or SC-W containing 50 μM $CdCl_2$ and incubated at 30° for 3 days. (C) *rkr1Δ* (KY2309) and *rkr1Δ sup35-Y351C* (KY2306) strains were used for 10-fold dilution analysis on YPD and YPD containing 50 μM $CdCl_2$ and incubated at 30° for 3 days.

nonstop reporter, *rtf1Δ rkr1Δ* double mutants had reduced nonstop protein A levels compared to *rkr1Δ* cells (Figure 7C). These results argue against a simple model in which a combinatorial increase in nonstop protein levels causes *rtf1Δ rkr1Δ* synthetic growth defects.

Discussion

We investigated the genetic relationship between the Paf1C subunit Rtf1 and the ubiquitin-protein ligase Rkr1 to better understand the interaction between their transcription and protein quality-control functions. A transposon mutagenesis screen for genetic suppressors of *rtf1Δ rkr1Δ* synthetic lethality identified a mutation in the gene encoding Hsp104. Further investigation into the suppression mechanism of an *hsp104* mutation showed that *rtf1Δ rkr1Δ* strains are inviable only in the presence of [*PSI*$^+$]. The overexpression, deletion, or GuHCl-mediated inactivation of *HSP104*, as well as the overexpression of the prion-coding genes *URE2* and *LSM4*, all rescue *rtf1Δ rkr1Δ* synthetic lethality and clear cells of [*PSI*$^+$]. [*PSI*$^+$], the prion aggregate of Sup35, a translation termination factor necessary for proper stop codon recognition, results in readthrough of normal stop codons (Paushkin *et al.* 1996; Wilson *et al.* 2005). In turn, Rkr1 is required for the efficient ubiquitylation and degradation of nonstop proteins by recognition of a polylysine tract resulting from translation through the poly(A) tail (Bengtson and Joazeiro 2010). We have shown that [*PSI*$^+$] and the resulting nonsense suppression conditions exacerbate *rkr1Δ* phenotypes, suggesting that the presence of [*PSI*$^+$] and excess nonstop proteins is detrimental in the absence of *RKR1*. However, it is only in the absence of Rtf1 that this increased burden on protein quality-control machinery causes inviability.

Improper recognition of stop codons leads to readthrough of both normal and premature stop codons, resulting in nonstop proteins and nonsense suppression, respectively, and explaining the multiple phenotypic effects of [*PSI*$^+$] (True *et al.* 2004; Wilson *et al.* 2005). In addition, because degradation of many aberrant mRNAs depends on proper translation termination, the presence of [*PSI*$^+$] also affects the degradation of these transcripts (Wilson *et al.* 2005). Therefore, in combination, the presence of [*PSI*$^+$] and the absence of *RKR1* likely results in increased levels of nonstop proteins that cannot be efficiently recognized and degraded, as well as in an increased burden on mRNA quality control. Although we have shown that [*PSI*$^+$] influences the phenotypic consequences of deleting *RKR1* in yeast, added stress to protein or mRNA quality-control systems could also impact the severity of *rkr1* mutations in higher eukaryotes and contribute to the development of diseases, such as neurodegeneration (Chu *et al.* 2009).

In addition to mechanisms that recognize and degrade aberrant proteins, such as that involving Rkr1, several mRNA surveillance pathways also prevent the translation of erroneous transcripts. Two such quality-control pathways are nonsense-mediated decay, which recognizes transcripts with premature termination codons, and nonstop decay, which recognizes transcripts without stop codons (reviewed in Fasken and Corbett 2005). Cells deficient in Paf1C exhibit transcript-specific increases or decreases in mRNAs targeted for quality control, presumably due to errors in RNA processing (Penheiter *et al.* 2005; Strawn *et al.* 2009). For example, mutations in yeast *PAF1* result in shortened poly(A) tails

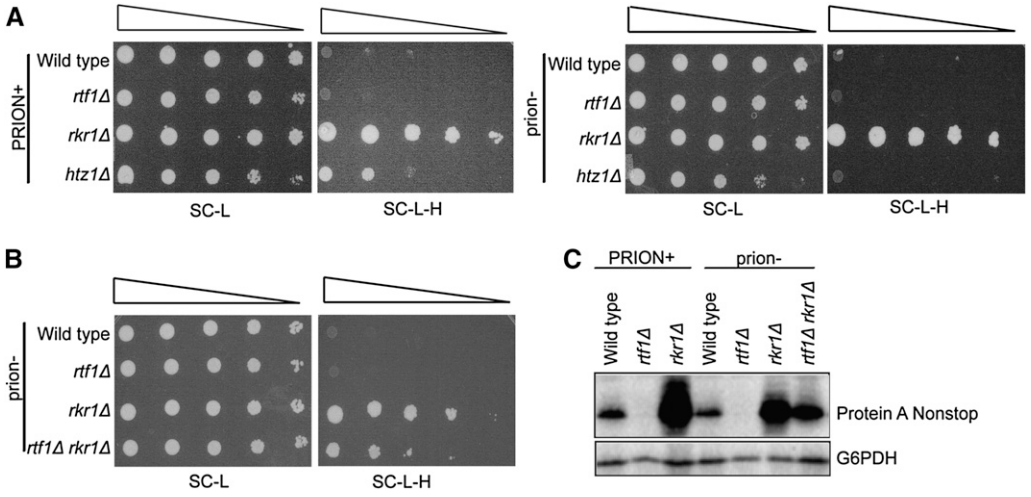

Figure 7 *rtf1Δ rkr1Δ* strains exhibit a decrease in nonstop reporter proteins. (A and B) Wild-type (KY1030), *rtf1Δ* (KY564), *rkr1Δ* (KY2202), *rtf1Δ rkr1Δ* (KY2210), and *htz1Δ* (KY1404) strains were transformed with a *LEU2*-marked plasmid containing a *his3* nonstop reporter. Ten-fold serial dilutions were plated on SC-L or SC-L-H medium and incubated at 30° for 2 or 3 days, respectively. Prion− strains were generated prior to transformation by curing on medium containing 2.5 or 5 mM GuHCl. (C) Wild-type (KY307), *rtf1Δ* (KY2211), *rkr1Δ* (KY2236), and *rtf1Δ rkr1Δ* (KY2209) were transformed with a *URA3*-marked plasmid containing a protein A nonstop reporter and grown to early log phase in SC-U containing 2% galactose. prion− strains were generated as in A. Trichloroacetic acid extracts were analyzed by Western blotting using antibodies against protein A or G6PDH, which served as a loading control. "PRION+" indicates uncured cells; "prion−" indicates passage on medium containing GuHCl.

and in altered poly(A) site utilization, which produce substrates for mRNA surveillance pathways (Mueller *et al.* 2004; Penheiter *et al.* 2005; Nordick *et al.* 2008), and defects in hPaf1C give rise to aberrant transcripts, which are inefficiently processed or exported (Farber *et al.* 2010; Nagaike *et al.* 2011). In agreement with these earlier observations, we have shown that deletion of *RTF1* results in decreased protein product from two nonstop reporters (Figure 7), presumably because the absence of Rtf1-dependent histone modifications leads to transcriptional alterations and/or effects on RNA export, stability, or translation.

The mechanism by which Rtf1 and H2B ubiquitylation protects cells against the combined lethal effects of [*PSI*$^+$] and *rkr1*Δ is not clear. Our initial hypothesis was that deletion of *RTF1* elevates nonstop protein synthesis or stability to levels that are intolerable in [*PSI*$^+$] *rkr1*Δ strains; however, our nonstop reporter assays detected decreased, not increased, nonstop protein levels in *rtf1*Δ strains. Therefore, the synthetic lethality between *rtf1*Δ and *rkr1*Δ is not easily explained by an elevation in nonstop protein levels. An alternative explanation, based on the importance of Paf1C in transcription and RNA processing (reviewed in Jaehning 2010 and Crisucci and Arndt 2011), is that *rtf1*Δ leads to a spectrum of aberrant transcripts that may impose stress on the cell or impair the expression of specific genes whose products play a role in RNA surveillance or protein quality control. These products would be especially important in cells lacking Rkr1 and containing [*PSI*$^+$]. Our observation that *rtf1*Δ and *rkr1*Δ mutants have similar stress-related phenotypes (Figure 6) further supports a role for both proteins in preventing the accumulation of quality-control substrates. Finally, our data do not preclude the possibility that Rkr1 possesses alternate activities, which remain to be identified, and it is the absence of these functions that elevates the need for Paf1C and its associated histone modifications. Regardless of the precise mechanism, our results reveal a previously unrecognized requirement for a functional Paf1C and its associated histone modifications in protecting cells from the adverse effects of [*PSI*$^+$] in the context of impaired protein quality control.

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The Paf1 Complex Subunit Rtf1 Buffers Cells Against the Toxic Effects of [*PSI*$^+$] and Defects in Rkr1-Dependent Protein Quality Control in *Saccharomyces cerevisiae*

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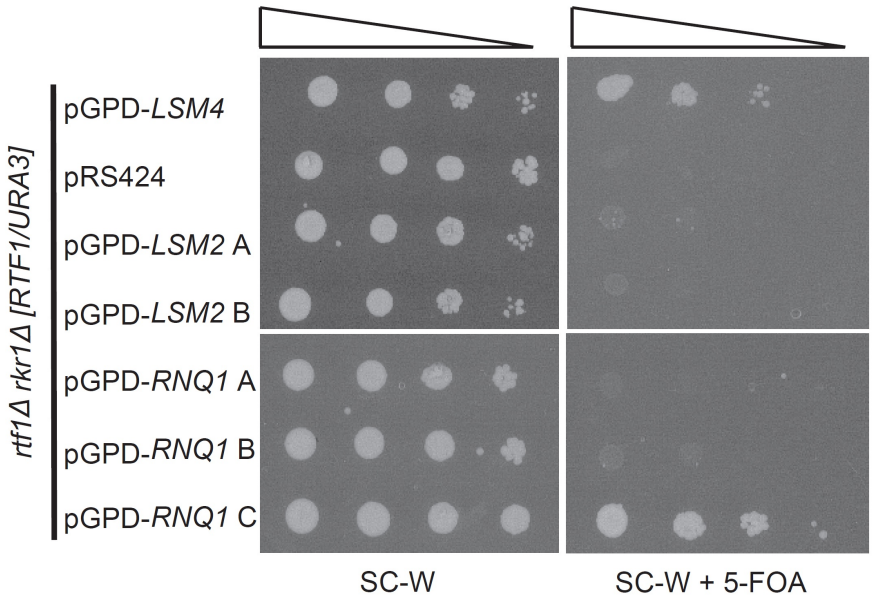

Figure S1 Effect of *LSM2 or RNQ1* overexpression on *rtf1Δ rkr1Δ* synthetic lethality. An *rtf1Δ rkr1Δ* strain (KY2205) carrying a *URA3*-marked *RTF1* plasmid was transformed with 2μ *TRP1*-marked p*GPD-LSM4*, p*GPD-LSM2*, p*GPD-RNQ1*, or empty vector. Ten-fold serial dilution assays were performed on SC-W or SC-W + 5-FOA media and plates were incubated at 30^{o}C for 2 days. Overexpression of *RNQ1* resulted in 9 out of 23 transformants that showed rescue of *rtf1Δ rkr1Δ* lethality (representative transformants depicted as A, B, or C). No variation in the lack of rescue of *rtf1Δ rkr1Δ* lethality by overexpression of *LSM2* was observed in 25 transformants (representative transformants depicted as A or B).

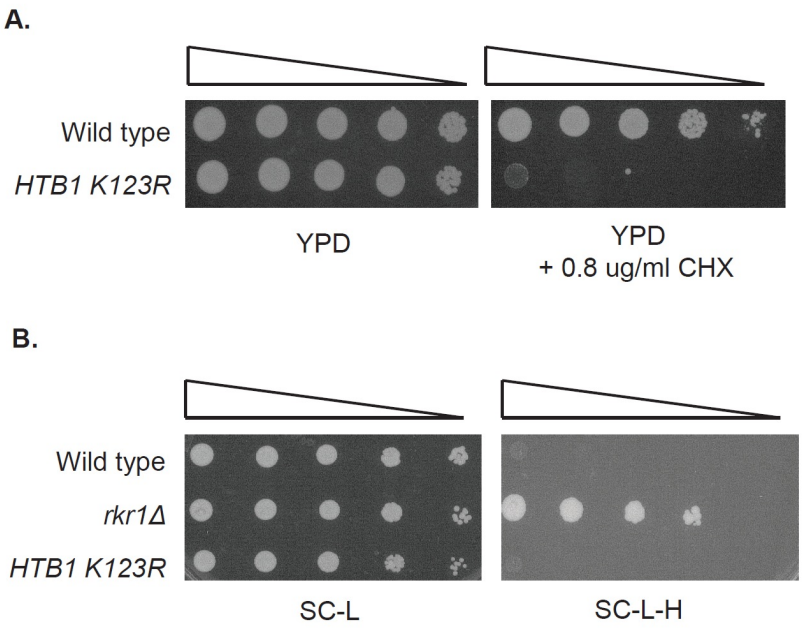

Figure S2 An H2B K123R mutant strain is sensitive to cycloheximide but does not exhibit increased expression of a nonstop reporter. (A) *HTB1* (KY2043) and *htb1-K123R* (KY2044) strains were grown to saturation and diluted to 3 x 10^8 cells/ml. Ten-fold serial dilutions were performed on YPD or YPD containing 0.8 µg/ml cycloheximide (CHX) and incubated at 30°C for 2 or 6 days, respectively. (B) *HTB1* (KY2043) and *htb1-K123R* (KY2044) strains were transformed with a *LEU2*-marked plasmid containing a *his3* nonstop reporter. Strains were then grown to saturation and diluted to 1 x 10^8 cells/ml. Ten-fold serial dilutions were performed on SC-L or SC-L-H and plates were incubated at 30°C for 3 days.

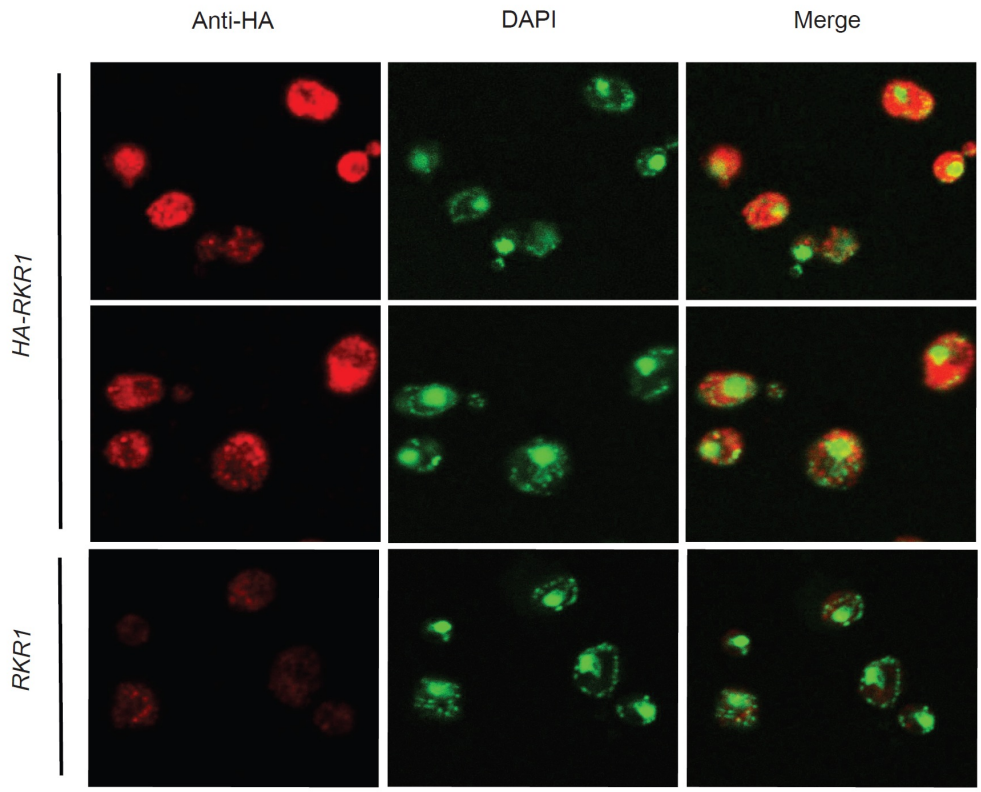

Figure S3 Localization of HA-Rkr1 is predominantly cytoplasmic. A *rkr1Δ* strain (KY2289) was transformed with either an H*A-RKR1* (pMB11) or untagged *RKR1* (pPC65) plasmid and grown to mid log phase before fixation and incubation with anti-HA antibody primary (Roche) and Alexa Red 647 secondary (Molecular Probes). Cells were mounted with ProLong DAPI stain (Invitrogen) and visualized using confocal microscopy.

Table S1 ***Saccharomyces cerevisiae*** **strains**

Strain	Genotype
KY307	*MAT*α *his3Δ200 ura3-52 trp1Δ63 lys2Δ202*
KY453	*MAT*α *rtf1Δ100::URA3 his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-173R2*
KY564	*MAT*α *rtf1Δ102::ARG4 his3Δ200 leu2Δ1 ura3-52 trp1Δ63 lys2-173R2 arg4-12*
KY607	*MAT*α *rtf1Δ101::LEU2 his3Δ200 leu2Δ1 ura3-52 lys2-128δ*
KY761	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY958	*MAT*α *rtf1Δ101:LEU2: his3Δ200 leu2Δ1 ura3Δ0 trp1Δ63 lys2-128δ*
KY1030	*MAT*α *his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY1404	*MAT*α *htz1Δ::KanMX his3Δ200 leu2Δ0 ura3Δ0 lys2Δ0*
KY1663	*MAT*α *rtf1Δ102::ARG4 rkr1Δ::KanMX leu2Δ1 ura3-52 arg4-12 [pKA69: RTF1/URA3/C/A]*
KY1898	*MAT*α *chl1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY1899	*MAT***a** *chl1Δ::KanMX his3Δ200 leu2Δ1 ura3-52*
KY2043	*MAT***a** *hta2-htb2Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2044	*MAT***a** *HTA1-htb1K123R hta2-htb2Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2202	*MAT***a** *rkr1Δ::HIS3 his3Δ200 leu2Δ1 ura3-52*
KY2203	*MAT***a** *hta1-htb1Δ::LEU2 hta2-htb2Δ::TRP1 his3Δ200 leu2Δ1 ura3-52 trp1Δ63* [*URA3/HTA1-HTB1/C/A*] [*HIS3/HTA1-FLAG-htb1-K123R/C/A*]
KY2204	*MAT***a** *hta1-htb1Δ::LEU2 hta2-htb2Δ::TRP1 rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63* [*URA3/HTA1-HTB1/C/A*] [*HIS3/HTA1-FLAG-htb1-K123R/C/A*]
KY2205	*MAT***a** *rtf1Δ102::ARG4 rkr1Δ::KanMX leu2Δ1 ura3-52 arg4-12 trp1Δ63 [pKA69: RTF1/URA3/C/A]*
KY2206	*MAT***a** *chl1Δ::KanMX rtf1Δ101::LEU2 rkr1Δ::HIS3 his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2207	*MAT***a** *chl1Δ::KanMX rtf1Δ101::LEU2 his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2208	*MAT*α *chl1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63 arg4-12*
KY2209	*MAT*α *rtf1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63* [*psi*$^-$]
KY2210	*MAT***a** *rtf1Δ::KanMX rkr1Δ::KanMX his3Δ200 leu2Δ1 trp1Δ63* [*psi*$^-$]
KY2211	*MAT*α *rtf1Δ::KanMX his3Δ200 leu2Δ1 ura3Δ0 trp1Δ63*
KY2212	*MAT***a** *ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2213	*MAT***a** *rtf1Δ101::LEU2 ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2214	*MAT*α *chl1Δ::KanMX ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2215	*MAT*α *chl1Δ::KanMX rtf1Δ101::LEU2 ade1-14 leu2Δ1 ura3-52 trp1Δ63*
KY2236	*MAT*α *rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 trp1Δ63*
KY2286	*MAT*α *rtf1Δ101::LEU2 rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 ade1-14* [*psi*$^-$]
KY2289	*MAT***a** *rkr1Δ::HIS3 his3Δ200 leu2Δ1 trp1Δ63 lys2-128δ*
KY2292	*MAT***a** *rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 ade1-14* [*psi*$^-$]
KY2306	*MAT***a** *rkr1Δ::KanMX sup35-Y351C his3Δ200 leu2Δ1 ura3-52 trp1Δ63 ade1-14* [*psi*$^-$]
KY2309	*MAT***a** *rkr1Δ::KanMX his3Δ200 leu2Δ1 ura3-52 ade1-14* [*psi*$^-$]
L2261	*MAT***a** *kar1 ura2 leu2 his* [*PIN*$^+$]
L2265	*MAT***a** *kar1 ura2 leu2 his* [*PSI*$^+$]