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# Nonsense Suppression in Yeast Cells Overproducing Sup35 (eRF3) Is Caused by Its Non-heritable Amyloids*

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**The [*PSI*$^+$] prion determinant of *Saccharomyces cerevisiae* causes nonsense suppressor phenotype due to a reduced function of the translation termination factor Sup35 (eRF3) polymerized into amyloid fibrils. Prion state of the Rnq1 protein, [*PIN*$^+$], is required for the [*PSI*$^+$] *de novo* generation but not propagation. Yeast [*psi*$^-$] [*PIN*$^+$] cells overproducing Sup35 can exhibit nonsense suppression without generation of a stable [*PSI*$^+$]. Here, we show that in such cells, most of Sup35 represents amyloid polymers, although the remaining Sup35 monomer is sufficient for normal translation termination. The presence of these polymers strictly depends on [*PIN*$^+$], suggesting that their maintenance relies on efficient generation *de novo* rather than inheritance. Sup35 polymers contain Rnq1, confirming a hypothesis that Rnq1 polymers seed Sup35 polymerization. About 10% of cells overproducing Sup35 form colonies on medium selective for suppression, which suggests that the proportion of Sup35 monomers to polymers varies between cells of transformants, allowing selection of cells deficient for soluble Sup35. A hybrid Sup35 with the N-terminal domain replaced for 66 glutamine residues also polymerizes and can cause nonsense suppression when overproduced. The described polymers of these proteins differ from the [*PSI*$^+$] polymers by poor heritability and very high frequency of the *de novo* appearance, thus being more similar to amyloids than to prions.**

The Sup35 protein of *Saccharomyces cerevisiae* belongs to the family of eRF3, one of the two key factors required for the termination of translation in eukaryotes (1). This protein has a three-domain structure, but only its C-terminal part (C domain) of 432 amino acids is responsible for the function in translation termination and conserved (2). The N-terminal (N) Sup35 domain of 123 amino acids allows protein to switch into the aggregated prion-like state. This aggregation reduces the efficiency of translation termination, which manifests as nonsense-suppressor cytoplasmically inherited determinant [*PSI*$^+$]. The middle domain (M, residues 124–253) is inessential for [*PSI*$^+$], although it may be important for its stability (3) and is involved in the [*PSI*$^+$] strain variation (4). Reduction of the Sup35 function due to its mutations or underproduction also causes nonsense suppression. Surprisingly, overproduction of Sup35 may have the same effect. Transient overproduction of Sup35 can cause its rare conversion into the prion form, which could be revealed by the appearance of a small proportion of cells carrying the nonsense suppressor [*PSI*$^+$] determinants, which are then able to propagate at the normal levels of Sup35 (5). Continuous Sup35 overproduction causes frequent appearance of cells with the suppressor phenotype without generation of [*PSI*$^+$]. Such cells differ from the [*PSI*$^+$]-carrying cells since their suppressor phenotype is manifested only at increased Sup35 levels (6). The molecular basis of such "multicopy" suppression is not clear, although it appears to be related to [*PSI*$^+$] since the suppressor effect of Sup35 overdose depends both on the presence of Sup35 prion-forming domain (2) and on the prion form of the Rnq1 protein ([*PIN*$^+$] determinant), which allows induction of [*PSI*$^+$] upon Sup35 overproduction (7), probably by seeding its polymerization (8).

Here, we show that in [*psi*$^-$] [*PIN*$^+$] cells, overproduced Sup35 is present mostly as amyloid aggregates, which differ from the Sup35 prion amyloids by much higher frequency of appearance and lower efficiency of Sup35 polymerization. Due to this, such cells show normal nonsense codon readthrough but easily develop the suppressor phenotype upon selective pressure. Similar properties were observed for the hybrid Sup35, in which the N domain was replaced for a sequence of 66 glutamine residues. Thus, yeast Sup35 can form non-heritable amyloid structures, which may have phenotypic manifestation.

## MATERIALS AND METHODS

*Strains and Genetic Methods*—In most cases, the 74-D694 (MAT**a** *ura3–52 leu2–3,112 trp1–289 his3-Δ200 ade1–14*) strain (9) and its derivative 74-D694/Q66 (obtained by replacing *SUP35* for the chimerical *SUP35-Q66* allele encoding glutamines 1–66 instead of amino acids 1–120 of Sup35 under control of *MET17* promoter, see below) were used. Since the *ade1–14* UGA mutation carried by this strain caused accumulation of red pigment, its suppression (suppressor phenotype, Sup$^+$) could be easily detected by the appearance of colonies with lighter color (white or pink, depending on the suppression efficiency). The 5V-H19 (MAT**a** *ura3–52 leu2–3,112 ade2–1 SUQ5*) and 1–5V-H19 (same, but with the *SUP35-C* allele, which lacks a region encoding the N and M domains of Sup35) strains were also used in some experiments (10). Yeast were grown at 30 °C in rich (YPD, 10 g of yeast extract, 20 g of peptone, 20 g of glucose/liter) or synthetic (6.7 g of yeast nitrogen base, 20 g of glucose supplemented with the required amino acids) medium. Selective media lacking leucine, uracil, or adenine are designated as −Leu, −Ura, or −Ade, respectively. To select cells that have lost *URA3* plasmids, corresponding transformants were grown on 5-fluoroorotic acid medium (11). To cure cells of the [*PSI*$^+$] determinants, corresponding strains were grown from single cells to colonies on medium containing 3 mM guanidine hydrochloride (12).

*Plasmids*—YEplac181-SUP35 was constructed by inserting the XhoI-XbaI fragment of *SUP35* into the SalI and XbaI sites of YEplac181. The HpaI-XbaI fragment of YEplac181-SUP35 encoding the

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Sup35 C domain was replaced for the *eGFP* gene as SmaI-XbaI fragment from peGFP (Clontech). The plasmid yex4Sup35NM6His was obtained from yex4-SUP35 (2) by removing the ClaI fragment encoding *LEU2* and replacing the BclI-XbaI fragment encoding the Sup35 C domain with oligonucleotides encoding 6 histidines. yex4MetQ66MC35 also derives from yex4-SUP35. The *SUP35* promoter starting from the MluI site (position −383) was replaced by the PCR-amplified *MET17* promoter starting from position −429. The expressed open reading frame encodes (MPSHFGGSETS, the first amino acids of Met17)-Met-(Glu, 66 residues)-(Sup35 starting from serine 121). To replace the chromosomal *SUP35* gene for the *SUP35-Q66* allele, the 74-D694 [*PSI*$^+$] strain was co-transformed with the XhoI-XbaI fragment of yex4MetQ66MC35 and the YEplac181 vector. The transformants with replaced *SUP35* gene were distinguished by red colony color (since this eliminated [*PSI*$^+$]) and then checked by immunoblotting for the presence of altered Sup35 protein. In this strain, the *MET17* promoter provided expression of the hybrid protein at a level slightly exceeding that of the native *SUP35* promoter (data not shown). Plasmid yex4MetQ66MC35ΔS was obtained from yex4MetQ66MC35 by deletion of SalI fragment encoding amino acids 483–685 of Sup35. To obtain pBC-RNQ1, the *RNQ1* gene, including 487 bp upstream and 186 bp downstream of it, was amplified from yeast genomic DNA and inserted into pBC KS+ by XhoI and EclI36II sites. To disrupt *RNQ1*, the plasmid pBC-rnq1::HIS3 was constructed by inserting the NsiI-BamHI fragment from pBC-HIS3 (BamHI genomic fragment, containing the *HIS3* gene, was inserted into the BamHI site of pBC KS+) into the pBC-RNQ1 plasmid cut by NsiI and BglII. Yeast cells containing Sup35 or Q66-Sup35MC aggregates were transformed by the EcoRV-SnaBI fragment of the pBC-rnq1::HIS3 plasmid carrying the *RNQ1* gene disrupted by *HIS3*. *RNQ1* disruption events were confirmed by immunoblot analysis of cell lysates for Rnq1.

*Preparation and Analysis of Yeast Cell Lysates*—Yeast cultures were grown in liquid media to an $A_{600}$ of 1.5. The cells were harvested, washed in water, and lysed by glass beads in buffer A: 25 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM dithiothreitol. To prevent proteolytic degradation, 10 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and Complete™ protease inhibitor mixture (Roche Applied Science) were added. Cell debris was removed by centrifugation at 10,000 × *g* for 3 min. To equalize the amount of protein in electrophoretic samples, the protein concentration was determined according to Ref. 13. To determine the size distribution of Sup35, the cell lysates were fractionated by centrifugation through 20% sucrose cushion in the SW-50 rotor (Beckman) for 30 min at 35,000 rpm (170,000 × *g*). The *in vitro* conversion of Sup35 into prion-like aggregated form was performed according to Ref. 14. To isolate the Sup35 amyloid-like polymers, the 1–5V-H19 strain was transformed with multicopy plasmids YEplac181-SUP35 and yex4Sup35NM6His and grown in 2 liters of synthetic media. Lysates were obtained as described above and centrifuged in Ti-75 rotor (Beckman) at 40,000 rpm for 2 h. The pellets were dissolved in buffer A containing 2% SDS and centrifuged through 1 ml of 0.1% SDS 30% sucrose cushion in Ti-75 for 10 h at 45,000 rpm, 13 °C. The top of the pellet, containing GFP,[1] was dissolved in buffer A containing 0.05% SDS and incubated with Talon resin (Clontech) overnight at 13 °C. Then the Talon beads were washed with the same buffer, eluted with 50 mM EDTA, and analyzed by Western blotting for Sup35 and Rnq1. The developed blots were scanned and quantified to determine the proportions between Rnq1 and Sup35.

*Electrophoresis*—Protein electrophoresis in 9% polyacrylamide gels was performed according to Ref. 15 except that, where indicated, boiling of the samples was avoided, and agarose was used for the concentrating gel. For separation of prion particles, we used horizontal 1.8% agarose gels in the Tris-acetate-EDTA buffer (16) with 0.1% SDS. Samples were incubated in the sample buffer (0.5× Tris-acetate-EDTA, 2% SDS, 5% glycerol, and 0.05% bromphenol blue) for 5 min at 37 °C (17). After the electrophoresis, proteins were transferred from gels to Immobilon-P polyvinylidene difluoride sheets (Millipore) by vacuum blotting overnight (agarose gels) or electrophoretically (acrylamide gels). Bound antibody was detected using the Amersham Biosciences ECL system.

*Determination of the Nonsense Readthrough*—The levels of nonsense codon readthrough were determined as a ratio of β-galactosidase activity in cells transformed with pUKC817, -818, and -819 plasmids to that in cells with pUKC815 as described previously (1). The 74-D694 [*psi*$^-$] [*PIN*$^+$] strain was transformed with *LEU2* multicopy vectors lacking or containing *SUP35* and with the *URA3* pUKC plasmids. Transformants were incubated overnight in liquid −Ura −Leu medium either containing or lacking adenine. When necessary, Sup$^+$ cells grown in the latter medium were transferred to −Ura −Leu medium containing adenine and allowed to grow for about 20 generations.

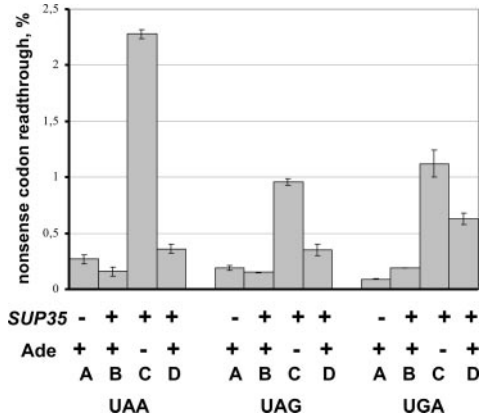


FIG. 1. **The efficiency of nonsense codon readthrough in cells overproducing Sup35.** In the *SUP35* row, + indicates extra copies of *SUP35* (multicopy YEplac181-SUP35 plasmid), and − indicates no extra copies of *SUP35* (empty vector YEplac181). In the *Ade* row, + and − indicate that the medium contained or lacked adenine, respectively. The strain 74-D694 [*psi*$^-$] [*PIN*$^+$] contained the pUKC815, pUKC817, pUKC818, or pUKC819 *URA3* plasmids in combinations with either one of the *LEU2* plasmids YEplac181 or YEplac181-SUP35. *A* and *B*, transformants with YEplac181 or YEplac181-SUP35, respectively, incubated in −Ura −Leu medium. *C*, transformant with YEplac181-SUP35 grown in −Ura −Leu −Ade medium. *D*, the latter cells returned to −Ura −Leu medium containing adenine. The levels of readthrough of UAA, UAG, or UGA nonsense codons were determined as described under "Materials and Methods." Values are the mean of three independent assays ± S.D.

## RESULTS

*Most of Sup35 Overproduced in [psi*$^-$*] [PIN*$^+$*] Cells Is Found in Amyloid Form*—To analyze the mechanism of *SUP35* multicopy suppression, the strain 74-D694 [*psi*$^-$] [*PIN*$^+$] was transformed with the multicopy plasmids YEp181-SUP35 or YEp181-SUP35NM-GFP expressing Sup35 or Sup35NM-GFP, respectively, and transformants were selected on −Leu medium. The transformants were red, indicating the absence of suppression of *ade1–14* UGA mutation. Quantitative assay confirmed that nonsense codon readthrough in these cells was not increased (Fig. 1). Surprisingly, the analysis of Sup35 by electrophoresis in SDS-agarose gel (SDD-AGE (17)) revealed the presence of a significant proportion of polymerized Sup35 (Fig. 2) and Sup35NM-GFP (data not shown). These polymers should be of amyloid nature since insolubility in SDS at room temperature represents a characteristic property of Sup35 amyloids obtained *in vitro* (18) and Sup35 prion particles from yeast lysates (17). Analysis of Sup35 sedimentation and Sup35NM-GFP fluorescence (data not shown) also confirmed their aggregated state. Another characteristic property of amyloids and prions is the ability to seed polymerization of the same protein. Sup35NM-GFP aggregates from the transformants overproducing this protein could seed the aggregation of wild type Sup35 *in vitro*, although such aggregation proceeded considerably less efficiently than the aggregation seeded by the [*PSI*$^+$] Sup35 aggregates (Fig. 2). The aggregation of Sup35 *in vivo* depended on the presence of [*PIN*$^+$] determinant and was not observed in the [*psi*$^-$] [*pin*$^-$] cells. Furthermore, [*PIN*$^+$] elimination via disruption of *RNQ1* in the [*psi*$^-$] [*PIN*$^+$] cells possessing these aggregates caused the disappearance of the aggregates (Fig. 2). [*PIN*$^+$] is known to be required for the *de novo* [*PSI*$^+$] generation but not for its propagation (7). Thus, in contrast to [*PSI*$^+$], maintenance of these aggregates depended on [*PIN*$^+$], which suggests that they were inherited poorly at

[1] The abbreviations used are: GFP, green fluorescent protein; SDD-AGE, semidenaturing detergent-agarose gel electrophoresis.

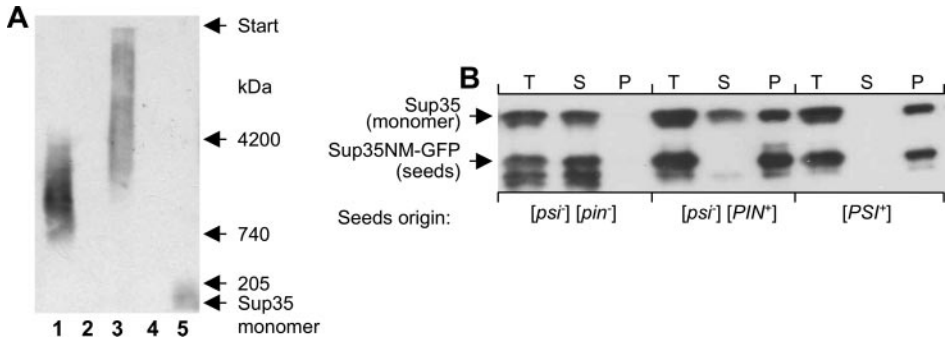


FIG. 2. **Overproduced Sup35 forms SDS-insoluble polymers able to seed Sup35 polymerization *in vitro*.** *A*, analysis of the Sup35 polymer state. Cell lysates of the strain 74-D694 were analyzed by SDD-AGE and immunostained for Sup35. *Lane 1*, [*PSI*$^-$] cells; *lane 2*, [*psi*$^-$] [*PIN*$^+$] cells with empty vector YEplac181; *lanes 3–5*, transformants of the cells with following the genotypes bearing the multicopy plasmid YEplac181-SUP35: *3*, [*psi*$^-$] [*PIN*$^+$]; *4*, [*psi*$^-$] [*pin*$^-$]; *5*, [*psi*$^-$] [*PIN*$^+$] with *RNQ1* being disrupted after transformation. Molecular mass standards are as follows: titin (4200 kDa), nebulin (740 kDa), and myosin heavy chain (205 kDa). *B*, *in vitro* polymerization of Sup35 caused by Sup35NM-GFP seeds. Lysates of the 5V-H19 [*psi*$^-$] [*pin*$^-$] strain were mixed with lysates of 1–5V-H19 cells overproducing Sup35NM-GFP and having the indicated prion status (see "Materials and Methods"). The reactions were incubated for 2 h, fractionated by centrifugation, and analyzed by immunoblotting for Sup35. Centrifugation fractions are as follows: *T*, total mixed lysates; *S*, supernatant; *P*, pellet.

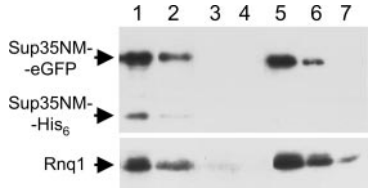


FIG. 3. **Rnq1 is present in Sup35NM polymers.** *Lane* 1, Sup35NM polymers isolated via $His_6$ affinity tag in the presence of SDS; *lane* 2, same as *lane 1* but ⅕ load; *lanes* 3 and *4*, control (preparations were made similarly to *lanes 1* and *2*, but Sup35NM polymers lacked $His_6$ tag); *lanes* 5–7, sequential 5-fold dilutions of a total protein extract of the cells used.

cell divisions but were constantly generated *de novo*.

The levels of total and soluble Sup35 in the [*psi*$^-$] [*PIN*$^+$] strain overproducing this protein exceeded the total Sup35 levels in the non-transformed [*psi*$^-$] strain 20- and 3-fold, respectively (data not shown). Thus, 15% (3⁄20) of Sup35 in transformant cells was soluble and 85% polymerized. A minimum of 85% cells is required to accommodate this amount of polymers, basing this on the assumption that these cells contain no Sup35 monomers, whereas others contain no polymers. However, the lack of suppressor phenotype suggests that all cells contained some amount of soluble Sup35 and, therefore, Sup35 polymers were present in more than 85% of cells, probably in almost all cells.

*The Sup35 Polymers Contain Rnq1*—The dependence of the Sup35 polymerization on [*PIN*$^+$] suggested, in agreement with the earlier data (8), that the Sup35 polymerization is seeded by Rnq1 polymers. To confirm this idea, we tried to find Rnq1 in the Sup35 polymers. For this, the strain 1–5V-H19 [*PIN*$^+$] (*SUP35C*) was transformed with multicopy plasmids encoding Sup35NM-GFP and Sup35NM-$His_6$. Control transformant expressed only Sup35NM-GFP. The amyloid polymer fraction was isolated as described under "Materials and Methods." Sup35NM-$His_6$ polymers were affinity-purified from this fraction in the presence of SDS, an agent that dissolves nonspecific protein interactions but leaves intact amyloid polymers (17). Analysis of the obtained polymers showed the presence of Rnq1, although its proportion to Sup35 was ~5-fold lower than in total cell extracts (Fig. 3). Since most of Sup35 was in polymer form, this means that about 20% of Rnq1 was incorporated into Sup35 polymers.

*Nonsense Suppression Caused by Sup35 Overproduction*—Transformants of the 74-D694 [*psi*$^-$] [*PIN*$^+$] strain overproducing Sup35 were able to grow on −Ade −Leu medium, which indicates suppression of the *ade1–14* nonsense mutation. Overproduction of Sup35-NM-GFP caused even better suppression of *ade1–14* (Fig. 4), presumably because this molecule is similar to Sup35 in its polymerization ability but non-functional in translation and was present in a large excess over Sup35. To analyze the appearance of Sup$^+$ cells, cell suspensions of the transformants overproducing Sup35 grown on −Leu medium were spread to single colonies on either −Leu or −Ade −Leu plates. About 1% of colonies (7 out of 712 checked clones) growing on −Leu medium were light pink. They became red

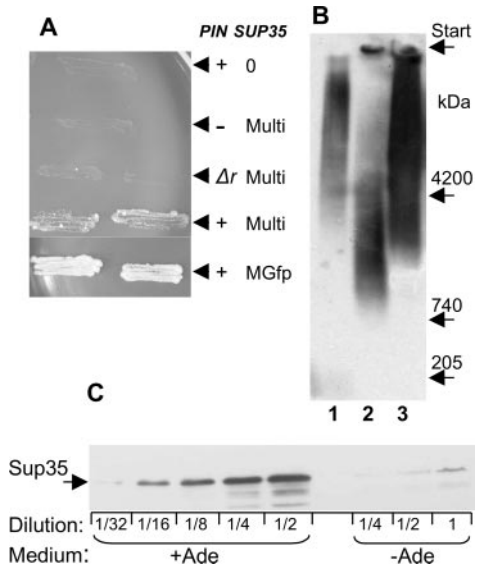


FIG. 4. **Nonsense suppression due to overproduction of Sup35 or Sup35NM-GFP.** *A*, growth of various transformants of 74-D694 [*psi*$^-$] on −Ade −Leu medium. For *PIN*, + indicates [*PIN*$^+$]; − indicates [*pin*$^-$], and Δ*r* indicates that [*PIN*$^+$] cells were transformed with YEplac181-SUP35 and then *RNQ1* was disrupted by *HIS3* insertion. For *SUP35*, *SUP35* indicates alleles in addition to genomic *SUP35*; *0* indicates empty vector YEplac181; *Multi* indicates multicopy *SUP35* vector YEplac181-SUP35; *MGfp* indicates multicopy vector YEplac181-SUP35NM-GFP with the Sup35NM-GFP-encoding allele. Plates were incubated at 30 °C for 7 days. *B*, analysis of the Sup35 polymer size. 74-D694 [*psi*$^-$] [*PIN*$^+$] cells overproducing Sup35 were grown in −Leu medium containing (*lane 1*) or lacking (*lane 2*) adenine. The latter cells were then returned to the medium lacking leucine but supplemented with adenine (*lane 3*). *C*, the determination of the levels of soluble Sup35 in the 74-D694 [*psi*$^-$] [*PIN*$^+$] transformants with the *SUP35* multicopy plasmid incubated in −Leu medium selective (−*Ade*) or non-selective (+*Ade*) for suppression. Cell extracts were equalized for the total amount of protein and then diluted as shown, mixed with the SDS-PAGE sample buffer, subjected without boiling to SDS-PAGE, and analyzed for Sup35 by Western blotting.

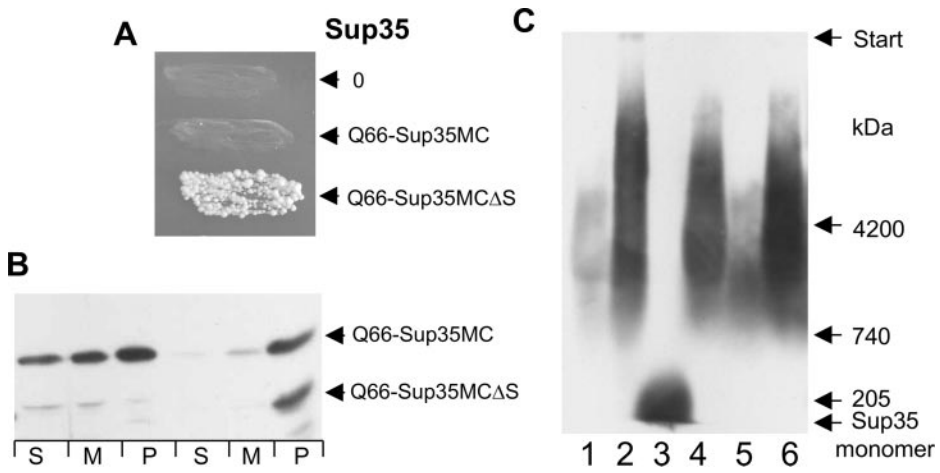


FIG. 5. **Hybrid Q66-Sup35MC protein can cause nonsense suppression due to its polymerization.** The 74-D694 [*PIN*$^+$] strain was used in which genomic *SUP35* was altered for the Q66-SUP35MC construct under the control of *MET17* promoter. *A*, cell growth on −Ade −Ura medium. The cells were transformed with the *URA3* pEMBLyex4 (25) and yex4MetQ66MC35 or yex4MetQ66MC35ΔS multicopy plasmids encoding either no Sup35 (0) or its indicated variants, respectively. Plates were incubated at 30 °C for 7 days. *B*, overproduced Q66-Sup35MCΔS promotes polymerization of Q66-Sup35MC. Cell lysates of the 74-D694/Q66 strain transformed with an empty vector or a multicopy plasmid encoding Q66-Sup35MCΔS grown on −Ura medium containing adenine were centrifuged and analyzed by Western blotting for Sup35. Centrifugation fractions are as follows: *S*, supernatant; *M*, middle; *P*, pellet. *C*, analysis of the Q66-Sup35MC polymer state by SDD-AGE and immunoblotting for Sup35. Lysates of the 74-D694/Q66 [*PIN*$^+$] cells were transformed with multicopy plasmids encoding the following Sup35 hybrids and incubated in the indicated media: *lane 1*, no plasmid (−Ura); *lane 2*, Q66-Sup35MC (−Ura); *lane 3*, same, but in the Δ*rnq1* strain (−Ura); *lane 4*, Q66-Sup35MCΔS (−Ura); *lane 5*, same (−Ura and −Ade); *lane 6*, same cells returned to −Ura medium containing adenine.

after growth in the presence of guanidine hydrochloride, indicating that these clones were [*PSI*$^+$]. These [*PSI*$^+$] represented novel variants since conventional [*PSI*$^+$] are incompatible with Sup35 overproduction (19). Furthermore, they all depended on Sup35 overexpression and could not propagate at standard Sup35 levels. A relatively high proportion, ~13% of cells, were able to produce colonies on medium lacking both adenine and leucine; equal aliquots of the cell suspension produced 340 colonies on −Leu plate and 44 on −Ade −Leu plate. The *SUP35* plasmid was never lost from these clones under conditions selective for suppression but non-selective for the plasmid marker. This suggests that suppression required continuous Sup35 overproduction.

The levels of soluble Sup35 were strongly decreased in the cells grown in −Ade −Leu medium (Fig. 4), which shows that the cause of suppression was the lack of soluble Sup35. In addition, the average size of Sup35 polymers in these cells was decreased in comparison with the cells incubated in medium lacking only leucine (Fig. 4). Such a decrease should lead to an increase in the number of polymer particles, faster polymerization, lower proportion of soluble Sup35 and, therefore, stronger suppression (17).

Quantitative assay confirmed that the nonsense codon readthrough was increased upon selection for suppression and showed that it returned to almost original levels when only the plasmid marker was selected (Fig. 1). The disappearance of Sup$^+$ cells proceeded gradually; spreading of the transformant cells, grown on −Ade −Leu medium, on medium lacking only leucine, produced stable red clones, and unstable light pink clones segregating red sectors (data not shown). It is important that the decrease of suppression efficiency in this medium correlated with an increase in the levels of soluble Sup35 and in the size of Sup35 SDS-insoluble polymers. This suggests that the suppressor phenotype observed upon incubation of transformant cells on −Ade −Leu medium was not due to enrichment by cells with unconventional [*PSI*$^+$] (see above), which could be then spontaneously lost in the absence of selective pressure.

*Amyloid-like Aggregation of Sup35 with the N Domain Replaced for a Polyglutamine Sequence*—The [*psi*$^-$] [*PIN*$^+$] strain 74-D694/Q66 was obtained by the replacement of chromosomal *SUP35* for the hybrid *SUP35-Q66* allele encoding Sup35 with its N domain replaced for a stretch of 66 glutamine residues instead of its N domain (Q66-Sup35MC). This strain showed non-suppressor phenotype, but in contrast to wild type Sup35, a significant proportion of the hybrid Q66-Sup35MC protein formed SDS-insoluble polymers (Fig. 5). Despite this, extra copies of *SUP35-Q66* did not cause nonsense suppression; such transformants had red color and did not grow on −Ade −Ura medium. As for Sup35, disruption of *RNQ1* in these transformants caused the disappearance of SDS-insoluble polymers (Fig. 5). This is consistent with the observation that proteins containing expanded polyQ stretches aggregate upon overproduction in yeast in a [*PIN*$^+$]-dependent manner (20, 21). Suppressor phenotype was, however, observed for the transformants overproducing Q66-Sup35MC without 202 C-terminal amino acids (Q66-Sup35MCΔS), which is inactive in translation termination (Fig. 5). Overproduction of Q66-Sup35MCΔS caused aggregation of both plasmid-encoded Q66-Sup35MCΔS and its chromosomally encoded functional version with the intact C domain, decreasing the levels of soluble Q66-Sup35MC (Fig. 5) and thus stimulating nonsense codon readthrough. As for Sup35, the loss of plasmid expressing Q66-Sup35MCΔS caused the immediate disappearance of the suppressor phenotype, whereas the transfer of such transformants to a medium selective only for plasmid reduced the proportion of Sup$^+$ cells gradually, mainly causing the appearance of pink/red sectored colonies (data not shown). The size of Q66-Sup35MCΔS polymers decreased in medium selective for suppression and increased almost to the original values after return to medium selective only for plasmid (Fig. 5).

In contrast to Sup35, Q66-Sup35MCΔS was unable to generate prions. Spreading cells of the transformants overproducing Q66-Sup35MCΔS on medium selective only for plasmid maintenance did not produce colonies with the suppressor phenotype; no white or pink colonies appeared among about 30,000 of checked clones. We also failed to observe the appearance of clones with the suppressor phenotype upon transient overproduction of Q66-Sup35MCΔS. No such clones were observed among about 5000 of clones that have lost the Q66-Sup35MCΔS-encoding multicopy plasmid on 5-fluoroorotic acid-containing medium.

## DISCUSSION

Overproduction of the Sup35 protein in yeast may cause nonsense suppression, an effect that suggests a reduced function of this protein. Although it is known that Sup35 overproduction can induce the *de novo* generation of nonsense suppressor [*PSI*$^+$] prion determinants (5), this does not explain the suppressor effect caused by excess Sup35 since [*PSI*$^+$] prions appear with very low frequency and, in contrast to [*PSI*$^+$], the *SUP35* multicopy suppressor effect strictly depends on the presence of inducing multicopy plasmid (6).

Here, we have found that Sup35 overproduced in [*psi*$^-$] [*PIN*$^+$] cells forms in the majority of those cells SDS-insoluble amyloid-like polymers similar to the [*PSI*$^+$]-related Sup35 prion polymers. The presence of such polymers strictly depends on [*PIN*$^+$], and they rapidly disappear when [*PIN*$^+$] disappears due to disruption of *RNQ1*. This allowed us to conclude that these Sup35 polymers are poorly inherited but reappear with very high frequency in [*PIN*$^+$] cells. Such dependence on [*PIN*$^+$] suggests that Sup35 polymers may be seeded by Rnq1 polymers, and indeed, we found that about ⅕ of total Rnq1 was bound to Sup35 polymers. This bond was resistant to cold SDS, and thus, this Rnq1 belonged to the Sup35 polymers. It appears unlikely that Rnq1 was dispersed along the Sup35NM polymers since homotypic polymerization should be much more efficient than heterotypic. So most likely, this Rnq1 represented Rnq1 seeds attached to the Sup35NM polymers, which they initiated.

Similar properties, low transmissibility and the high frequency of appearance, distinguish mammalian amyloids from prions. Thus, these Sup35 polymers may be considered as non-prion amyloids. The likely reason for the poor inheritance of the polymers was their large size, presumably due to low accessibility to the fragmenting activity mediated by the Hsp104 chaperone (22). It was proposed that larger polymers have a higher propensity to form higher order aggregates, which unproportionally decreases the number of heritable units (17). Another likely consequence of the large polymer size was low efficiency of Sup35 polymerization, which resulted in relatively high levels of monomer Sup35 and the lack of suppressor phenotype.

Despite the lack of suppression, the presence of Sup35 polymers was a prerequisite for such a phenotype to appear upon selective pressure. Analysis of the population of [*psi*$^-$] [*PIN*$^+$] cells overproducing Sup35 revealed two cell categories capable of suppression. About 1% of these cells showed stable suppressor phenotype (pink color of colonies) on −Leu medium containing adenine, whereas 13% were able to form colonies on −Leu −Ade medium. The former group represented unconventional [*PSI*$^+$] variants, which, in contrast to common [*PSI*$^+$], were compatible with Sup35 overproduction. The latter group represented cells with an unstable suppressor phenotype, which acquired increased levels of nonsense codon readthrough under selection for suppression but returned gradually to the original levels under non-selective conditions. We propose that the suppressor phenotype may appear due to non-heritable variations in the efficiency of Sup35 polymerization and selection of cells with fastest Sup35 polymerization.

A similar property, the ability to cause suppression under selective conditions, was observed for the overproduced polyglutamine fusion Q66-Sup35MCΔS. In this case, no stable [*PSI*$^+$]-like containing clones appeared, and thus, the effect was solely due to the formation of non-prion amyloids of this protein.

Thus, Sup35 overproduction in the [*psi*$^-$] [*PIN*$^+$] cells caused appearance of a spectrum of its prion/amyloid variants, which differed greatly by their frequency of appearance and the polymerization rate. The most abundant of these variants, non-prion amyloids, are normally phenotypically silent, but the random non-heritable fluctuations of the polymerization process allow the selection of cells capable of nonsense suppression. It is also important that the results obtained show the absence of a sharp difference between the prion and non-prion amyloids. In fact, the gradual disappearance of suppressor phenotype of transformants overproducing Sup35 or Q66-Sup35MCΔS after their transfer to medium non-selective for suppression shows that this phenotype can be inherited, albeit inefficiently. This makes such transformants similar to strains with weak and poorly heritable [*PSI*$^+$] variants.

Expansion of polyglutamine domains in some human proteins is known to cause several fatal neurodegenerative diseases, for example, Huntington's disease (for a review, see Refs. 23 and 24). Earlier, a yeast model establishing a direct link between aggregation of expanded polyglutamine domain and its cytotoxicity was developed. Exploiting this model allowed Merrin *et al.* (21) to identify chaperones critical for the formation and expansion of aggregates (21) and to reveal a defect of endocytosis as one of the reasons for their cytotoxicity (25). Nonsense suppressor phenotype developing upon overproduction of Sup35 demonstrates that this protein can be used as a convenient reporter for the detection of amyloids in the yeast cell. This provides an additional approach for the identification of the host factors involved in amyloid formation and suggests that yeast can be used for the study of mechanisms of both prion and amyloid diseases.

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