Mms21 SUMO Ligase Activity Promotes Nucleolar Function in *Saccharomyces cerevisiae*

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**ABSTRACT** The budding yeast E3 SUMO ligase Mms21, also known as Nse2, is a component of the Smc5/6 complex, which regulates sister chromatid cohesion, DNA replication, and repair. Our study shows that the mms21RINGD mutant exhibits (1) reduced ribosomal RNA production; (2) nuclear accumulation of ribosomal proteins; (3) elevated Gcn4 translation, indicating translational stress; and (4) upregulation of Gcn4 targets. Genes involved in ribosome biogenesis and translation are downregulated in the mms21RINGD mutant. We identified RPL19A as a novel genetic suppressor of the mms21RINGD mutant. Deletion of RPL19A partially suppresses growth defects in both smc5-6 and mms21RINGG mutants as well as nuclear accumulation of ribosome subunits in the mms21RINGD mutant. Deletion of a previously identified strong suppressor, MPH1, rescues both the accumulation of ribosome subunits and translational stress. This study suggests that the Smc5/6 complex supports nucleolar function.

**KEYWORDS** MMS21; NSE2; MPH1; nucleolus; RPL19A; Smc5/6; translational stress

MMS21 is a SUMO ligase that is a subunit of the Smc5/6 complex. The Smc5/6 complex has been shown to regulate sister chromatid segregation, DNA replication, and DNA damage repair (Lindroos et al. 2006; Rai et al. 2011; Almedawar et al. 2012; McAleenan et al. 2012; Wu et al. 2012; Bermudez-Lopez et al. 2015). The structural maintenance of chromosome (SMC) complexes are conserved from prokaryotes to humans and they promote chromosome integrity. Previous reports have shown that Mms21 SUMO ligase activity is required for the Smc5/6 complex to function in those cellular processes (Zhao and Blobel 2005; Murray and Carr 2008; Takahashi et al. 2008; Potts 2009; Stephan et al. 2011; Bermudez-Lopez et al. 2015).

One of the striking phenotypes of loss of Mms21 sumoylation activity is an aberrant nucleolus (Zhao and Blobel 2005). The nucleolus is the nuclear body where ribosomal DNA (rDNA) resides and ribosomes begin to be assembled from their RNA and protein components. Mms21 has been shown to target some nucleolar proteins for sumoylation (Albuquerque et al. 2013). The Smc5/6 complex binds to the rDNA repeats, suggesting it could play a role in the formation of the nucleolus (Torres-Rosell et al. 2005b). Furthermore, the Smc5/6 complex is required for rDNA integrity (Torres-Rosell et al. 2005a) and segregation (Torres-Rosell et al. 2005b). The aberrant nucleolar structure observed in cohesin loss-of-function mutants was a harbinger of defects in ribosome biogenesis (Bose et al. 2012). Given the defect in nucleolar structure in MMS21 mutants, we speculated that the sumoylation activity of Mms21 might be required for nucleolar function.

Faithful regulation of ribosome biogenesis is important for translation and cell proliferation. In proliferating cells, there is a high demand for protein production. To meet this demand, cells must produce ribosomes at a rapid rate (Montanaro et al. 2008; Lempiainen and Shore 2009). Defects in ribosome biogenesis have been shown to correlate with reduced or altered protein translation and growth defects (Zanchin et al. 1997; Yamada et al. 2007; Jack et al. 2011; Bose et al. 2012; Xu et al. 2013). More importantly, deregulation of ribosome biogenesis has been correlated with tumorigenesis and developmental syndromes, including cohesinopathies and ribosomopathies (Montanaro et al. 2008; Ruggiero 2013; Zakari et al. 2015), demonstrating that proper ribosome biogenesis is critical for human health.
Ribosome biogenesis requires the assembly and transport of many different RNA and protein components. This process includes ribosomal RNA (rRNA) transcription, processing, ribosome assembly, and export. The budding yeast 35S rRNA precursor is transcribed by RNA polymerase I in the nucleolus and undergoes a series of cleavages and modifications to generate the 25S, 18S, and 5.8S rRNAs. The 25S, 5.8S, and 5S (transcribed by RNA polymerase III) rRNAs and the 18S rRNA assemble the 60S and the 40S preribosome particles, respectively, with both large and small ribosomal proteins and transacting factors (Strunk and Karststein 2009; Kressler et al. 2010; Woolford and Baserga 2013). After assembly of the preribosome particles, the 60S and the 40S preribosome particles undergo maturation and are exported to the cytoplasm to form functional ribosomes. We find that mutations in the Smc5/6 complex allow ribosomal proteins to accumulate in the nucleus. In addition, rRNA is produced at a reduced rate and translational stress is detected in the mms21RINGΔ mutant. The gene expression profile in the mms21RINGΔ mutant is consistent with the idea that translation could be negatively affected. Deletion of RPL19A or the previously identified suppressor MPH1 partially rescues growth and the accumulation of ribosomal proteins in the nucleus. Deletion of the gene encoding the Mph1 helicase also partially rescues rRNA production and translational stress in the mms21RINGΔ mutant. Our study suggests that the Smc5/6 complex supports nucleolar function.

Materials and Methods

Spot growth assay
Strains (all listed in Supplemental Material, Table S2) were grown to midlog phase at 30°C and spotted in 10-fold serial dilutions onto YPD plates. The plates were incubated at 30°C and 37°C for 1–3 days.

Confocal microscopy
Images of live cells were taken with a 100× Plan Apochromat 1.46 N.A. oil objective using a confocal microscope (Perkin Elmer Ultraview Spinning Disk) and the Volocity 6.3 software program. Scale bars were created using ImageJ software.

RNA-sequencing analysis
Both WT and mms21RINGΔ cells were grown in triplicate to OD600 = 0.6 at 30°C. Total RNA was isolated using the hot phenol method, subjected to ribodepletion, and libraries were made using the library construction kit Ribo-Zero (Epigen) for sequencing. Reads were aligned to sacCer3 using TopHat v2.0.8 with default alignment parameters. The resulting binary alignment/map (BAM) files were sorted and indexed using SAMtools. Because there were >25 million reads per sample, the BAM files were downsampled to take 5 million reads at random for expression analysis.

Gcn4 site enrichment
Promoters consisting of 600 bases upstream of the transcription start site were extracted for all genes from sacCer3 in R. The consensus Gcn4 binding site TGASTCW (Natarajan et al. 2001) was used to query each promoter for the presence or absence of sites. The International Union of Pure and Applied Chemistry codes are S = G,C and W = A,T. The number of promoters in the differentially expressed gene sets scoring positive for a Gcn4 binding site was tabulated (Table S1). The degree of enrichment in the up- and downregulated genes compared to the background set (genes not differentially expressed) was compared and tested for statistical significance using a Fisher exact test.

Gene group enrichment
Various gene groups were examined for higher rankings in terms of differential expression relative to random groups of genes using a mean-rank gene set test (Wu and Smyth 2012).

3H-uridine incorporation assay
Strains were grown in SD – Ura medium supplemented with 6.7 ng/µl uracil in triplicate to OD600 = 0.3 at 30°C. Five µCi of 3H-uridine was added to the cultures and incubated at 30°C for 5 min. Samples were treated with 2.5 ml of 10% trichloroacetic acid (TCA) containing 2.5 mg/ml uridine. The samples were filtered onto nitrocellulose membrane using a vacuum and washed with 5% TCA. The membranes were air dried and analyzed via scintillation counting (Beckman LS 6500).

Gcn4-lacZ reporter assay
Strains transformed with a p180–Gcn4–lacZ reporter plasmid and were grown in SD – Ura medium to midlog phase at 30°C. Strains with the Gcn4–lacZ reporter integrated at TRP1 were grown in YPD. The cells were lysed in breaking buffer (100 mM Tris-Cl, Ph 8.0, 1 mM DTT, 20% glycerol, 1 mM PMSF) by vortexing with glass beads for 4 min at 4°C. The supernatant was incubated with O-nitrophenyl-β-D-galactoside (ONPG) for 60 min. β-Galactosidase activity was measured at OD420.

Rps2–GFP and Rpl25–GFP quantification
Strains were transformed with either pRS315–Rps2–GFP plasmid or pRS315–Rpl25–GFP plasmid. Cells were grown in SD – Leu or SD – Ura medium containing 0.02 mg/ml adenine to midlog phase at 30°C. Mean peak GFP fluorescence intensity was measured using the MACSQuant analyzer (Miltenyi Biotec). The distances between biological replicates vs. the distances between samples with different genotypes was determined as previously described and the Kolmogorov–Smirnov (KS) statistic test was applied (Bose et al. 2012; Kim 2016).

Strain and data availability
Strains are available upon request. Table S2 contains a complete list of all strains used in this study. The Gene Expression Omnibus accession number for RNA-sequencing (RNA-seq) data is GSE69826. Original data underlying this manuscript can be accessed from the Stowers Original Data Repository at http://www.stowers.org/research/publications/libpb-1027.
Results

**RPL19A is a genetic suppressor of the mms21RINGΔ mutant**

In order to gain insight into the function of Mms21 SUMO ligase activity, we performed a synthetic genetic array (SGA) screen for novel genetic suppressors of the mms21RINGΔ mutant in which its SUMO ligase activity is deficient. Since the mms21RINGΔ mutant exhibits a growth defect at 37°C, we took advantage of this temperature sensitivity to identify potential genetic suppressors. From this SGA analysis, we found that deletion of RPL19A partially suppresses the temperature-sensitive phenotype of the mms21RINGΔ mutant.

RPL19A is a ribosomal protein gene and has a paralog, RPL19B. In order to test the specificity of RPL19A as a genetic suppressor of the mms21RINGΔ mutant, we compared the growth rate of the mms21RINGΔ mutant to the mms21RINGΔ rpl19aΔ double mutant at both 30°C and 37°C. As shown in Figure 1A, the growth defect of the mms21RINGΔ mutant at 37°C was partially rescued by rpl19aΔ but not by rpl19bΔ. In our previous report, we showed that a mutant in the cohesin acetyltransferase (eco1–W216G) exhibits a growth defect at the nonpermissive temperature of 37°C (Lu et al. 2010). Therefore, we wanted to test whether rpl19aΔ can also suppress the temperature sensitivity of the eco1–W216G mutant via a spot growth assay (Figure 1B). This assay showed that the deletion of RPL19A does not suppress the growth phenotype of the eco1–W216G mutant at 37°C, suggesting that genetic suppression by rpl19aΔ is specific to the mms21RINGΔ mutant. Then, we examined whether deletion of RPL19A could also suppress the MMS sensitivity of the mms21RINGΔ mutant (Figure 1C). Interestingly, the MMS sensitivity of the mutant was not rescued by deletion of RPL19A, indicating that this genetic suppression must act by a mechanism that does not rescue DNA damage. For growth curves, see Figure S1.

SMC5 encodes another subunit of the Smc5/6 complex. We next checked whether deletion of RPL19A would rescue the temperature-sensitive phenotype of the smc5-6 mutant. The growth of this mutant was also partially rescued by the deletion of RPL19A in both the W303 and BY4741 strain backgrounds (Figure 1, D and E), suggesting that rpl19aΔ may be a suppressor for Smc5/6 loss of function in general and is not specific to the mms21RINGΔ mutant.

**Genes involved in ribosome biogenesis and protein translation are downregulated in the mms21RINGΔ mutant**

It has been previously shown that nucleolar morphology of the mms21RINGΔ mutant is aberrant (Zhao and Blobel 2005). Recent work from our lab has shown that the SMC complex
known as cohesin is important for gathering the rDNA repeats into a functional nucleolar structure (Bose et al. 2012; Harris et al. 2014). Like cohesin, the Smc5/6 complex binds to rDNA repeats (Torres-Rosell et al. 2005b). Since the nucleolus serves as a ribosome factory, we hypothesized that the mms21RINGΔ mutant may have defects in ribosome biogenesis. In order to gain insight into the physiology of the mms21RINGΔ mutant, we performed RNA-seq on cells grown in YPD at 30°C, the permissive temperature. The number of differentially expressed genes for the mms21RINGΔ mutant

Figure 2. Genes involved in ribosome biogenesis and protein translation are downregulated in the mms21RINGΔ mutant. (A) The top 20 biological process terms for upregulated and downregulated genes in mms21RINGΔ mutant are shown. (B) Groups of genes involved in ribosome biogenesis and protein translation are downregulated in the mms21RINGΔ mutant. Various gene groups were examined for differential expression relative to random groups of genes using a mean-rank gene set test (Wu and Smyth 2012). The P-values for each gene group are shown as follows: Gcn4 P-value = 0.0049, size = 699; C/D snoRNAs P-value = 5.17E-09, size = 42; H/ACA snoRNAs P-value = 0.0038, size = 34; Processome P-value = 6.64E-21, size = 63; RPL P-value = 6.79E-36, size = 78; and RPS P-value = 4.65E-25, size = 56. (C) Gene expression profiles of the eco1–W216G and mms21RINGΔ mutants are significantly correlated. Genes common to both experiments are compared. A total of 6763 genes exhibit a Pearson correlation of 0.70. The log2 ratios for mms21RINGΔ/WT and eco1–W216G/WT were compared to each other. Values with a z score of >5 in the data set were excluded.

A

| Term                        | p-val | enrich | Term                        | p-val | enrich |
|-----------------------------|-------|--------|-----------------------------|-------|--------|
| oxidation-reduction process | 1.3E-14 | 2.1    | translation                 | 0     | 5.0    |
| cellular carbohydrate metab. process | 1.6E-10 | 2.9    | gene expression              | 0     | 2.6    |
| carbohydrate metabolic process | 4.6E-09 | 2.0    | cellular macromolec. biosynthetic process | 0     | 2.4    |
| catabolic process          | 0.00000031 | 1.5    | macromolecule biosynthetic process | 0     | 2.4    |
| carbohydrate biosynthetic process | 3.30E-07 | 3.0    | cellular biosynthetic process | 5.10E-30 | 2.0    |
| carbohydrate catabolic process | 1.70E-06 | 2.7    | biosynthetic process         | 9.60E-29 | 2.0    |
| small molecule metabolic process | 2.30E-06 | 1.4    | cellular protein metabolic process | 2.10E-24 | 2.2    |
| small molecule catabolic process | 3.10E-06 | 2.8    | cellular macromolec. metabolic process | 3.30E-23 | 1.7    |
| monosaccharide metabolic process | 3.10E-06 | 2.5    | macromolecule biosynthetic process | 6.50E-22 | 1.6    |
| glycogen metabolic process | 4.60E-06 | 4.9    | primary metabolic process    | 9.10E-22 | 1.5    |
| energy reserve metabolic process | 4.60E-06 | 4.9    | cellular metabolic process   | 2.70E-20 | 1.4    |
| polysaccharide metabolic process | 6.00E-06 | 2.9    | protein metabolic process    | 2.70E-20 | 2.0    |
| cellular carbyd. biosynthetic process | 1.00E-05 | 3.3    | ribosome biogenesis          | 2.90E-19 | 4.2    |
| glutamine family amino acid metab. pr... | 2.20E-05 | 2.7    | metabolic process            | 1.10E-18 | 1.4    |
| glutamate metabolic process | 2.30E-05 | 5.2    | ribonucleoprotein complex biogenesis | 4.10E-18 | 3.9    |
| hexose metabolic process | 3.20E-05 | 2.4    | ncRNA processing             | 2.30E-13 | 3.2    |
| cofactor metabolic process | 3.70E-05 | 1.9    | rRNA metabolic process       | 4.50E-13 | 4.1    |
| glucan biosynthetic process | 4.60E-05 | 4.4    | ncRNA processing             | 4.60E-13 | 3.5    |
| fungal-type cell wall organization | 4.60E-05 | 2.1    | rRNA processing              | 5.90E-13 | 4.1    |
| cellular glucan metabolic process | 5.30E-05 | 3.4    | cellular process             | 1.60E-12 | 1.2    |

Gene Class

- GCN4
- C/D snoRNA
- H/ACA snoRNA
- Processome
- RPL
- RPS

B

C


duo1-W216G VS mms21RINGΔ

6763 genes within ratio of 4 to 4

cor = 0.70

-3 -2 -1 0 1 2 3 4

mms21RINGΔ/WT

6-D. H. Kim et al.
at an adjusted $P$-value of $\leq 0.05$ was 1921. However, this set contains genes with ratios as low as 1.2. After imposing an additional criteria of fold change $> 1.5$, the number of genes decreases to 1191. We carried out Gene Ontology term analysis on 657 upregulated genes and 337 downregulated genes (Figure 2A). The most significantly upregulated biological processes were carbohydrate metabolism and oxidation reduction. The most significantly downregulated biological processes in the mms21RING$\Delta$ mutant were translation, ribosome biogenesis, and related processes.

In order to further analyze the up- and downregulated genes, we examined individual gene groups (Figure 2B). We found that transcripts for both the large and small ribosomal proteins were significantly downregulated in the mms21RING$\Delta$ mutant (mean rank gene set test; large 6.79E-36, small 4.65E-25). We also observed a significant downregulation in small nucleolar RNAs (snoRNAs) (box CD 5.17E-09 and box H/ACA 0.0038). The box CD snoRNAs are hosted in introns of the ribosomal protein genes, so their downregulation correlates with the expression of these genes. Many genes involved in the processing steps of ribosome biogenesis and the post-transcriptional processing of noncoding RNAs (ncRNAs) were also downregulated (Processome 6.64E-21).

Gcn4 is a transcriptional activator that is translated when cells are under stress or nutritional starvation. Many targets of Gcn4 were upregulated in the mms21RING$\Delta$ mutant (mean rank gene set test; large 6.79E-36, small 4.65E-25). We also observed a significant downregulation in small nucleolar RNAs (snoRNAs) (box CD 5.17E-09 and box H/ACA 0.0038). The box CD snoRNAs are hosted in introns of the ribosomal protein genes, so their downregulation correlates with the expression of these genes. Many genes involved in the processing steps of ribosome biogenesis and the post-transcriptional processing of noncoding RNAs (ncRNAs) were also downregulated (Processome 6.64E-21).

Gen4 is a transcriptional activator that is translated when cells are under stress or nutritional starvation. Many targets of Gcn4 were upregulated in the mms21RING$\Delta$ mutant (Figure 2B). Of the 699 genes with a Gcn4 binding site, 99 were present in the upregulated gene group, while only 40 were present in the downregulated gene group (Fisher exact test 0.0049, see Materials and Methods and Table S1). We previously characterized a mutant in the cohesin ECO1 acetyltransferase (eco1–W216G) that had similarities in its gene expression profile (Bose et al. 2012). For example, many targets of Gcn4 were upregulated (Bose et al. 2012). The Pearson correlation between eco1–W216G and mms21RING$\Delta$ gene expression is 0.7 (Figure 2C). These results are consistent with the idea that the Smc5/6 complex is necessary for nucleolar function and suggest that Mms21 is important for a normal gene expression profile with respect to ribosome biogenesis and translation.

**The mms21RING$\Delta$ mutant exhibits defects in ribosome subunit export**

We next examined rRNA synthesis in the mms21RING$\Delta$ mutant. We measured rRNA synthesis in the mms21RING$\Delta$ mutant by performing a $^3$H-uridine incorporation assay. As shown in Figure 3A, total rRNA production was significantly reduced in the mutant. This result is consistent with defects in rRNA biogenesis in the mutant. Despite the reduced level of rRNA and messenger RNAs (mRNAs) encoding ribosomal proteins, we could not detect a reduction in global protein synthesis in the mutant (data not shown).

Gcn4 translation is upregulated to promote the transcription of its target genes such as genes involved in amino acid biosynthetic pathways. Gcn4 translation has been extensively used as an indicator of translational stress (Hinnebusch 1997). Thus, we decided to analyze whether Gcn4 translation was affected in the mms21RING$\Delta$ mutant at permissive temperature. The translation of Gcn4 was assessed from both a WT strain and the mms21RING$\Delta$ mutant harboring the p180–Gcn4–lacZ plasmid by measuring $\beta$-galactosidase activity. This assay demonstrated that the $\beta$-galactosidase activity in the mms21RING$\Delta$ mutant was elevated by threefold, compared to WT (Figure 3B), similar to previous observations in the eco1–W216G mutant (Bose et al. 2012). Consistent with translational stress, we found that Sui2 (eif2α) was more phosphorylated in the mms21RING$\Delta$ mutant as compared to WT (Figure 3C).

We were concerned that the elevation in Gcn4 might be due to the constitutive activation of the S-phase checkpoint in the mms21RING$\Delta$ mutant, so we examined $\beta$-galactosidase activity in a WT strain treated with either 150 mM HU or 0.005% MMS. In neither case did the treatment elevate the levels of $\beta$-galactosidase activity (Figure 3D), suggesting that DNA damage or replication slow down alone is not sufficient to promote Gcn4 translation. A previous study with a higher concentration of MMS found that MMS could induce Gcn4, but independently of the DNA damage checkpoint, suggesting Gcn4 translational activation can be separated from checkpoint activation (Natarajan et al. 2001). Furthermore, $\gamma$-irradiation, which causes DNA damage, does not affect Gcn4 levels, further suggesting that DNA damage and Gcn4 activation are not necessarily linked (Jelinsky et al. 2000). Therefore, the translational stress in the mms21RING$\Delta$ mutant is not necessarily due to the activation of the DNA damage checkpoint.

Next, we monitored the localization of both the large and small subunits of ribosomal proteins in both WT and the mms21RING$\Delta$ mutant at permissive temperature. The Rps2– and Rpl25–GFP reporter plasmids have been previously used to visualize the localization of the 40S and 60S subunit ribosomal proteins, respectively (Bose et al. 2012). Both Rps2– and Rpl25–GFP proteins are evenly distributed throughout the cytoplasm in WT cells. Accumulation of these proteins into foci is often indicative of a defect in ribosome biogenesis. Although endogenous ribosomal protein transcripts are downregulated overall, Rps2–GFP and Rpl25–GFP expressed from the plasmids are present at normal levels in whole cell extracts. These proteins accumulate in the nucleus in the mms21RING$\Delta$ mutant (Figure 3E), suggesting that ribosome assembly or export might be impaired in this mutant. The Rpl25–GFP protein colocalized with Sik1–RFP in this mutant, indicating that Rpl25, a 60S ribosomal protein, accumulates in the nucleolus. Interestingly, the Rps2–GFP protein did not colocalize with Sik1–RFP, a nucleolar marker, in the mutant; but instead seemed to be predominantly nuclear. In order to quantify the ribosomal protein accumulations in the mms21RING$\Delta$ mutant, we carried out cytometry analysis for peak fluorescence for the reporters (Kim 2016). These results showed that the accumulation of both the 40S and the 60S ribosomal proteins in the nucleus
Figure 3 The *mms21*RINGΔ mutant exhibits ribosome biogenesis defects. (A) rRNA production is reduced in *mms21*RINGΔ mutant. Both WT and *mms21*RINGΔ mutant cells were grown to midlog phase in SD –Ura medium containing 6.7 ng/μl uracil. The cultures were labeled with 3H-uridine for 5 min at 30°C. Total rRNA level was measured by scintillation counting. Error bars indicate the standard deviation from three independent experiments, *P* = 0.0034. (B) Gcn4 translation is increased in the *mms21*RINGΔ mutant. Both WT and *mms21*RINGΔ cells were transformed with the p180–Gcn4–LacZ reporter plasmid and were grown to midlog phase at 30°C. β-Galactosidase activity was measured using ONPG as a substrate. Error bars indicate the standard deviations from three independent experiments, *P* = 0.0012. (C) Sui2 is more phosphorylated in the *mms21*RINGΔ mutant compared to WT. The indicated strains were grown to midlog phase at 30°C. Phosphorylation level of Sui2-HA, in both WT and *mms21*RINGΔ strains was analyzed using
and nucleolus was significantly increased in the mms21RINGΔ mutant (Figure 3E), suggesting a defect in ribosome biogenesis. These results suggest that Mms21 supports ribosome biogenesis and prevents translational stress.

**Dosage of RPL19A regulates ribosomal protein accumulation in the mms21RINGΔ mutant**

Since the mms21RINGΔ mutant accumulates both the 40S and the 60S ribosomal subunits in the nucleus and the nucleolus (Figure 3E), we examined whether this phenotype was rescued by deletion of RPL19A. The cytometry analysis, which was also verified by microscopy, indicated that nuclear/nucleolar accumulation of the 40S and the 60S ribosomal proteins in the mms21RINGΔ mutant were significantly reduced by the deletion of RPL19A (Figure 4). Taken together, these data suggest that the deletion of RPL19A suppresses the defective localization of the two ribosomal proteins examined in the mms21RINGΔ mutant.

Given that the deletion of RPL19A suppresses the accumulation of both the 60S and the 40S subunits in the mms21RINGΔ mutant, we wondered whether overexpression of RPL19A would exacerbate the accumulation of ribosomal proteins in the mutant. To test this idea, RPL19A was overexpressed under the GAL1-10 promoter at permissive temperature. Under these conditions, there is no additional growth defect in the mutant and levels of β-galactosidase from the Gcn4–lacZ reporter are not further elevated (not shown). Rps2– and Rpl25–GFP mean peak fluorescence intensity was measured in WT and the mms21RINGΔ strains by conducting a cytometry assay. Overexpression of RPL19A did not increase the peak fluorescence intensity of Rps2–GFP in WT cells (Figure 5A). However, RPL19A overexpression further increased the mean fluorescence intensity of Rps2–GFP in the mms21RINGΔ mutant, suggesting that the 40S ribosomal subunit accumulation is exacerbated. We also examined the effect of the RPL19A overexpression on the Rpl25–GFP protein in both WT and the mms21RINGΔ mutant (Figure 5B). Unlike the strong effect on Rps2 accumulation, the overexpression of RPL19A only modestly increased Rpl25–GFP accumulation in the mms21RINGΔ mutant. These experiments indicate that overproduction of a ribosomal subunit can exacerbate the nuclear accumulation of ribosome components in the mms21RINGΔ mutant, suggesting that export of some ribosomal proteins may become more compromised.

The smc5-6 mutant accumulates both the small and large subunits of ribosomal proteins

Our data show that the mms21RINGΔ mutant, in which SUMO ligase activity is deficient, exhibits defects in the export of ribosomal subunits. A recent report suggests that the SUMO ligase activity of Mms21 is required for Smc5/6 complex-mediated DNA repair (Bermudez-Lopez et al. 2015). Thus, we wanted to test whether other subunits of the Smc5/6 complex also contribute to regulation of ribosome biogenesis. We examined the localization of both small and large subunits of ribosomal proteins in the smc5-6 mutant at permissive temperature. Both Rps2– and Rpl25–GFP proteins accumulated in the smc5-6 mutant compared to WT, but not in the smc6-9 mutant (Figure 6). The phenotype of the smc5-6 mutant was milder than in the mms21RINGΔ mutant and elevated β-galactosidase levels were not detected using the Gcn4–lacZ reporter (not shown). Unlike the mms21RINGΔ mutant, the smc5-6 mutant has no detectable growth defect at 30°, which may partially explain the mild phenotype of this mutant at 30°. However, the accumulation of ribosomal proteins in the smc5-6 mutant is consistent with the idea that Smc5/6 complex function may be required for normal ribosome biogenesis. While it is possible that the Mms21 SUMO ligase acts independently from the Smc5/6 complex for nuclear function, it seems more likely that deletion of the RING domain of MMS21 may provide a surgical removal of the SUMO ligase activity compared to temperature-sensitive mutations in SMCM5 or SMCM6, accounting for why the RING deletion provides the strongest phenotype.

Deletion of MPH1 partially rescues ribosomal protein accumulation, rRNA production, and translational stress in the mms21RINGΔ mutant

Deletion of MPH1 has previously been shown to rescue the MMS and temperature sensitivity of the mms21RINGΔ mutant (Chen et al. 2009). MPH1 encodes a helicase that operates during replication-associated recombination. The deletion of MPH1 in the context of the mms21RINGΔ mutation has been shown to reduce the accumulation of unresolved toxic recombination intermediates. We verified that deletion of MPH1 or RAD54, a DNA-dependent ATPase, suppresses the mms21RINGΔ mutant growth phenotypes, with MPH1 having a stronger effect (Figure S2). In addition, we
found that deletion of MPH1 could rescue the nuclear accumulation of Rps2–GFP and Rpl25–GFP (Figure 7).

We further examined whether the deletion of RPL19A or MPH1 could rescue rRNA production in the mms21RINGΔ mutant at permissive temperature. Intriguingly, rRNA synthesis in the rpl19aΔ mutant was also reduced, compared to WT. rRNA production in the mms21RINGΔ rpl19aΔ double mutant was slightly but significantly decreased relative to the rpl19aΔ and the mms21RINGΔ single mutants, suggesting that the deletion of RPL19A does not rescue the rRNA production.
Figure 5 Overexpression of RPL19A exacerbates Rps2–GFP accumulation in the mms21RINGΔ mutant. (A) Accumulation of Rps2–GFP in the mms21RINGΔ mutant is enhanced by overexpression of RPL19A. Both WT and mms21RINGΔ cells were transformed with either empty plasmid or pBY011–RPL19A plasmid containing the GAL1,10 promoter. The indicated strains were used to measure mean peak GFP fluorescence intensity as shown in Figure 3E. *P = 6.10E-05, **P = 0.3972. (B) Overexpression of RPL19A does not significantly increase accumulation of Rpl25–GFP in the mms21RINGΔ mutant. The indicated strains were transformed with either empty plasmid or pBY011–RPL19A plasmid carrying the GAL1,10 promoter. Rpl25–GFP mean peak fluorescence intensity was measured as shown in Figure 3E. *P = 0.0437, **P = 0.3751.
Figure 6  Both Rps2–GFP and Rpl25–GFP accumulate in the smc5-6 mutant. (A and B) Both the 40S and 60S subunits of ribosomal protein reporters accumulate in the smc5-6 mutant but not the smc6-9 mutant. Strains were transformed with either the Rps2–GFP or Rpl25–GFP reporter plasmid. Images were taken as described in Figure 3E. For either Rps2–GFP (A) or Rpl25–GFP (B) quantification, the strains carrying either Rps2–GFP or Rpl25–GFP were grown to midlog phase at 28°C in dropout medium supplemented with 0.02 mg/ml adenine. Mean peak Rps2–GFP (A) or Rpl25–GFP (B) fluorescence intensity was measured by performing cytometry analysis as described in Figure 3E. P-values were measured using a t-test.
production defect observed in the mms21RINGΔ mutant (Figure 8A). Deletion of MPH1 alone appears to increase rRNA synthesis and provides a slight but significant rescue of rRNA production in the context of the mms21RINGΔ mutant (Figure 8C).

We next focused on translational stress. We examined Gcn4 translation in the mms21RINGΔ rpl19aΔ and mms21RINGΔ mph1Δ double mutants to determine whether either deletion could suppress the elevated β-galactosidase activity in the mms21RINGΔ mutant. The mms21RINGΔ rpl19aΔ double
mutant had even higher levels of β-galactosidase activity than observed in the mms21RINGΔ mutant (Figure 8B), suggesting that translational stress was not relieved, even though the ribosomal proteins accumulate less. In contrast, we found that the mms21RINGΔ mph1Δ double mutant had significantly lower levels of β-galactosidase activity compared to the mms21RINGΔ mutant strain (Figure 8D), suggesting that in this case, nuclear accumulation of ribosomal proteins, rRNA production, and translational stress are partially relieved by MPH1 deletion. This suggests that the elimination of toxic recombination intermediates helps to partially restore normal translation in the mms21RINGΔ mutant.

Discussion

Previous reports have shown that Smc5/6 and its SUMO ligase activity provided by Mms21 are crucial for the roles of the complex in sister chromatid cohesion, restart of stalled DNA replication forks, and DNA damage repair (Rai et al. 2011; Almedawar et al. 2012; McAleenan et al. 2012; Wu et al. 2012; Bermudez-Lopez et al. 2015). While the mms21RINGΔ mutant had been previously reported to have aberrant nucleolar morphology (Zhao and Blobel 2005), and Mms21 had been reported to target some nucleolar proteins for sumoylation (Albuquerque et al. 2013), we now provide evidence that the Smc5/6 complex significantly contributes to nucleolar function. In this study, we have shown that Smc5/6, and in particular the SUMO ligase activity of the Mms21 subunit, are important to promote rRNA production, assembly and/or export of ribosomal proteins, and normal translation.

Our study identified RPL19A as a novel genetic suppressor of the mms21RINGΔ mutant. We observed reduced rRNA production, nuclear accumulation of ribosomal proteins, and elevated Gcn4 translation in the mms21RINGΔ mutant. We speculate that deletion of RPL19A may partially relieve an imbalance between ribosomal RNA and ribosomal protein–rRNA complexes in the nucleus in the mms21RINGΔ mutant. We speculate that overexpression of RPL19A increases this imbalance. However, it appears that the rescue provided by deletion of RPL19A is not sufficient to restore the production of rRNA, or to rescue translational stress.

We have previously shown that the related SMC complex cohesin is important for ribosome biogenesis. Mms21-dependent sumoylation of cohesin has been shown to be important for cohesion (Takahashi et al. 2008; McAleenan et al. 2012; Wu et al. 2012). Thus, one possible explanation for our findings is that mutations affecting Smc5/6 function are disrupting cohesion, which has already been shown to be important for nucleolar function. Other mechanisms are also possible,
including that (1) Mms21 sumoylation of nucleolar proteins is critical for nucleolar function; (2) the role of Smc5/6 in DNA replication and DSB repair, in particular at the rDNA, is important for nucleolar function; or (3) the role of Mms21 in nuclear–cytoplasmic trafficking affects nucleolar function (Rothenbusch et al. 2012). Lending support to the second mechanism, deletion of MPH1, which blocks the formation of toxic recombination intermediates (Chen et al. 2009), partially suppressed translational stress in the mms21RINGΔ mutant. This result is reminiscent of the rescue provided by deletion of FOB1 in the eco1–W216G strain (Lu et al. 2014). Deletion of FOB1 allows bidirectional replication of the ribosomal DNA, which partially rescued low rRNA production and translational stress associated with the cohesin acetyltransferase mutant. In the future, it will be important to further determine the molecular mechanisms by which Smc5/6 contributes to nucleolar function.

Given that both cohesin and Smc5/6 promote nucleolar function, a picture starts to emerge in which stress at the rDNA may trigger a translational stress response regulated by this ancient group of SMC complexes. Interestingly, the role of cohesion in nucleolar function and translation appears to be evolutionarily conserved and an important part of the etiology of a group of developmental syndromes known as the cohesinopathies (Xu et al. 2013; Xu et al. 2015). Increasing translation in zebrafish models for the cohesinopathies had dramatic rescue effects on developmental phenotypes. A recent report demonstrated that mutations in human NSMCE2/MMS21 lead to primordial dwarfism and insulin resistance (Payne et al. 2014). We speculate that nucleolar dysfunction caused by mutations in MMS21 might contribute to this human syndrome.

Acknowledgments

We thank Malcolm Moses for his technical contributions to the experiments and Richard Shrock for assistance with manuscript preparation and submission. We also thank the Molecular Biology Core at the Stowers Institute for Medical Research for RNA-sequencing libraries and for sequencing and Hua Li for the Kolmogorov–Smirnov statistic test.

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Communicating editor: O. Cohen-Fix
Table S1. Gcn4 site enrichment

|          | mms21 up | background | Total | mms21 down | background | Total |
|----------|----------|------------|-------|------------|------------|-------|
| Gcn4 site| 99       | 600        | 699   | 40         | 659        | 699   |
| absent   | 679      | 5748       | 6427  | 373        | 6054       | 6427  |
| Total    | 778      | 6348       | 7126  | 413        | 6713       | 7126  |
### Table S2. Strains used in this study

| Strain     | Genotype                                           | Reference                           |
|------------|----------------------------------------------------|-------------------------------------|
| W303       | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ | (BOSE et al. 2012; Lu et al. 2014) |
| JG2059     | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 | This study                          |
| DHKY300    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 | This study                          |
| DHKY348    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19bΔ::KanMx6 | This study                          |
| DHKY304    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 rpl19aΔ::KanMx6 | This study                          |
| DHKY349    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 rpl19bΔ::KanMx6 | This study                          |
| JG2087.1   | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ eco1 W216G::MX6-Hyg-MX6 | (BOSE et al. 2012; Lu et al. 2014) |
| DHKY357    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ eco1W216G::HygMX6 rpl19aΔ::KanMx6 | This study                          |
| DHKY313     | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ SUI2-3HA::KanMX6 carrying 2FLAG-SMT3 CEN GAL URA3 plasmid | This study |
|------------|---------------------------------------------------------------------------------------------------------------|-----------|
| DHKY315    | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3MX6 SUI2-3HA::KanMX6 carrying 2FLAG-SMT3 CEN GAL URA3 plasmid | This study |
| DHKY430    | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ SIK1-RFP::KanMX6 carrying Rps2-GFP CEN LEU plasmid | This study |
| DHKY431    | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ SIK1-RFP::KanMX6 carrying Rpl25-GFP CEN LEU plasmid | This study |
| DHKY432    | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3MX6 SIK1-RFP::KanMX6 carrying Rps2-GFP CEN LEU plasmid | This study |
| DHKY433    | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3MX6 SIK1-RFP::KanMX6 carrying Rpl25-GFP CEN LEU plasmid | This study |
| DHKY7.1    | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying Rps2-GFP CEN LEU plasmid | This study |
| Strain     | Genetic Background                                                                 | Notes               |
|------------|------------------------------------------------------------------------------------|---------------------|
| DHKY13.1   | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3Mx6 carrying Rps2-GFP CEN LEU plasmid | This study          |
| DHKY316    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 carrying Rps2-GFP CEN LEU2 plasmid | This study          |
| DHKY317    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3Mx6 rpl19aΔ::KanMx6 carrying Rps2-GFP CEN LEU2 plasmid | This study          |
| DHKY8.1    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying Rpl25-GFP CEN LEU plasmid | This study          |
| DHKY14.1   | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3Mx6 carrying Rpl25-GFP CEN LEU plasmid | This study          |
| DHKY318    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 carrying Rpl25-GFP CEN LEU plasmid | This study          |
| DHKY319    | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3Mx6 rpl19aΔ::KanMx6 carrying Rpl25-GFP CEN LEU2 plasmid | This study          |
| Code   | Description                                                                 | This study |
|--------|-----------------------------------------------------------------------------|------------|
| DHKY276| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying p180-GCN4-LacZ CEN URA3 plasmid | This study |
| DHKY278| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 carrying p180-GCN4-LacZ CEN URA3 plasmid | This study |
| DHKY331| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 carrying p180-GCN4-LacZ CEN URA3 plasmid | This study |
| DHKY332| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 rpl19aΔ::KanMx6 carrying p180-GCN4-LacZ CEN URA3 plasmid | This study |
| DHKY402| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying pBY011 CEN GAL URA3 plasmid Rps2-GFP CEN LEU2 plasmid | This study |
| DHKY403| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying pBY011-RPL19A CEN GAL URA3 plasmid Rps2-GFP CEN LEU2 plasmid | This study |
| DHKY404| MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3MX6 carrying pBY011 CEN GAL URA3 plasmid Rps2-GFP CEN LEU2 plasmid | This study |
| Strain   | Genetic Information                                                                 | Cited By          |
|----------|-------------------------------------------------------------------------------------|-------------------|
| DHKY405  | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3MX6 carrying pBY011-RPL19A CEN GAL URA3 plasmid Rps2-GFP CEN LEU2 plasmid | This study        |
| DHKY392  | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying pBY011 CEN GAL URA3 plasmid Rpl25-GFP CEN LEU2 plasmid | This study        |
| DHKY393  | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ carrying pBY011-RPL19A CEN GAL URA3 plasmid Rpl25-GFP CEN LEU2 plasmid | This study        |
| DHKY394  | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3MX6 carrying pBY011 CEN GAL URA3 plasmid Rpl25-GFP CEN LEU2 plasmid | This study        |
| DHKY395  | MATα ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21 RINGΔ::HIS3MX6 carrying pBY011-RPL19A CEN GAL URA3 plasmid Rpl25-GFP CEN LEU2 plasmid | This study        |
| BY4741   | MATα his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 bar1Δ                                                |                   |
| YBR084C-A| MATα his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 bar1Δ rpl19aΔ::KanMx6                             | Winzeler et al., 1999 |
|   | Strain Code | Genotype | Source                  |
|---|-------------|----------|-------------------------|
| JG2343 | MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 bar1Δ rpl19aΔ::KanMx6 smc5-6::natMx6 | This study |
| DHKY703 | MATa his3Δ0 leu2Δ0 met15Δ0 ura3Δ0 bar1Δ smc5-6::KanMx6 | Li et al., 2011 |
| DHKY706 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ smc5-6::KanMx6 | This study |
| DHKY707 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 smc5-6::KanMx6 | This study |
| BH913.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ADE2 can1-100 NUP49-mCherry::HygMX6 Rps2-GFP CEN LEU2 plasmid | This study |
| BH914.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ADE2 can1-100 NUP49-mCherry::HygMX6 Rpl25-GFP CEN LEU2 plasmid | This study |
| BH915.2 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::NatMX6 NUP49-mCherry::HygMX6 Rps2-GFP CEN LEU2 plasmid | This study |
| BH916.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::NatMX6 NUP49-mCherry::HygMX6 Rpl25-GFP CEN LEU2 plasmid | This study |
| BH917.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 NUP49-mCherry::HygMX6 Rps2-GFP CEN LEU2 plasmid | This study |
| BH918.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rpl19aΔ::KanMx6 NUP49-mCherry::HygMX6 Rps2-GFP CEN LEU2 plasmid | This study |
| BH919.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 rpl19aΔ::KanMx6 NUP49-mCherry::HygMX6 Rps2-GFP CEN LEU2 plasmid | This study |
| BH920.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 rpl19aΔ::KanMx6 NUP49-mCherry::HygMX6 Rpl25-GFP CEN LEU2 plasmid | This study |
| BH922.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ rad54Δ::KAN | This study |
| BH923.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 rad54Δ::KAN | This study |
| BH924.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mph1Δ::KAN | This study |
| BH925.1 | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 mph1Δ::KAN | This study |
| Strain   | Genetic Background                                                                 | Notes     |
|----------|------------------------------------------------------------------------------------|-----------|
| JG2131   | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ GCN4-LacZ::TRP1          | This study|
| JG2129   | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ GCN4-LacZ::TRP1 mms21RINGΔ::HIS3Mx6 | This study|
| BH939.1  | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ GCN4-LacZ::TRP1 mph1Δ::KAN | This study|
| BH938.1  | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ GCN4-LacZ::TRP1 mms21RINGΔ::HIS3Mx6 mph1Δ::KAN | This study|
| BH934.1  | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mph1Δ::KAN Rps2-GFP CEN LEU2 plasmid | This study|
| BH935.2  | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mph1Δ::KAN Rpl25-GFP CEN LEU2 plasmid | This study|
| BH936.1  | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 mph1Δ::KAN Rps2-GFP CEN LEU2 plasmid | This study|
| BH937.1  | MATa ura3-1 leu2,3-112 his3-1 trp1-1 ade2-1 can1-100 bar1Δ mms21RINGΔ::HIS3Mx6 mph1Δ::KAN Rpl25-GFP CEN LEU2 plasmid | This study|
