Genetic Interactions Show the Importance of rRNA Modification Machinery for the Role of Rps15p during Ribosome Biogenesis in S. cerevisiae

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Abstract

Rps15p, an essential ribosomal protein, was previously shown to be critical for nuclear export of small subunit pre-particles. We have designed a synthetic lethal screen in Saccharomyces cerevisiae to identify its genetic partners and further elucidate its role during ribosomal biogenesis. Our screen revealed interactions with mutants affected at various stages during ribosome biogenesis, from early nucleolar steps to nuclear export. Mutations were identified in genes encoding proteins involved in early ribosome biogenesis steps, like the small subunit processome component Utp15p, the 90S pre-ribosome factor Sla9p and the H/ACA snoRNP core protein Nhp2p. In addition, we found a synthetic lethality with BUD23, a gene encoding a methyltransferase involved both in rRNA modification and small subunit nuclear export. Interestingly, deletion of snR36 or snR85, two H/ACA snoRNAs that direct modifications close to Rps15p’s binding site on the rRNA, produces mild and opposite effects on growth in an rps15 hypomorphic background. These data uncover an unreported link between a ribosomal protein and rRNA modification machinery.

Introduction

Ribosome biogenesis in eukaryotes is a complex process that takes place, for most of it, in the nucleolus, a specialized domain of the nucleus. RNA polymerase I synthesizes a large ribosomal RNA precursor (pre-rRNA) that includes three of the four ribosomal RNAs constituting the ribosomal subunit, i.e. the 18S, 5.8S and 25S rRNAs in yeast. Conversion of this large precursor to the mature species involves sequential removal of flanking and internal sequences, the external (ETS) and internal transcribed spacers (ITS), through action of endonucleases and exonucleases (Fig. 1; for a review, see [1]). In addition to cleavage, the pre-rRNA is subjected to a series of nucleotide modifications, mostly ribose 3’-O-methylations and pseudo-uridylations. These modifications are catalyzed by small nucleolar ribonucleoparticles (snoRNPs) through specific base-pairing between their RNA component (snoRNA) with the surrounding of the position to be modified. These RNA processing steps are intimately coupled to the assembly of diverse proteins with the precursor RNAs, which starts as soon as transcription is initiated. These proteins include the 79 ribosomal proteins, which remain associated to the mature subunits, as well as a large number of trans-acting factors. The large ribonucleoproteinic particle thus assembled in the early part of the pathway, or 90S pre-ribosome, is composed mainly of so-called UTP proteins (U-Three Particle), which associate to the U3 snoRNP [2]. After participating in the early 18S rRNA maturation steps, these factors are released from pre-ribosomal particles when paths to form the precursors to the 40S and the 60S ribosomal subunits separate after A2 cleavage (Fig. 1).

The multiple steps of this process are highly organized in the cell nucleus as indicated by the dynamics of the nucleolus, the formation and morphology of which strictly depend on the activity of ribosome biogenesis [3]. The late steps of ribosome biogenesis, however, sequentially take place in the nucleoplasm and in the cytoplasm. For instance, the 40S ribosomal subunit, when exported from the nucleus, contains the 2OS pre-ribosomal RNA which 3’-end maturation in the cytoplasm yields the mature 18S rRNA. The determinants of the nuclear export of the 40S subunit are still poorly known. The exportin Crm1p is necessary [4], but although potential interactors of this exportin in the pre-40S particles were described, no critical binding site or essential adapter with the pre-40S particles has been found yet [5]. By screening yeast strains expressing sub-optimal levels of specific ribosomal proteins, we identified the ribosomal protein Rps15p as a particular actor of nuclear export of the pre-40S particles [6], a function conserved in mammalian cells [7]. Depletion of Rps15p provokes retention of the pre-40S particles in the nucleus without affecting the upstream RNA processing steps. It is tempting to speculate that Rps15p interacts with proteins involved in nuclear export of the pre-40S particles, like nuclear export factors or nucleoporins. Alternatively, shielding of a particular domain in the ribosomal RNA could be critical for nuclear domain, as already...
Complementation Cloning of the RPS15 gene

Screening of RPS15 genetic partners

To ascribe precise functions to Rps15p and identify its partners, we designed a synthetic lethal genetic screen based on a yeast hypomorphic mutant rps15-1, thermosensitive at 37°C. At 25°C, this mutant has a generation time doubled relative to the WT strain, indicating a defect even at permissive temperature. We identified mutants worsening the phenotype associated with the rps15-1 allele, by isolating synthetic lethal mutants at 25°C.

Permissive conditions are provided by a conditional expression of the rps15-1 allele, by isolating synthetic lethal mutants at 25°C. To ascribe precise functions to Rps15p and identify its partners, we designed a synthetic lethal genetic screen based on a yeast hypomorphic mutant rps15-1, thermosensitive at 37°C. At 25°C, this mutant has a generation time doubled relative to the WT strain, indicating a defect even at permissive temperature. We identified mutants worsening the phenotype associated with the rps15-1 allele, by isolating synthetic lethal mutants at 25°C. Permissive conditions are provided by a conditional expression of wild-type RPS15 from a galactose-inducible/glucose repressible promoter, while the mutant rps15-1 allele is located at the chromosomal locus, expressed via its own constitutive promoter (Fig. 2). After UV mutagenesis, we isolated 8 strains bearing a mutation synthetic lethal with rps15-1. The two mutated alleles ins9 and ins15 were thus renamed nhp2ins9 and utp15ins15. When co-expressed from a centromeric plasmid, nhp2ins9 and utp15ins15 were actually able to suppress growth defects on galactose in the Ins9 (ins9, rps15-1, GAL::RPS15) and Ins15 (ins15, rps15-1, GAL::RPS15) strains respectively, albeit much less efficiently than the wild-type allele (data not shown).

To confirm that these mutations are indeed responsible for the synthetic lethality with rps15-1, we crossed the Ins9 and Ins15 strains to either the Δnhp2::kanMX4, rps15-1 or Δnhp2::kanMX4, rps15-1 null mutants respectively. The resulting Δnhp2=:kanMX4, rps15-1 diploid strain was viable in the absence of a complementing wild-type copy of RPS15, while the backcross with the NHP2+ strain yielded a viable diploid strain. Similar results were obtained with utp15ins15. We thus conclude that nhp2ins9 and utp15ins15 are indeed mutations synthetically lethal with rps15-1.

Complementation Cloning of the ins mutants

With the exception of Ins3, which happened to be sterile, all Ins mutants were backcrossed with an rps15-1 strain and proved to be recessive. By transforming a yeast genomic library (kindly provided by P. Thuriaux) and selecting on glucose at 25°C, reproducibly complementing clones were obtained for Ins9 and Ins15. Plasmids complementing Ins9’s growth on glucose, and remaining thermosensitive, all contained genomic regions encompassing the NHP2 gene (YDL208W). Nhp2p is essential for function of H/ACA-type snoRNPs [10], which serve as guides to pseudouridylation about 46 U residues on rRNAs, including thirteen in the 18S rRNA. Plasmids complementing synthetic lethality in strain Ins9 contained UTP15 (YMR093W), an essential gene coding for a component of the U-Three Particle, as defined by Baserga and co-workers [2]. Sequencing of nhp2 in the Ins9 strain identified a TG deletion near the end of nhp2 ORF, leading to a frameshift mutation 20 codons before the end of the coding sequence. This is predicted to change the C-terminal sequence, and further lengthen the protein by 11 amino-acids. In the Ins15 strain, we found that utp15 translation start codon was mutated from AUG to AUU. As UTP15 is an essential gene, translation initiation is likely to proceed in some other unidentified way, which might still support poor growth in the presence of WT Rps15p, but become lethal in an rps15-1 background. The two mutated alleles ins9 and ins15 were thus renamed nhp2ins9 and utp15ins15. When co-expressed from a centromeric plasmid, nhp2ins9 and utp15ins15 were actually able to suppress growth defects on glucose in the Ins9 (ins9, rps15-1, GAL::RPS15) and Ins15 (ins15, rps15-1, GAL::RPS15) strains respectively, albeit much less efficiently than the wild-type allele (data not shown).

To confirm that these mutations are indeed responsible for the synthetic lethality with rps15-1, we crossed the Ins9 and Ins15 strains to either the Δnhp2::kanMX4, rps15-1 or Δnhp2::kanMX4, rps15-1 null mutants respectively. The resulting Δnhp2=:kanMX4, rps15-1 diploid strain was viable in the absence of a complementing wild-type copy of RPS15, while the backcross with the NHP2+ strain yielded a viable diploid strain. Similar results were obtained with utp15ins15. We thus conclude that nhp2ins9 and utp15ins15 are indeed mutations synthetically lethal with rps15-1.

Complementation cloning in the other INS genes was unsuccessful. However, since Ins7 and Ins9 showed very similar molecular phenotypes (see below), we sequenced the NHP2 locus in Ins7, and found the exact same nhp2ins9 mutation. For
simplification, these strain will be referred to as Ins7/9, bearing the nhp2<sup>2<sup>n</sup></sup> mutation.

**Phenotypic analysis**

Ribosome biogenesis is arguably a well-ordered and compartmentalized process, as depicted in Figure 1. Early 35S maturation takes place in the nucleolus, where A2 cleavage separates large and small pre-subunit maturation pathways. Pre-40S particles are rapidly exported to the cytoplasm where an endonucleolytic cleavage at site D releases the ITS1 from the 18S rRNA, thus completing maturation. Rps15p was shown to be involved in nuclear export, a late step in ribosome biogenesis [6]. Since mutations synthetic lethal with rps15-1 were identified in NHP2 and UTP15, two genes coding for proteins known to have a role in the early steps of pre-RNA maturation, we sought to characterize the rRNA sub-cellular distribution in the double mutant strains to understand the origin of the synthetic lethal relationship. By using a D-A2 (ITS1) probe in fluorescence in situ hybridization (FISH) experiments, it is possible to visualize all 18S rRNA precursors, as depicted in Figure 1. In wild-type yeast cells, for instance, there is a mild ITS1 signal in both cytoplasm and nucleoplasm, along a strong nucleolar signal (Fig. 3). At 25°C in the presence of glucose, the rps15-1 reference strain presents essentially a wild-type phenotype (Fig. 3), with lower cytoplasmic accumulation of the D-A2 signal in a small minority of cells. At 37°C, this strain shows a strong nucleolar and nucleoplasmic accumulation (Fig. 3), similar to Rps15p depletion [6]. We analyzed the distribution for the 18S rRNA precursors in mutant cells grown in permissive (Gal) and restrictive (Glc) conditions. Representative pictures for some mutants in restrictive conditions are shown in Fig. 3, and our observations are summarized in Table 1. There is a strong nucleolar signal in restrictive conditions for Ins7/9 and Ins15 consistent with defects in early processing steps. However, persistence of a cytoplasmic signal similar to the one in the rps15-1 reference strain indicates that pre-40S particles export is still taking place. In contrast, all other Ins strains are devoid of a cytoplasmic ITS1 signal, and display retention of pre-40S particles in the nucleoplasm, which indicates that pre-40S particles are not exported to the cytoplasm.

We then characterized defects at the molecular level by Northern Blot, probing whole cellular RNAs with various oligonucleotides depicted in Fig. 4. rRNA signals were normalized to 25S rRNA or 18S rRNA (Fig. 1). These observations are summarized in Table 1. In both permissive and restrictive conditions, the Ins7/9 strains show a strong decrease of the 32S pre-rRNA, while 35S pre-rRNA accumulates. This is paralleled by 23S RNA accumulation and 27S precursor RNA decrease. This phenotype indicates that early cleavages A0, A1, A2 are defective. Regarding Ins15, all rRNAs except 25S rRNA drastically under-accumulate, irrespective of their size, including 35S pre-rRNA, suggesting a defect in rDNA transcription. These pre-rRNA processing defects are consistent with the nucleolar accumulation observed with the D-A2 probe by FISH, and resembles the molecular phenotypes observed upon depletion of Nhp2p or Utp15p: Utp15p was proposed to be required for optimal rRNA synthesis [2,11], whereas Nhp2p depletion led to an A0-A1-A2 cleavage defect [10]. In glucose, the depleted GAL-nhp2 strain presented a decreased 20S rRNA level [10] while the Ins7/9 strains described here displayed a steady 20S rRNA level in permissive conditions and an actual increase in restrictive conditions. This suggests a maturation defect additive to that induced by Nhp2 loss of function, likely due to the rps15-1 mutation, as 20S pre-rRNA accumulation is also observed for the rps15-1 reference strain (Fig. 4, lane 1 and 2). Similar results are observed with utp15<sup>ins15</sup>.

None of the other mutant stains showed remarkable processing phenotypes, except Ins11 which displayed abnormal 21S RNA in parallel with a disappearance of 27S RNA, indicative of defective cleavage at site A2. The Ins17 strain, and to a lesser extent Ins13, showed a much stronger level of 20S rRNA when normalized to 18S (Fig. 4) or 25S rRNAs. However, in contrast to Ins7/9 and 15, pre-40S particles in Ins3, 5, 11, 13 and 17 were...
strongly retained in the nucleoplasm, which suggests that the mutations involved affect the export competence of these particles, either in cis or in trans.

Relative to the parental strain, all Ins strains under-accumulated 18S rRNA, while the levels of 25S rRNA did not seem to be affected, proving our screen based on an rps mutant to be small subunit specific. We observed however a very unusual form of rRNA with the 25S probe in mutants Ins7/9, building up to high levels in restrictive conditions. We probed this RNA with oligos 25S-01 (complementary to 2347-2377 in 25S rRNA), 25S-02

Table 1. Phenotype summaries for Ins strains.

|       | D-A2 FISH (a) | Northern Blot (b) |
|-------|---------------|-------------------|
|       | nucleolus    | nucleoplasm       | cytoplasm | 35S | 32S | 27SA2 | 23S | 21S | 20S |
| Ins3  | +             | ++                | -         | + (c) | =   | =     | + (d) | =   | =   |
| Ins5  | +             | ++                | -         | =   | =   | =     | =   | =   | =   |
| Ins7  | ++            | +                 | +         | +   | -   | =     | + (d) | =   | =   |
| Ins9  | ++            | +                 | +         | +   | -   | =     | + (d) | =   | =   |
| Ins11 | +             | ++                | -         | =   | =   | =     | + (d) | =   | +   |
| Ins13 | +             | ++                | -         | =   | =   | =     | =   | =   | =   |
| Ins15 | ++            | +/-               | +/-       | -   | -   | -     | -   | =   | -   |
| Ins17 | +             | ++ (e)            | -         | =   | =   | =     | =   | =   | =   |

(a) same as rps15, ++ accumulation, - absence, +/- intermediate. Restrictive conditions except where noted (f).
(b) = comparable to rps15, + accumulation, - reduction. Permissive and restrictive conditions, except where noted (g).
(c) only in restrictive conditions.
(d) only in restrictive and permissive conditions.

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**Figure 4.** Northern blot of whole rRNAs. Schematic representation and names of the identified RNAs are listed on the right. Pre-rRNAs were detected with probes D-A2 (upper panel), E-C2 (middle panel) and 18S and 25S (lower panel). These probes are depicted as black squares along the identified rRNA species (see table 3 for sequence). Cells were grown as in Fig. 3 in medium containing galactose (G) or glucose (D).

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(1431–1452) and 25S-03 (638–658), of which only 25S-01 and 02 were positive. With an estimated size of around 2.1 Kb, this new rRNA species spans the 3′ two-thirds of the mature 25S rRNA and we therefore named it 25S2/3. It is known that absence of some pseudouridylation guide H-ACA snoRNAs can actually perturb rRNA cleavages [12,13], but we do not know at this point if 25S2/3 rRNA is an aberrant processing by-product or a partial 25S rRNA degradation product.

**bud23 and slk9 are synthetic lethal with rps15-1**

As we were not able to identify the other ins mutations by complementation experiments due to high reversion rates, we looked at phenotypic analogy with mutations described in the literature. For instance, Ins17, which shows the strongest 20S rRNA accumulation in the nucleolus, is linked to the MAT locus as evidenced by analyzing tetrads obtained from the initial backcross to *tps15-1* (data not shown). *BUD23* has been described as being a non-essential methyltransferase responsible for modifying 18S rRNA residue G1575. *BUD23* is closely linked to the MAT locus, and deletion for *bud23* leads to a small subunit export phenotype [14] very similar to the one observed for the Ins17 strain even in permissive conditions. This strongly pointed towards *ins17* to be a *BUD23* allele. We first decided to examine whether *bud23 deletion is synthetic lethal with rps15-1*. A *Abud23:kanMX4* strain was crossed with an *rps15-1* strain, and the resulting diploid was set to sporulate. G418 resistance thermosensitive spores were incapable of forming colonies, indicating that a *Abud23, rps15-1* genetic combination is synthetically lethal. Sequencing of *BUD23* in the Ins17 strain showed no mutation in the ORF or 250 nucleotides surrounding. Thus *BUD23* is an additional genetic interactant with *RPS15*.

Similarly, Ins11 stood out during our phenotypic analysis, as it showed accumulation of both 23S and 21S rRNA. Based on this phenotype, which was not often described in the literature, we sequenced some candidates in the Ins11 strain, like *RPS15A/B, RPS18A/B, RPS1, YAR1, ENP1, LTV1*, and SLX9, but found that none of them was mutated (data not shown). Because deletion of the non-essential gene SLX9 shows a striking phenotypic resemblance with Ins11, with the same rRNA precursors accumulating [15], we wondered if it would also present genetic interactions with *RPS15*. We crossed a *Als9:kanMX4* mutant with our *rps15-1* mutant. After sporulation, *Als9, rps15-1* spores were incapable to grow, indicating synthetic lethality. Thus, we identified two genes which deletion is synthetic lethal with *rps15-1*, in addition to the mutations identified in our synthetic lethal screen. When attempting to construct a *Abud23, rps15-1* [pFL38-GAL::RPS15] strain, recombinant descendants were unviable in Galactose, suggesting that a *Abud23 deletion is lethal when Rps15p is over-expressed with a GAL promoter. This could explain why no *BUD23* mutant was isolated in our screen. Moreover, our screen was clearly not saturated, as no additional lethal mutation was selected for in *rps15-1*.

**Are specific rRNA modifications responsible for synthetic lethality?**

*NHP2* and *BUD23*, two of the mutated genes showing synthetic lethality with *rps15-1*, are involved in rRNA modifications, suggesting a functional link between Rps15p and rRNA modifications. Nhp2p is required for synthesis and stability of H/ACA snoRNAs, which are responsible for targeting pseudouridylation [10]. We examined the influence of the *nhp2* mutation on the state of the H/ACA snoRNAs accumulation (Fig. 5). Abundance of snoRNAs in *nhp2* strains was clearly affected, with very low levels of all the H-ACA snoRNA tested, except snR30 and snR37, even in a *RPS15* background (in galactose, G, in Fig. 5), while snR190, a C/D box snoRNA involved in rRNA methylation through a different type of snRNP, remained unaffected.

Interestingly, a few pseu-uridines lie near Rps15p’s binding site (extrapolated from the bacterial 30S subunit structure [16]), the closest being at positions U1181 (red in Fig. 6A, 6B) and U1187 (blue in Fig. 6A, 6B), guided by snR36 and snR36 respectively. Bud23p is a methyltransferase responsible for 18S rRNA modification, the m’G1575 methylation [14]. It is noteworthy that G1575 is located near helix 29 (H29, shown in yellow in Fig. 6A, 6B), while Rps15p’s binding to rRNA, as deduced from the position of its bacterial homolog S19 in the prokaryotic 30S crystal structure [16] (green in Fig. 6A, 6B), is located at the H30-H31-H32 junction, only a few A away.

Remarkably, snR36 and snR36 were almost undetectable in the Ins17 strains (Fig. 5). While we do not know if under-accumulation of these snoRNA greatly reduces or abolishes 18S rRNA modifications, we directly questioned whether synthetic lethality between *nhp2+/-* and *rps15-1* was due to specific absence of these snoRNAs. We constructed double mutants *tps15-1, Asmr36* and *tps15-1, Asmr85*, as well as the triple *tps15-1, Asmr36, Asmr85* mutant. All genetic combinations were viable, indicating that the synthetic lethality relationship between *tps15-1* and *nhp2+/-* cannot be explained by the absence of the snR36 and snR85 guided modifications in the vicinity of Rps15p’s binding site. However, although deletion of these snoRNAs displayed no measurable growth defect in a wild-type background (Fig. 6C, bottom row), we observed faster growth when snR85 was deleted in the *tps15-1* background, while the *snr36 deletion had a synthetic sickness effect with *tps15-1* (Fig. 6C, top row). In liquid culture, generation times were consistent, ranging from 3.8 h for *Asmr85, tps15-1* to 4.5 h for *Asmr36, tps15-1*, with the parental *tps15-1* strain doubling every 4.1 h (data not shown). When *snr36* and *snr85* deletions were combined, both synthetic suppression and synthetic sickness
appear to neutralize each other, as this triple mutant grew like the rps15-1 parental strain (Fig. 6C, top row).

Similar to deletion of snR36 and snR85, a BUD23 allele encoding an enzymatically inactive protein (G57E or D77K from [14]) was not synthetic lethal with rps15-1 (data not shown), indicating that the absence of methylation m'G1575 is not sufficient to explain synthetic lethality between Bud23 and rps15-1.

Thus, as anticipated from the structure of the 40S subunit, these data suggest a complex interplay between Rps15p and the pseudo-uridylation at position U1181 or U1187. However, synthetic lethality between nhp2ins9 and rps15-1 appear to be due to a wider impairment of the H/ACA snoRNP function.

Discussion

We designed a novel approach to perform a synthetic lethal screen aimed at uncovering more precisely the function of Rps15p in ribosome biogenesis. We identified 8 mutants in a specific screening process based on conditional (galactose induced/glucose repressed) expression of the WT allele. Upon functional complementation on glucose (Fig. 2), two mutations were identified in NHP2 and UTP15. The rps15-1 allele was also found to be synthetic lethal with deletions of BUD23 or SLX9. The mutants were assigned to two main classes: those retaining pre-40S particles in the nucleoplasm, which could be genuine mutants in the export pathway, like Ins3, Ins5, Ins13, Ins17, and Abud23; and mutants affected in early, nucleolar steps in ribosome biogenesis, such as Ins11, nhp2<sup>20s</sup> and rps15-1, utp15<sup>20s</sup>. 20S pre-rRNA is readily detected in the cytoplasm, indicating that pre-40S particles are exported from the nucleus despite upstream defects, even in restrictive conditions. It is possible that overall rRNA processing is slowed down, but can still be pursued up to the final cytoplasmic D cleavage; lethality could arise from a trivial “flow” defect in ribosome production, due to two independent bottlenecks. But more specific hypotheses for the observed synthetic lethality can be envisioned. It has been reported that Utp15p depletion triggers cell cycle arrest [17]. The Ins15 strain stops growth almost immediately in restrictive conditions, merely undergoing one additional division. In this case, it is conceivable that the two hypomorphic mutations rps15-1 and utp15<sup>20s</sup> provoke a cell cycle arrest in restrictive synthetic conditions, when their defects are additive. Regarding NHP2 synthetic lethality, numerous snoRNAs deletions have been described as yielding defects in ribosome biogenesis defects causing translational defects [12], and the rps15-1...

Figure 6. Positions of Rps15p and rRNA modifications on 18S rRNA, and growth phenotypes for snoRNA-deleted mutants. A. 2D representation for yeast 18S rDNA sequence. Red : Helix 30, Blue : H31 and loop, Grey : H32 and distal unpaired nucleotides, Yellow : H42 and surrounding unpaired nucleotides. Rps15p contacts (green) with 18S rRNA are extrapolated from those between S19 and 16S rRNA [16]. 2D representation obtained from CRW http://www.rna.ccbu.utexas.edu/ [30] for Genebank sequence #U53879. B. 3D representation of the ribosomal region surrounding prokaryotic S19, in green (Rps15p homolog). Colors as above. Equivalences for eukaryotic 18S rRNA U1181 (red, target of snr85), U1187 (blue, target of snr36) and G1575 (yellow, target of Bud23p) are indicated in colored spheres. Obtained using Pymol and PDB #1FJG. C. Yeast spotted as single cells. Relevant genotype are indicated at the top concerning snoRNAs and right for RPS15 background.
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Rps15p could be involved in several steps during ribosome accumulation early 21S or 23S rRNA precursors, while Rps15p. Moreover, a good part of the synthetic lethal mutants isolated here accumulate early 21S or 23S rRNA precursors, while Rps15p depletion was shown to result in 20S pre-rRNA accumulation. Rps15p could be involved in several steps during ribosome biogenesis. Rps15p depletion [6] or the rps15-1 mutant at permisive temperature affect the export function, but early nuclear steps could be affected in the rps15-1 mutant especially at restrictive temperature, as suggested by the accumulation for 23S rRNA precursor in the rps15-1 strain at restrictive temperature (Fig. 4, lane 4). Such phenotypic discrepancies have already been revealed by studies on RPS14, showing a strikingly different phenotype for deletion of the whole protein causing an early small subunit processing blockage [19], or point mutations leading to late high-level 20S cytoplasmic accumulation [20]. However, RPS15/15' deletion induces a late maturation defect, while the rps15-1 recessive mutant would display an early processing phenotype, which seems counter-intuitive. So reciprocally, it is possible that although hypomorphic mutations in Utp15p and Nhp2p, whose deletion results in early ribosome biogenesis defects, rather perturb downstream steps in the pathway. For instance, specific snoRNAs shortage in the abhp2+ mutant might have consequences more downstream in conjunction with rps15-1. In this respect, we tried to identify specific synthetic lethality between rps15-1 and a subset of H-ACA snoRNA guided targets close to the binding site of Rps15p on 18S rRNA, driven by snr36 and snR85. This idea was strengthened by the finding of a synthetic lethal interaction between rps15-1 and absence of Bud23p, which methylates G1575 in 18S rRNA, close to Rps15p’s binding site. Although deletion of the two snoRNAs resulted either in synthetic sickness with rps15-1 or partial suppression of rps15-1 growth impairment, it could not explain the abhp2+ synthetic lethality with rps15-1.

Nevertheless, the synthetic phenotype presented by either snoRNA deletion is striking, for such a synthetic sickness (Δsnr36) or partial suppression (Δsnr85) has never been reported for a single snoRNA to our knowledge. Although additive effects were observed for combinations of snoRNA deletions [12,21], opposite phenotypes that suppress each other, as observed for Δsnr36 and Δsnr85 in the rps15-1 background, have never been shown before. This effect might be explained in part by the fact that the targets of these snoRNAs overlap on the 18S rRNA, making it impossible for both snoRNAs to intervene simultaneously. Also, in a bacterial in vitro reconstituted system, the binding of S19 to 16S rRNA was reported to sustain a conformational switch, which involves H30 and H31 [22]. These two helices are targeted in yeast by snR85 and snR36, which could affect in opposed way this conformational rearrangement, triggered by S19 homolog, Rps15p. Our data thus give hints on the interplay between a ribosomal protein and snoRNA mediated modifications that significantly impair ribosome biogenesis and/or function. Such an approach could be extended to identify other specific interactions between ribosomal protein mutants and snoRNA deletions to help understand the role of RNA modifications, which are still largely unknown.

Finally our most intriguing result is the accumulation of an aberrant large subunit 25S 2/3 rRNA form in the abhp2+ mutant, when Rps15p is mutated and thus limits small subunit availability. It raises the possibility that a 60S/40S imbalance leads to degradation of the large subunit. In an abhp2+ mutant, such a degradation process could be stalled on the hypomodified 25S rRNA. This degradation pathway could involve the TRAMP complex and the exosome [23] or the recently described ubiquitine-mediated NRD (Non-functional Ribosome Decay) [24]. How absence of RNA modifications could perturb degradation remains to be understood. Accumulation at high levels of this stalled degradation product could actually be an explanation for synthetic lethality between rps15-1 and abhp2+ and to be a genetic starting point to identify actors in this pathway.

Table 2. Yeast strains used in this work.

| Strain | Relevant Genotype | Source |
|--------|------------------|--------|
| BY4741 | MATa his3Δ11 leu2Δ10 met15Δ0 ura3Δ0 | Euroscarf WT |
| ME14-3 | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 rps15Δ1 | This work<sup>1</sup> |
| Ins x  | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 rps15Δ1 ins [pFL38-GAL::RPS15] | ME14-3 UV-mutagenized |
| ME17-F4 | MATa Δhis3Δ11 leu2Δ10 ura3Δ0 lys2Δ10 Δnhp2::kanMX4 [pCM180-NHP2] | Euroscarf Y23906 spore |
| ME17-F7 | MATa Δhis3Δ11 leu2Δ10 ura3Δ0 lys2Δ10 Δutp15::kanMX4 [pCM189-UTP15] | Euroscarf Y26228 spore |
| YO7184 | MATa his3Δ11 leu2Δ10 met15Δ0 ura3Δ0 Δbub2Δ3::KanMX4 | Euroscarf |
| YO4711 | MATa his3Δ11 leu2Δ10 met15Δ0 ura3Δ0 Δbub2Δ3::KanMX4 | Euroscarf |
| LM4458 | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 snr85::KAN | Kindly provided by A. Jacquier and M. Fromont-Racine [28] |
| ME17-1S | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 snr85::KAN rps15Δ1 | ME14-3 x LM4458 spore |
| ME17-1G | MATa his3Δ11 leu2Δ10 met15Δ0 ura3Δ0 snr36::URA3 | BY4741<sup>1</sup> |
| ME17-1B | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 snr36::URA3 rps15Δ1 | ME14-3<sup>1</sup> |
| ME18-a1 | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 snr36::KAN snr36::URA3 | LM4458<sup>2</sup> |
| ME18-d6 | MATa his3Δ11 leu2Δ10 lys2Δ10 ura3Δ0 snr36::KAN snr36::URA3 rps15Δ1 | ME17-1S x ME17-1B spore |

<sup>1</sup>strain GAL-RPS15 [6] transformed with a PCR holding the rps15-1 allele (kindly from P. Milkereit, unpublished). A thermo-sensitive clone was isolated and cured from pFL38-GAL::RPS15 plasmid, rps15-1 locus was sequenced for confirmation.
<sup>2</sup>transformed with PCR product amplified from the Δsnr36::URA3 strain from [29], kindly provided by E. Fayet-Lebaron. Absence of SnR36 was checked by Northern blotting.

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**Materials and Methods**

**Yeast strains and media**

Regular yeast genetic methods were used [25]. Relevant *Saccharomyces cerevisiae* strains are listed in Table 2 and can be obtained on demand to the corresponding author. Cells were grown either in YP medium (1% yeast extract, 1% peptone) supplemented with 2% galactose or 2% glucose as the carbon source, or in YNB medium (0.17% yeast nitrogen base, 0.5% (NH₄)₂SO₄) supplemented with 2% galactose or 2% glucose and the required amino acids and bases. When required, G418 and doxycyclin were added at 0.2 mg/ml and 30 μg/ml final concentrations, respectively.

**Plasmids**

pFL38-RPS15wt or pFL38-rps15-1 contain the promoter and coding sequence for RPS15 or rps15-1, followed by a PGK terminator (Valérie Choesmel-Cadamuro, unpublished). pFL38-Gal:RPS15wt was described earlier [6]. pCM189-NHP2 and terminator (Vale`rie Choesmel-Cadamuro, unpublished). pFL38-m were precipitated for 10 min at room temperature in a fresh tube

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### Table 3. Oligonucleotide probes used in this work.

| Name | Sequence (5’→3’) |
|------|------------------|
| D-A2 | TTG CAC AGA AAT CTC TCA CCG TTG GGA GTA GCA AGA AGG AAA CTT ACA AGC TT |
| 18S-01 | TTG TTC CTC GGT AA GGA TTA TTG ACA TTG TAC TCA TTC C |
| 25S-01 | CCC GTT CCC TTG GCT GTT TCG CTA GAT A |
| 25S-02 | TGC TAC TAC CAC CAA GAT CTG C |
| 25S-03 | CTT GGT CCG TGT TTC AAG CCG |
| E-C2 | ATA GCC CAG GAA TTT CCA GAT AAG TCC AAA GAG GAT CAC TC |
| snR10 | CAT GGG TCA AGA AGG CCC CGG AGG GG |
| snR11 | GAC GAA TCG TGA CTC TG |
| snR30 | GAA GCG CCA TCT AGA TG |
| snR31 | GTAGAAAGAATCATGACC |
| snR36 | TTA CTC GAG TGA TAT GAG ACG TTC TAA TTA |
| snR37 | GAT AGT ATT AAC CAC TAC TG |
| snR44 | GCC CGA TCA CAC CAT CTA GAT ATC AGC CCG |
| snR85 | GAC ATA TGT GCT AGT ATG CG |
| snR190 | CGA GGA AAG AAG AGA CAC CAT TAT C |

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**Author Contributions**

Conceived and designed the experiments: FG PG GS. Performed the experiments: CB PC FG GS. Analyzed the data: CB PC PEG GS. Contributed reagents/materials/analysis tools: GS. Wrote the paper: PEG GS.
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