THE INFLUENCE OF NONSENSE MUTATIONS IN THE *SUP35* GENE ON THE [*PSI*$^+$] PRION PROPERTIES IN YEAST *SACCHAROMYCES CEREVISIAE*

N.P. Trubitsina[1], O.M. Zemlyanko[1,2], S.A. Bondarev[1,2], G.A. Zhouravleva[1,2]
[1]Department of Genetics and Biotechnology, Saint Petersburg State University
[2]The laboratory of amyloid biology, Saint Petersburg State University
n.trubitcina@gmail.com

Introduction.

Various changes in the protein structure can lead to its abnormal aggregation. Recently several cases of amyloidosis or new prion diseases caused by truncated proteins were found. Such non-functional protein fragments may arise by different mechanisms one of which is a preliminary termination of translation due to nonsense mutations in the corresponding gene. Therefore, the study of this phenomenon may be of great interest.

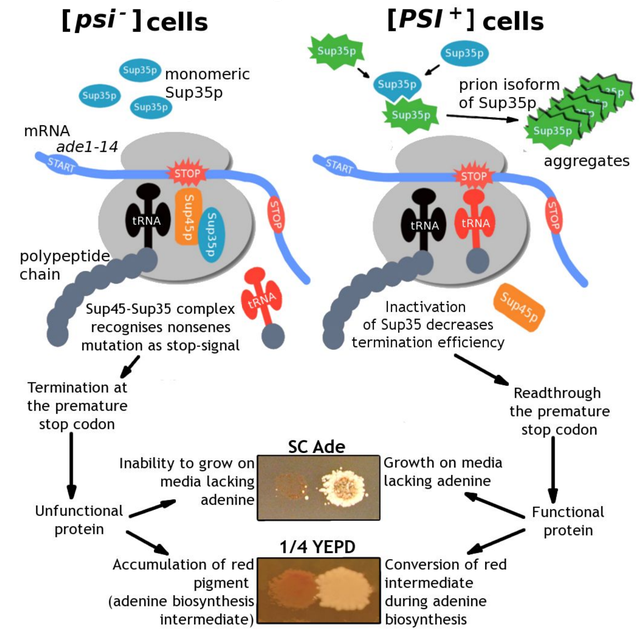

Figure 1. [*PSI*$^+$] cells show nonsense-suppressor phenotype. The protein Sup35 (release factor eRF3) may exist in the yeast *S.cerevisiae* cells in two isoforms: soluble and aggregated. The last one leads to [*PSI*$^+$] prion formation. The efficiency of translation termination reduces when a part of Sup35p is sequestered in prion aggregates. Different mutations in genes *SUP45* and *SUP35* have the same effect. In yeast cells, this phenomenon can be detected by suppressing a nonsense mutation in genes that control the biosynthesis of adenine (for example *ADE1*). Yeast strains harboring *ade1-14* (TGA) nonsense mutation can not grow on media without adenine and have red colonies on medium ¼ YEPD. Reduction in translation termination efficiency caused by [*PSI*$^+$] or mutations in *SUP35* or *SUP45* allows such cells to growth.

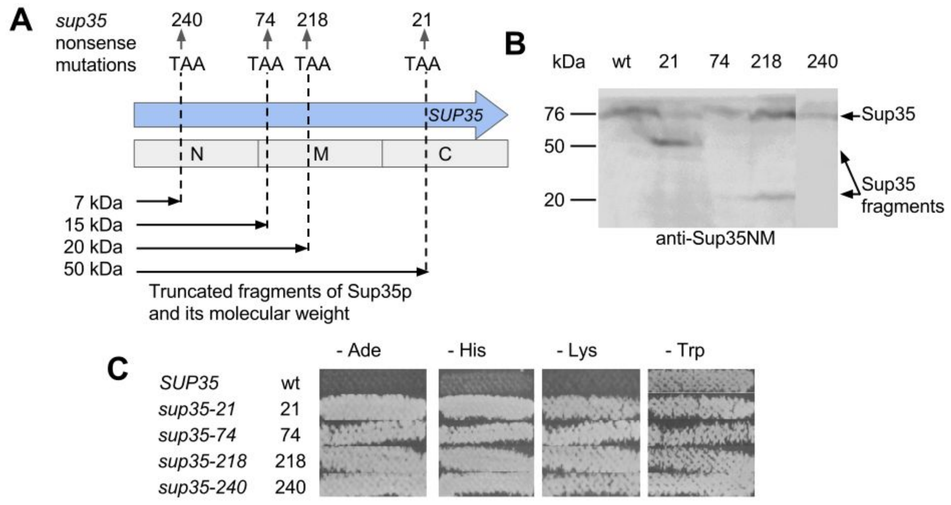

Figure 2. Manifestation of nonsense mutations in the gene *SUP35*. **A.** Localization of studied nonsense mutation in the gene *SUP35*. N, M, C designate corresponding Sup35 protein domains. Short abbreviations of *sup35* mutations are indicated on the top. Truncated fragments sinthesized from nonsense mutations and its molecular weight are presented on the bottom of panel. **B.** All studied *sup35* nonsense mutations lead to a decrease in the amount of full-length Sup35p. Lysates of cells bearing the mutations *sup35-21* and *sup35-218* contained a truncated protein with a molecular mass that approximately corresponded to the predicted one. **C.** Nonsense-mutations in *SUP35* have omnipotent nonsens-suppressor phenotype. Entire and short *SUP35* alleles names are indicated on the left of panel. Types of synthetic culture media (SC) are listed on the top of panel.

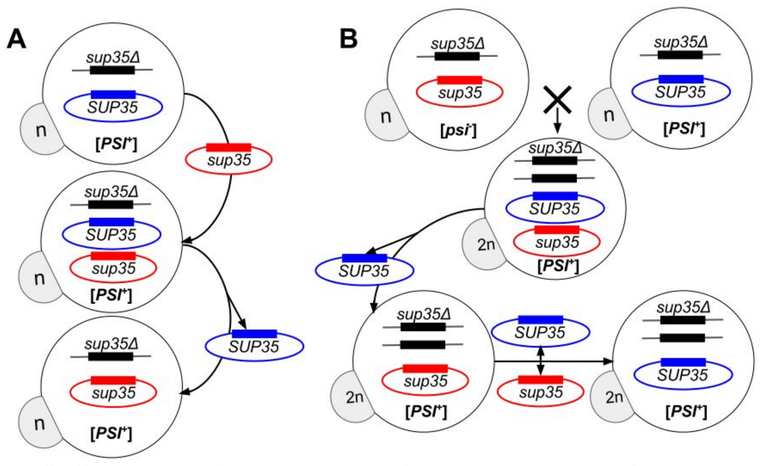

Figure 3. Schemes of experiments. In our work we analysed interplay between *sup35* nonsense mutations and [*PSI*$^+$] in haploid (**A**) and diploid yeast strains (**B**). “n” or “2n” inside the bud designate ploidy of strain. Bold black lines inside cell denote chromosomal deletion of *SUP35* gene. Blue and red circles are plasmids carrying wild type and mutant *SUP35* gene, respectively.

Results.

Figure 4. Incompatibility of *sup35* nonsense mutations with the [*PSI*$^+$] prion depends on strains ploidy. Transformants and diploid cells with *sup35* mutation and [*PSI*$^+$] prion are viable in the presence of wild-type *SUP35* gene copy (SC -Ura -Leu media). Nevertheless *sup35-21*, *sup35-74* and *sup35-218* mutations are incompatible with [*PSI*$^+$] prion in haploid strains in the absence of *SUP35* (FOA media). At the same time diploid cells with prion and *sup35* mutation have no deffects in growth on FOA media. Next, we focused on the study of the possible *sup35* effects on the properties of [*PSI*$^+$] prion in diploid strains.

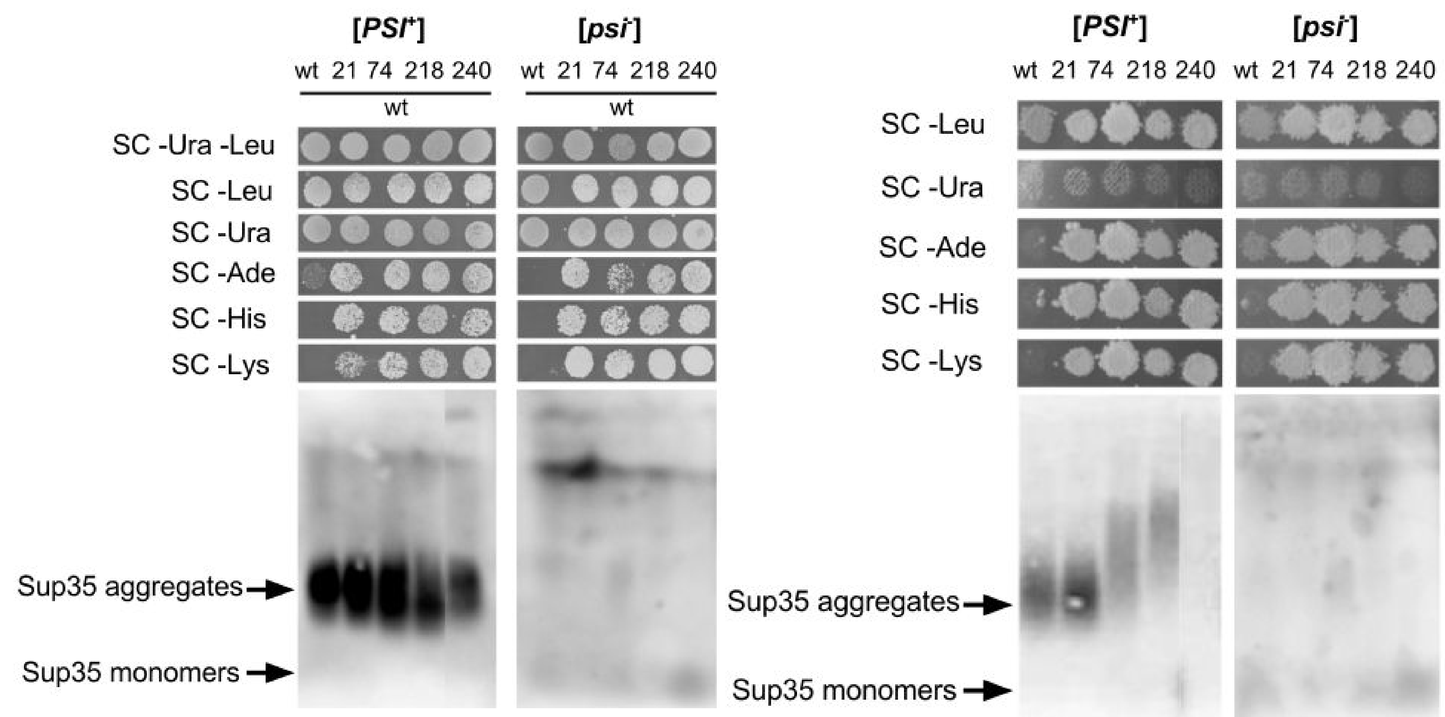

Figure 5. Size of Sup35p aggregates are affected in strains with *sup35* nonsence mutations. Effects of mutations on [*PSI*$^+$] prion properties (size of aggreegates) were observed only in abcence of wild type *SUP35* gene. It is assumed that the wild-type gene has a masking effect for mutations in these case. Aggregates were absent in diploid strains with *sup35-240* mutation. In the case of strains with *sup35-74* and *sup35-218* mutations sizes of aggregates are increased over the wild type; *sup35-21* allele does not change the sizes of the aggregates.

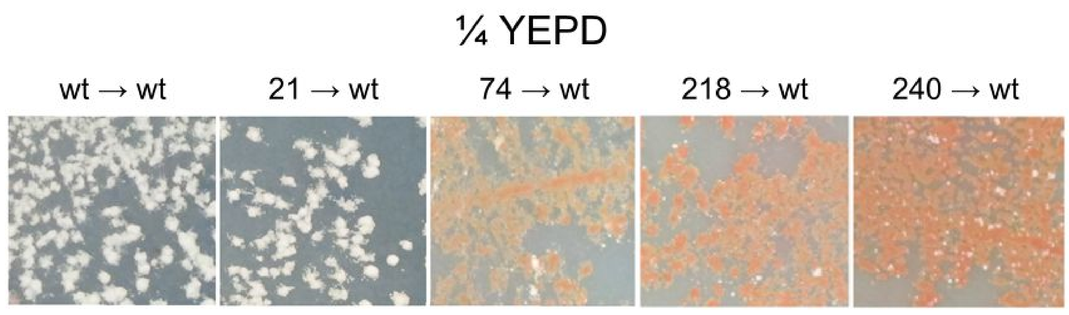

Figure 6. Nonsense mutations in *SUP35* change the [*PSI*$^+$] prion properties. We could not find any Sup35p aggregates in diploid [*PSI*$^+$] strains with *sup35-240* mutation. In order to verify prion loss in this case, we replaced *sup35* alleles with *SUP35* allele. As expected, nonsense suppressor phenotype has not been preserved in these cells that proved loss of the prion. In addition, we found weakening of prion nosense suppressor phenotype after the presence of *sup35-74* and *sup35-218* mutations, which corresponds to an increased sizes of aggregates. Thus these mutations lead to a changes in the properties of the [*PSI*$^+$] prion.

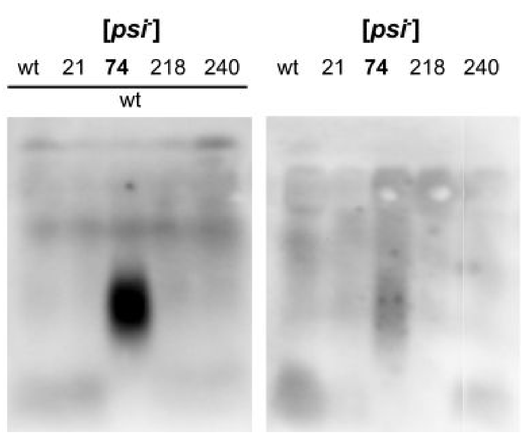

Figure 7. Truncated Sup35p fragments in cells with *sup35-74* may led to [*PSI*$^+$] prion induction. The Sup53p truncated fragments can serve as seeds for the *de novo* Sup35p aggregates formation in [*psi*$^-$] strain with *sup35-74*. The Sup35p fragment consists of entire N-domain and a little bit M-domain in these cells. It was previously shown in literature that N-domain is more prionogenic than entire Sup35 protein. Also presence of shortened M-domain leads to non-effective chaperones cleavage. That is [*psi*$^-$] cells with *sup35-74* mutation accumulate Sup35p aggregates.

Conclusions.

1. ~~*Sup35*~~*-74* and *sup35-218* mutations attenuate the [*PSI*$^+$] prion properties.
2. ~~*Sup35*~~*-240* mutation leads to the [*PSI*$^+$] prion loss.
3. *SUP35* wild-type gene has a masking effect to the [*PSI*$^+$] prion alterations for *sup35* mutations.
4. ~~*Sup*~~*35-74* mutation leads to the spontaneous formation Sup35p aggregates in diploid [*psi*$^-$] strains.

Acknowledgments.

This work was supported by research grants from Saint-Petersburg State University (1.50.1041.2014, 1.37.291.2015) and RFBR (16-04-00202, 16-34-60153), as well as by research resource center “Molecular and Cell Technologies” of Saint-Petersburg State University.