Research article

Inactivation of NMD increases viability of sup45 nonsense mutants in Saccharomyces cerevisiae

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Abstract

Background: The nonsense-mediated mRNA decay (NMD) pathway promotes the rapid degradation of mRNAs containing premature termination codons (PTCs). In yeast Saccharomyces cerevisiae, the activity of the NMD pathway depends on the recognition of the PTC by the translational machinery. Translation termination factors eRF1 (Sup45) and eRF3 (Sup35) participate not only in the last step of protein synthesis but also in mRNA degradation and translation initiation via interaction with such proteins as Pab1, Upf1, Upf2 and Upf3.

Results: In this work we have used previously isolated sup45 mutants of S. cerevisiae to characterize degradation of aberrant mRNA in conditions when translation termination is impaired. We have sequenced his7-1, lys9-A21 and trp1-289 alleles which are frequently used for analysis of nonsense suppression. We have established that sup45 nonsense and missense mutations lead to accumulation of his7-1 mRNA and CYH2 pre-mRNA. Remarkably, deletion of the UPF1 gene suppresses some sup45 phenotypes. In particular, sup45-n upf1∆ double mutants were less temperature sensitive, and more resistant to paromomycin than sup45 single mutants. In addition, deletion of either UPF2 or UPF3 restored viability of sup45-n double mutants.

Conclusion: This is the first demonstration that sup45 mutations do not only change translation fidelity but also acts by causing a change in mRNA stability.

Background

Two translation termination factors, eRF1 and eRF3, participate in termination of protein synthesis in eukaryotes (reviewed in [1]). In S. cerevisiae they are encoded by SUP45 and SUP35, respectively (reviewed in [2]). In eukaryotes, a single factor, eRF1 (Sup45 in yeast), decodes all three stop codons, while eRF3 (Sup35 in yeast) stimulates termination through a GTP-dependent mechanism by forming a complex with eRF1.

Eukaryotic cells possess a mechanism known as nonsense-mediated mRNA decay (NMD) to recognize and degrade mRNA molecules that contain premature termination codon (PTC) (reviewed in [3-5]). The NMD process is mediated by the trans-acting factors Upf1, Upf2 and Upf3.
that loss of Upf1 suppresses many of the pleiotropic phe-
tions have an inhibitory effect on NMD. Our observation
cuss the role of the Upf complex in translation
Here, we show that
mulation of
upf1 interaction experiments, it has been demonstrated that Upf2, Upf3 and eRF1 actually compete with each other for binding
mRNA is affected by NMD pathway [21]. In the present paper, we show that the accu-
mRNA is subject to degradation by NMD. The abundance of the precursor and mature
mRNA was 3.5 fold higher in the
upf1∆ cells (Fig. 1, upper panel). However, the
level of wild-type HIS7 mRNA was not affected by deletion
of UPF1 (see Additional file 1, figure 1A). The steady-
state level of ade1-14, another nonsense-containing tran-
script, did not depend on deletion of UPF1 (Fig. 1, middle
panel). Therefore, the deletion of UPF1 gene affects the
his7-1 transcript in precisely the same manner that it affects the CYH2 pre-mRNA demonstrating that his7-1 is a potential substrate for NMD. Earlier it was shown that deletion of UPF1 gene promotes suppression of some but not all nonsense mutations [7]. Indeed, we did not detect suppression of his7-1 mutation on SC-HIS medium in

The strain 1B-D1606 in which sup45 mutants were obtained, contains nonsense mutations in HIS7, LYS9 and TRP1 genes. As shown in Table 1, sequence analysis of the his7-1, lys9-A21 and trp1-289 alleles identified the presence of single nonsense mutations. It is known that destabilization of mRNAs by NMD depends on the position of the nonsense codon and the presence of a DSE (downstream destabilizing element) downstream of mutation (reviewed in [15]). Indeed, while mRNAs with nonsense codons occurring in the last 20 to 30% of the coding region retain their wild-type decay rates, mRNAs harboring PTC in the first two-third of the gene are subject to degradation by NMD. The his7-1 nonsense mutation is located at the beginning of HIS7 mRNA and is followed by two putative DSE (Table 1), suggesting that his7-1 transcript could be a substrate for NMD.

To check if his7-1 transcript is affected by NMD, two iso-
genic strains were constructed harboring the his7-1 allele together with UPF1 or upf1∆. For this purpose, the strain 5B-D1645 (his7-1 upf1∆) was transformed with prS316 and prS316/UPF1 plasmids. The mean steady-state level of the his7-1 mRNA was 3.5 fold higher in the upf1∆ cells than in UPF1 cells (Fig. 1, upper panel). However, the level of wild-type HIS7 mRNA was not affected by deletion
of UPF1 (see Additional file 1, figure 1A). The steady-
state level of ade1-14, another nonsense-containing tran-
script, did not depend on deletion of UPF1 (Fig. 1, middle
panel). Therefore, the deletion of UPF1 gene affects the
his7-1 transcript in precisely the same manner that it affects the CYH2 pre-mRNA demonstrating that his7-1 is a potential substrate for NMD. Earlier it was shown that deletion of UPF1 gene promotes suppression of some but not all nonsense mutations [7]. Indeed, we did not detect suppression of his7-1 mutation on SC-HIS medium in

**Table 1: Nonsense mutations sequenced in the present work**

| Mutant allele | Nucleotide substitution | Putative destabilizing motif | Position/ORF size | Codon change |
|---------------|-------------------------|-----------------------------|-------------------|--------------|
| his7-1        | AAA→TAA                | (TTGAT)TACAAG              | 229/1659          | TCA       |
| lys9-A21      | TTA→TAA                | (CGATG)TTCG                | 605/1341          | ND          |
| upf1-289      | CAG→TAC                | (ATGATG)TCTG               | 403/678           | ND          |

The downstream sequences were searched for the presence of DSE (TGYYGATGYYYY) [15]. The numbers above the sequences indicate the 5' boundaries of putative DSE (underlined sequences) in the corresponding open reading frame (ORF); ND – not detected. **Codon changes in the case of each mutation are shown, nucleotide changes are underlined.**
strain 5B-D1645 bearing upf1Δ (see additional file 1 Fig. 1B).

We studied NMD efficiency in sup45 mutant strains by examination of CYH2 and his7-1 mRNA levels. First, we have shown that the mRNA level of wild-type HIS7 mRNA in sup45-n mutants and upf1Δ mutants is not changed (see additional file 1 Fig. 1B). Next, we used strains harboring nonsense mutations sup45-102, 104, 105, 107, which lead to decrease of eRF1 full-length protein level [18] and the sup45-103 (L21S) missense-mutation [22]. Total RNA was isolated from mutants and analyzed by Northern blots using probes specific for CYH2 and his7-1 mRNA. Strains bearing sup45-n mutations had a significantly increased CYH2 pre-mRNA/RNA ratio (1.7 ± 0.1 to 2.1 ± 0.2) compared with that of wild-type SUP45 strain (ratio of 1). A similar increase was also observed for sup45-103 missense mutant (1.8 ± 0.1) (Fig. 2A). Similarly, the relative abundance of his7-1 mRNA in the sup45-n mutant strains, normalized using ACT1 mRNA, ranged from 1.8 ± 0.3 to 2.8 ± 0.4 compared with that of wild-type SUP45 strain (ratio of 1) (Fig. 2B). The strain harboring missense mutation sup45-103 was also characterized by the accumulation of the his7-1 transcript (1.8 ± 0.1). This effect was weaker than that observed for UPF1 deletion which causes 4 and 3.5 fold increases in the CYH2 pre-mRNA/RNA ratio and the accumulation of his7-1 mRNA, respectively (Fig. 1), however it appears specific for NMD substrates since the amounts of other nonsense-containing transcripts (ade1-14 and lys9-A21) were not significantly changed in sup45 mutants (see Additional file 2). We also observed a significant increase of the CYH2 pre-mRNA/RNA ratio and of his7-1 mRNA level in strains bearing sup35 nonsense and missense mutations (data not shown). Taken together, these results demonstrate that both nonsense and missense mutations in SUP45 decrease the efficiency of mRNA degradation by NMD thus leading to accumulation of mRNAs containing PTCs.

Figure 1

his7-1 mRNA accumulated when nonsense-mediated decay is inhibited. Northern blotting was used to assess the effect of UPF1 deletion on the accumulation of his7-1 mRNA. Total RNA was isolated from strain 5B-D1645 (his7-1 upf1Δ) transformed with plasmids pRS316 and pRS316/UPF1, designated as (upf1Δ) and (UPF1), respectively. Northern blots were hybridized with radiolabeled HIS7, ADE1, ACT1 and CYH2 probes. A. Representative hybridization signals specific to his7-1 mRNA (upper panel), ade1-14 mRNA (middle panel) and actin mRNA (ACT1) used as an internal control (lower panel) are shown. Numbers indicated under upper and middle panels represent the relative abundance of his7-1 and ade1-14 mRNA’s, respectively, in upf1Δ and UPF1 strains. (s.d.) – standard deviation. B. Accumulation of CYH2 precursor mRNA was used to control that NMD is altered in the upf1Δ strain. The CYH2 probe detects both precursor and mature CYH2 mRNA. The fold increase in CYH2 precursor/mature mRNA accumulation in upf1Δ strain relative to UPF1 strain is indicated with the standard deviation (s.d.).

Figure 2

Nonsense or missense alleles of SUP45 affect accumulation of his7-1 mRNA and CYH2 pre-mRNA. Northern blots were prepared with total RNA from wild-type strain 1B-D1606 (SUP45) and its sup45 mutant derivatives. Blots were hybridized with DNA probes that detected the his7-1, CYH2 and ACT1 transcripts. For each mutant the average CYH2 pre-mRNA/mRNA ratio (A) and the abundance of his7-1 mRNA (B) relative to the wild-type strain are shown with the standard deviation (s.d.). Following sup45 mutations were tested: 102, 104, 105, 107 (nonsense) and 103 (missense).
Increased viability of sup45 nonsense mutants in the absence of UPF1

Previously, we have shown that sup45 nonsense mutants are viable in different genetic backgrounds [18]. However, the efficiency of plasmid shuffle was significantly lower in the case of mutant sup45-n alleles compared to plasmid bearing wild-type SUP45 gene [18] indicating that sup45-n mutations imperfectly replace SUP45. To assess the effects of double sup45 upf1 mutations on viability of corresponding strains we performed plasmid shuffle analysis using strains bearing single sup45 mutations or sup45 in combination with upf1Δ (see Materials and Methods).

Two nonsense mutations resulting in different stop codons (sup45-102 (UAA) and sup45-107 (UGA)) and one missense mutation (sup45-103) were used to perform plasmid shuffle experiments. Two isogenic yeast strains 1A-D1628 (sup45Δ pRS316/SUP45) and 1-1A-D1628 (sup45Δ upf1Δ pRS316/SUP45) were transformed with the pRS315 plasmids bearing the wild-type SUP45 gene or different sup45 mutations. Transformants were then subjected to plasmid shuffle analysis to verify whether strains containing the sup45 alleles could lose the plasmid carrying the wild-type gene. In the sup45Δ strain, all transformants were able to grow in the presence of 5-FOA, indicating that all tested mutations can replace wild-type SUP45. However, as previously described [18], plasmid shuffle was less efficient with SUP45 indicating that all tested mutations can replace wild-type SUP45. Surprisingly, introduction of upf1Δ mutation lead to increased viability of sup45 mutants (Fig. 3, 5-FOA). We did not observe difference in growth between wild-type and sup45 mutants on medium selective for both plasmids (Fig. 3, -L-U). In order to check that deletion of UPF1 does not lead to higher production of eRF1 protein in double sup45 upf1Δ mutants which could explain the increased viability of these mutants, we analyzed eRF1 protein level by western blot. As shown in figure 3B, deletion of UPF1 does not affect the level of eRF1 protein in sup45 mutants.

The same experiment was repeated using two other strains, 3b-D1658 (sup45Δ pRS316/SUP45) and 3v-D1658 (sup45Δ upf1Δ pRS316/SUP45). These strains were transformed with the pRS315 plasmids bearing the wild-type SUP45 gene or different sup45 mutations, Leu+ Ura+ transformants were patched on 5-FOA medium to select against pRS316/SUP45 plasmid (see Additional file 3, Fig. 3AB). The same results were obtained. Again, introduction of upf1Δ mutation leads to increased viability of sup45 mutants (see Additional file 3, Fig. 3AB). Western blot analyses showed that deletion of UPF1 did not affect levels of eRF1 and eRF3 proteins in sup45 mutants (see additional file 3 Fig. 3C). Thus, deletion of UPF1 confers selective survival advantages to sup45 nonsense mutants.

Deletion of the UPF1 gene suppresses several sup45 phenotypes

It is known that mutations in the SUP45 gene lead to suppression of nonsense mutations and also to many phenotypic changes including high or low temperature sensitivity, respiratory efficiency and sensitivity to aminoglycoside antibiotics such as paromomycin (reviewed in [2]). It has been previously reported that loss the UPF1 gene results in the suppression of some but not all of nonsense mutations. In addition, deletion of UPF1 does not confer sensitivity to paromomycin [7], an aminoglycoside antibiotic that induces translational misreading. We therefore compared phenotypes of single

![Figure 3](page number not for citation purposes)

**Figure 3**
Deletion of UPF1 gene leads to increased viability of sup45 nonsense mutants. (A). Strains 1A-D1628 (sup45Δ) and 1-1A-D1628 (sup45Δ upf1Δ) all containing SUP45 deletion and pRS316/SUP45 plasmid were transformed with pRS315/SUP45-LEU2 plasmids carrying different sup45 mutant alleles. The growth of the transformants was tested by plating 10^2, and 10^3 serial dilutions of overnight cultures (left to right) onto 5-FOA plates. The extent of cell growth on 5-FOA plates indicates the ability of the sup45 mutant alleles to support cell growth in the presence and absence of UPF1 gene. The same serially diluted cultures were also spotted on synthetic complete plates lacking leucine and uracil (-L -U) to estimate the total number of cells analyzed. (B). Level of eRF1 protein in the clones selected on 5-FOA medium was analyzed by western blot. Tubulin was used as a loading control. Following sup45 mutations were tested: 102, 107 (nonsense) and 103 (missense).
sup45 and double sup45 upf1Δ mutants, previously obtained by plasmid shuffle, for suppression efficiency, temperature sensitivity and sensitivity to paromomycin. As previously described [18,22], nonsense sup45-102 and sup45-107 and missense sup45-103 mutations are temperature sensitive. We observed that deletion of UPF1 suppressed temperature sensitivity of both sup45-102 and sup45-107 nonsense mutants in rich medium. However UPF1 deletion did not suppress temperature sensitivity of sup45-103 missense mutant (Fig. 4A). The observed disparity in effect of UPF1 deletion on temperature sensitivity of nonsense and missense mutants can be connected with different nature of temperature sensitivity in the case of decreased level of eRF1 compared with mutated eRF1. Loss of Upf1 also restored growth of all sup45 mutants on paromomycin media (Fig. 4B). In addition, deletion of UPF1 had an allosuppressor effect on suppression of ade1-14 mutation by sup45 mutations. However deletion of UPF1 alone, in the presence of wild-type copy of the SUP45 gene had no suppressor effect on ade1-14 mutation (Fig. 4C). On YPD medium, all transformants grew indicating that they retained their growth capacity on rich medium (Fig. 4D). Therefore the analysis of sup45 upf1Δ double mutants shows that loss of Upf1 not only affects viability of sup45 mutants but also suppresses several sup45 phenotypes.

**Defects of NMD in double mutants sup45 upf1Δ**

We have previously shown that sup45 mutants affect NMD and that UPF1 deletion suppresses several sup45 phenotypes. Therefore, we next examined if UPF1 deletion would have an additional effect on NMD in sup45 mutants. For this purpose, we compared accumulation of CYH2 precursor mRNA in single sup45 mutants and sup45 upf1Δ double mutants. To this end, we transformed strain 3v-D1658 (sup45Δ upf1Δ pRS315/SUP45) and its derivatives (sup45Δ upf1Δ pRS315/sup45-n) with plasmids pRS316 or pRS316/UPF1. In the presence of upf1Δ and wild-type SUP45, accumulation of CYH2 precursor increased by 4.6 fold. We found that the presence of sup45 mutations increased the ratio of preCYH2 to mature CYH2 mRNA by 1.9 to 2.3-fold (Fig. 5A,B), in agreement with results obtained previously in a different genetic background (Fig. 2A). Combination of sup45-n mutations and upf1Δ slightly but reproducibly increased the ratio of preCYH2 to mature CYH2 mRNA from 4.6 to 6.0 fold. Therefore, these results indicate that the combination of sup45-n and upf1 mutations together increased accumulation of CYH2 precursor more than either single one.

To compare the effects of single sup45 mutations and the sup45 upf1Δ double mutations on the efficiency of suppression, we replica plated the same transformants that were used for Northern blots analysis (Fig. 5A) on adenine deprived medium. As shown in another genetic background (Fig. 4C), deletion of UPF1 does not promote suppression of ade1-14 mutation (Fig. 5C) and the combined effects of sup45 mutations and upf1Δ promote increase in the suppression efficiency compared with sup45 mutations alone. As shown in figure 5D, allosuppression of ade1-14 by deletion of UPF1 is not the result of stabilization of ade1-14 mRNA in double sup45 upf1Δ mutants. We also showed that deletion of UPF1 does not affect sup45-n mRNAs and eRF1 as well as eRF3 protein levels (Fig. 5D).

**Figure 4**

Deletion of the UPF1 gene suppresses several sup45 phenotypes. The growth of the transformants of two isogenic strains, 1A-D1628 (sup45Δ) and 1-IA-D1628 (sup45Δ upf1Δ), selected on 5-FOA medium (Fig. 3A) was tested by plating 10⁰, 10¹ serial dilutions of overnight cultures (left to right) onto YPD plates at 37°C (A), onto YPD plates with 1 mg/ml paromomycin (B) and onto synthetic complete -adenine -leucine plates (C). The same serially diluted cultures were also spotted on YPD plates at 25°C (D) to estimate the total number of cells analyzed. Following sup45 mutations were tested: 102, 107 (nonsense) and 103 (missense).
Deletion of either UPF2 or UPF3 increases viability of sup45 nonsense mutants

It has been described that functional NMD requires not only Upf1 but also Upf2 and Upf3 [7,9-11,23-25]. To test whether UPF2 or UPF3 deletion will have the same effect on viability of sup45 nonsense mutants as deletion of UPF1, we transformed strains 4a-D1659 (sup45Δ), 4b-D1659 (sup45Δ upf2Δ), 18a-D1660 (sup45Δ) and 3a-D1660 (sup45Δ upf3Δ), all containing pRS316/SUP45 [URA3] plasmid, with pRS315/sup45-n [LEU2] plasmids carrying sup45-102 or sup45-107 mutant alleles. Leu+ Ura+ transformants were selected and plated on leucine deprived medium containing 5-FOA to select against the pRS316/SUP45 [URA3] plasmid. Combining either UPF2 or UPF3 deletion with sup45 mutations results in an enhanced viability of double mutants (Fig. 6AB). Western blot analysis has shown, as in case of UPF1 deletion, that the level of eRF1 and eRF3 proteins was not dependent on the presence of functional UPF2 or UPF3 genes (Fig. 6CD). These results demonstrate that deletion of UPF2 or UPF3 as well as deletion of UPF1, all essential components of NMD pathway, leads to increase viability of sup45-n mutant strains.

Discussion

In the present work, we have shown that nonsense and missense mutations in the SUP45 gene lead to stabilization of PTC-containing mRNAs degraded by NMD. This is the first demonstration that sup45 mutations do not only change translation fidelity but also acts by causing a change in mRNA stability.

The CYH2 pre-mRNA which contains a premature termination codon was previously shown to be degraded by NMD pathway [21]. We also identified that NMD affects accumulation of his7-1 mRNA. A single A→T mutation in this allele leads to change of codon 229 for UAA. In addition, two imperfect putative DSE are found downstream
of this premature stop codon. Using a upf1Δ strain, we demonstrated that the his7-1 transcript is possibly under control of NMD pathway. In order to answer if changes in transcription of HIS7 gene could account for accumulation of his7-1 mRNA, we examined the mRNA level of wild-type HIS7 mRNA in sup45-n mutants and upf1Δ mutants. Deletion of upf1 as well as sup45 mutations leads to accumulation of his7-1 mRNA but do not affect mRNA level of wild-type HIS7 mRNA. Accordingly, a genome-wide analysis performed in strains depleted for NMD showed that wild-type HIS7 mRNA (and also ADE1 and LYS9 mRNAs) is not affected in strains deleted for upf1 [26].

Here, we demonstrate that accumulation of his7-1 and CYH2 precursor mRNAs in cells bearing sup45 mutations was much higher than that observed in the wild-type strain. But, sup45 mutations do not promote accumulation of other nonsense-containing transcripts, such as ade1-14 or lys9-A21, despite efficient suppression of these mutations, as well as his7-1 [18,22]. This result indicates that simply increasing read-through efficiency does not result in a general increase in the abundance of PTC-containing mRNAs but that sup45 mutations specifically affect PTC-containing mRNA subjected to NMD. We observed that the abundance of his7-1 and CYH2 precursor mRNAs in cells bearing sup45 mutations was lower compared to those in the upf1Δ strain. This difference between upf1Δ and sup45 mutants could be explained by the complete absence of Upf1 protein in the upf1Δ strain leading to complete inactivation of NMD and by the presence of some functional eRF1 protein in sup45 mutants which is necessary for cell viability [18,22]. Indeed, we previously reported that in the case of sup45-n, the level of eRF1 is decreased compared to wild-type and in the case of sup45 nonsense mutants the level of eRF1 is unchanged but its functionality is altered. These results demonstrate that eRF1 participates in NMD.

Recently, it was shown the importance of the second translation termination factor (eRF3) for NMD and interaction of both eRF1 and eRF3 with Upf proteins. Upf1 protein interacts with the polypeptide release factors eRF3 and eRF1 while they are still present in the ribosome-bound termination complex, providing a direct link between the termination complex and the NMD machinery [12,13]. Both Upf2 and Upf3 interact with eRF3, but not with eRF1; and Upf2, Upf3 and eRF1 compete with each other in vitro for binding to eRF3 [12,13]. eRF3 also interacts with poly(A)-binding protein (PABP) [27,28], furthermore, eRF3 regulates the initiation of normal mRNA decay at the poly(A) tail-shortening step through the interaction with PABP [29]. Thus, eRF3 can mediate normal and nonsense-mediated mRNA decay through its
association with Pab1 and Upf1 and therefore was proposed as a key mediator between translation termination and NMD [16]. Moreover, it was previously shown that a weak translation termination due to [PSI+] (a prion form of eRF3) antagonizes the effects of NMD [30]. A first indication for a link between translation termination and NMD came from observations that decay of PTC-containing mRNAs can be antagonized by tRNAs that suppress termination [31]. Data have shown that normal termination is distinct from premature termination and this difference is dependent upon the presence of Upf1 at the premature termination codon [32]. Our results together with data about eRF1-Upf1 interaction [12,13] demonstrate that eRF1 as well as eRF3 is an essential factor linking translation termination and NMD. Recognition of stop codons is a common event necessary for the two processes. Since it is established that eRF1 plays a crucial role in translation termination by directly recognizing stop codons (reviewed in [2]), eRF1 could have an identical function in NMD by recognition of PTC.

We observed that the combination of upf1Δ and sup45-n mutations leads to an increase in CYH2 precursor mRNA abundance that was higher than in upf1Δ and sup45-n single mutants. A similar additive effect on stabilization of nonsense-containing mRNA was shown for combination of upf1Δ and [PSI+] [30]. Therefore, a possible explanation for this additive effect of upf1Δ and sup45-n mutations could be that eRF1 is required for both normal and nonsense-mediated mRNA decay, as it was shown for eRF3 [16].

It has been shown that mutation in eRF3 which impairs eRF3 binding to eRF1 affected mRNA decay [16]. In the present paper, we show that the missense mutation sup45-103 (L21S) alters degradation of PTC-containing mRNAs by NMD. However, we have previously shown that this mutation does not affect the eRF1-eRF3 interaction [22], indicating that this allele has an inhibitory effect on NMD that is independent on eRF1-eRF3 binding. This result demonstrates that eRF1 mutation affecting PTC-containing mRNA decay by NMD does not obligatory alters the eRF1-eRF3 interaction.

A role for the Upf1 protein, essential for NMD, in translation termination first became evident when a set of mutations were isolated in the UPF1 gene that separated the mRNA decay function from its activity in modulating premature termination [33,34]. Subsequent studies have shown that deletion of either UPF2 or UPF3 can also lead to a nonsense suppression phenotype [7,9,11,13,35]. In addition, it was shown that upf1Δ mutation causes a general decrease in the efficiency of translation termination at UAG, UAA, and UGA stop codons [30].

In this work, we have shown that deletion of UPF1 does not affect ade1-14 mRNA level, but results in allosuppression of ade1-14 mutation in sup45 nonsense mutants therefore revealing that deletion of UPF1 has a synergistic effect with sup45-n mutants. Similar allosuppressor effect has also been shown for deletion of UPF1 in combination with [PSI+] [30,36]. Based on this additive effect, Keeling et al. [30] proposed that upf1Δ mutation and [PSI+] influence the termination process in distinct ways. Our results suggest that this could be also the case for upf1Δ and sup45 mutations.

We found that deletion of the UPF1 gene affects several other sup45 phenotypes, such as temperature sensitivity, paromomycin sensitivity and viability of sup45 mutants. It is known that deletion of UPF1 gene in yeast does not cause any detectable phenotypic effects except respiratory deficiency [37] and nonsense suppression [7,9,13,33-35]. Also telomere length is affected by deletions of UPF1-3 genes [38]. How UPF1 deletion could affect sup45 phenotypes? We can not exclude an indirect effect of UPF1 deletion on sup45 phenotypes. It has been reported that NMD controls the mRNA levels of several hundred of wild-type genes [24,26]. One can hypothesize that depletion of Upf1 could affect the expression of some translation apparatus components (e.g. tRNA genes) which themselves influence the viability of sup45 mutants. Indeed, the presence of SUQ5 mutation, a mutant suppressor tRNA<sup>sup45<sup> strand, increases the viability of sup45-n mutants [18]. Alternatively, since inactivation of the NMD pathway by upf1Δ mutation does not increase the steady-state levels of wild-type and mutant SUP45 mRNAs and does not cause a change in the amount of eRF1 protein, we propose that the effect of NMD on sup45 phenotypes is probably via a change in the stoichiometry of factors involved in translation termination and NMD. In contrast to mammals, Upf proteins of S. cerevisiae are present at very low intracellular concentrations [39]. Considering that in sup45-102 and sup45-107 nonsense mutants the amount of eRF1 was estimated as 8% and 17% of wild-type level, respectively [18], in mutant cells eRF1 and Upf1 proteins are probably present in stoichiometric amounts. Possibly, in wild-type cells, Upf1 is not preventing normal termination because its amount is ten times lower than eRF1, but in the case of their presence in stoichiometric amounts in sup45 nonsense mutants binding of Upf1 to eRF1 could result in a defective complex formation that blocks termination. This hypothesis is supported by finding that viability of sup45 nonsense mutants depends also on Upf2 or Upf3 proteins. There is a possibility that effect of Upf2 or Upf3 depletion is indirect and is under control of Upf1. It was shown that in mammalian cells a depletion of Upf2 or Upf3 reduces the amount of the phosphorylated form of Upf1 possibly preventing Upf1 dissociation from eRF3 and eRF1 [40]. Phosphorylation of Upf1 and Upf2 was
also shown in *S. cerevisiae* [41,42], an indication that this mechanism might operate in yeast cells as well.

From recent studies, it appears more and more clear that translation termination and mRNA stability are intimately linked and our results demonstrate that eRF1 is also an essential factor linking these two processes.

**Conclusion**

In the present work, we have shown that nonsense and missense mutations in *SUP45* gene lead to stabilization of *CYH2*, a PTC-containing pre-mRNA degraded by NMD, and to accumulation of *his7-1* mRNA. At the same time, *sup45* mutations do not promote accumulation of other nonsense-containing transcripts, such as *ade1-14* or *lys9-A21*, despite efficient suppression of these mutations. Thus *sup45* mutations specifically affect PTC-containing mRNA subjected to NMD. Deletion of *UPF1* results in allosuppression of *ade1-14* mutation in *sup45* nonsense mutants and leads to an increase in *CYH2* pre-mRNA abundance therefore revealing that deletion of *UPF1* has a synergistic effect with *sup45*-n mutants. This is the first demonstration that *sup45* mutations do not only change translation fidelity but also acts by causing a change in mRNA stability.

Models explaining increased viability of *sup45* nonsense mutants in the absence of Upf1, Upf2 or Upf3 proteins are proposed. First, the depletion of Upf1 could affect the expression of some translation apparatus components (e.g. tRNA genes) which themselves influence the viability of *sup45* mutants. Second, a change in the stoichiometry of factors involved in translation termination and NMD provides the effect of NMD on *sup45* phenotypes.

**Methods**

**Yeast strains, plasmids and growth conditions**

The *S. cerevisiae* strains used in this study are listed in Table 2. Previously characterized *sup45* mutations [18,22] were used in this study, among them the following nonsense mutations (*sup45*-n): *sup45*-102 (53 Tyr→TAA); *sup45*-104 (283 Leu→TAA); *sup45*-105 (385 Glu→TAA); *sup45*-107 (317 Leu→TGA); as well as missense mutation (*sup45*-m): *sup45*-103 (Leu21Ser). All *sup45* mutations were selected by spontaneous reversions of two nonsense mutations, *his7-1* and *lys9-A21* in strain 1B-D1606. Strains Y06214, Y01905, Y04702 are from Euroscarf; other strains were obtained during this study. Strain 5B-D1645 was recovered from the meiotic progeny of D1645, obtained by mating Y06214 and 16A-D1608. Strains D1658, D1659, D1660 were generated by mating of strains Y06214, Y01905, Y04702 with 1A-D1628. Tetrads were dissected and segregants were used for further study. Yeast strain 1-1A-D1628 was generated by using a one-step gene replacement method. The *UPF1* gene was deleted by the removal of the entire open reading frame and the insertion of the kanMX gene by using PCR-based gene deletion approach [43] with plasmid pFA6a-kanMX. The following primers were used for PCR: F1 (AATATACITTTTATAATTACATCAATCTATTGTCATTACCAACGCCATCCCGGGTTATAA) and R1 (AGGCAAGTTTACCTTTGAACACCGTCGACCGAAGATCAGGGAGCCCGAAGTCGTTTAAA). Yeast strain 1A-D1628 was transformed with the fragment generated by PCR. KanR transformants were screened by PCR. Yeast strains were grown either in standard rich or synthetic culture medium [44] at 25°C. Transformants were grown in the media selective for plasmid maintenance (SC-Trp, SC-Leu, SC-Ura). Suppression of nonsense mutations was estimated by growth at 25°C on synthetic medium lacking the corresponding amino acids. For plasmid shuffle, selective medium containing 1 mg/ml 5-fluoroorotic acid (5-FOA, Sigma) was used. Yeast transformation was performed as described [45]. Plasmid pRS316/UPF1 contains *UPF1* gene under its own promoter [46].

**Plasmid shuffle**

The haploid *SUP45::HIS3 [CEN URA3 SUP45]* and *SUP45::HIS3 UPF::kanMX4 [CEN URA3 SUP45]* strains were used in “plasmid shuffle”. These strains were transformed with [CEN LEU2 sup45] plasmids. Transformants, selected on -Ura-Leu medium, were velveteen replica plated onto 5-FOA medium, which counterselects against *URA3* plasmids [47]. Growth was also assayed using serial dilutions of overnight cultures with OD<sub>600</sub> = 1. Serially (10-fold) diluted yeast cell cultures were spotted on plates containing 5-FOA to determine the ability of the *sup45* mutant alleles to support cell growth in the presence and absence of any one of three *UPF* genes. The wild-type yeast *SUP45* gene carried on the *URA3* plasmid eliminates 5-FOA is toxic to cells expressing the *URA3* gene. The same serially diluted cultures were also spotted on plates lacking leucine and uracil to estimate the total number of cells analyzed.

**Sequencing of the alleles his7-1, lys9-A21 and trp1-289**

Yeast DNA was prepared using genomic DNA purification Kit (Promega). DNA fragments, corresponding to ORFs were amplified with the following primers:

*HIS7* (202 GGCAGCTATTGAAGTTGACGATCCACCG and 203 CCCTACTGACACCACCAATAACTCAACC),

*LYS9* (192 CAGCAATATAGATAGTACAAGGAGCTACGACAG and 193 CAAGCTTACCGAACCACCGTC),

*TRP1* (188 GAGGGAGGCATGGTGGCTTATTG and
189 GCACAACAAATACATAAATAAATACACTTC.

For each allele at least two independent PCR-products were sequenced using the following primers:

**HIS7** (195 GATGTCAGTACTAATGACCAAGGTG and 196 GTTACITTCATCCGCACCCTGTGGG),

**LYS9** (197 GCTAAATACAGAAAGCCGGAAG and 198 GATATCCAAATCTCAGTCTATGACCCG),

**TRP1** (177 GTCTGTTAATATTCAGGAGTTGTT).

**Analysis of mRNA steady-state levels**

Total RNA was prepared by hot-phenol extraction method from yeast culture grown in YPD medium to log phase OD_{600} = 0.5-0.8 as described [44]. For each RNA sample, 3% formaldehyde and transferred to nylon membrane, Z-probe (Bio-Rad), S1P45, HIS7, CYH2 and ACT1 transcripts were detected using gene-specific 32P-radio labelled DNA probes. Radioactive signals were detected using alkaline-phosphatase-coupled anti-mouse immunoglobulin G secondary antibodies (Jackson) (for tubulin) by Amersham ECF system (Amersham Pharmacia Biotech). Signals were quantified with STORM 840 Phosphor-Imager (Molecular dynamics, USA) and ImageQuant NT 5.2 software.

**Authors' contributions**

SC, carried out the molecular genetic studies and drafted the manuscript, VG, constructed all strains with UPF gene deletions, SM and CLG participated in the molecular genetic studies, GZ, designed the study, performed plasmid shuffle analysis and wrote the manuscript. All authors read and approved the final manuscript.

**Additional material**

### Additional file 1

**A. Deletion of UPF1 and mutation of SUP45 do not affect the wild-type HIS7 mRNA level.** Total RNA was isolated from strain 3v-D1658 (SUP45 and sup45) and its derivative (SUP45 upf1Δ) transformed with pRS316 and pRS316/UPF1. Blots were hybridized with DNA probes that detected the wild-type HIS7 and ACT1 transcripts (ACT1 was used as a loading control). The fold increase in HIS7/ACT1 mRNA accumulation relative to such in wild-type strain is shown. The HIS7/ACT1 ratio in wild-type strain was set as 1.0.

**B. Deletion of UPF1 does not suppress his7-1 mutation.** Strain 5B-D1645 (his7-1 upf1Δ) transformed with plasmids pRS316 and pRS316/UPF1, designated as (UPF1-) and (UPF1 +), respectively. The growth of the transformants was tested by plating 10^6, and 10^7 serial dilutions of overnight cultures on a synthetic complete medium without histidine (−HIS). This strain bears his7-1 mutation and is unable to grow on such medium except if his7-1 mutation is suppressed. Strain 1B-D1606 sup45-107 (UPF1 sup45-107) was used as a control of effective suppression of his7-1 mutation (as already reported [18]). sup45Δ strain did not grow on synthetic complete medium without histidine, demonstrating that there is no suppression of his7-1 in upf1Δ strain.

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### Additional file 2

**Nonsense or missense sup45 mutations do not affect the steady-state levels of nonsense-containing lys9-A21 and ade1-14 mRNAs.** Northern blots were prepared with total RNA from wild-type strain 1B-D1606 (SUP45) and its sup45 mutant derivatives (bearing missense sup45-103 or nonsense sup45-104 mutations). Blots were hybridized with DNA probes that detected the lys9-A21, ade1-14 and ACT1 transcripts (ACT1 was used as a control). The fold increase in lys9-A21/ACT1 (upper panel) and ade1-14/ACT1 (lower panel) mRNA accumulation relative to such in wild-type strain are shown. The lys9-A21/ACT1 and ade1-14/ACT1 ratio in wild-type strain was set as 1.0.

Click here for file [http://www.biomedcentral.com/content/supplementary/1471-2199-8-71-S2.jpeg](http://www.biomedcentral.com/content/supplementary/1471-2199-8-71-S2.jpeg)
Deletion of UPF1 gene leads to increased viability of sup45 nonsense mutants. Strains 3b-D1658 (sup45a pRS316/SUP45) and 3c-D1658 (sup45a upf1Δ pRS316/SUP45) all containing SUP45 deletion and pRS316/SUP45-URA3 plasmid were transformed with pRS315/SUP45-LEU2 plasmids carrying different sup45 mutant alleles. Following sup45 mutations were tested: 101, 102, 104, 105, 107 (nonsense) and 103 (missense). The growth of the transformants was tested by patching onto 5-FOA plates (A) or by plating 10³ and 10⁴ serial dilutions of overnight cultures (left to right) onto 5-FOA plates (B). The extent of cell growth on 5-FOA plates indicates the ability of the sup45 mutant alleles to support cell growth in the presence and absence of UPF1 gene. The same serially diluted cultures were also spotted on synthetic complete plates lacking leucine and uracil to estimate the total number of cells analyzed. (C) eRF1 and eRF3 protein levels in the clones selected on 5-FOA medium were analyzed by western blot. (*) indicates a non-specific band used as loading control.

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