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СБОРНИК ТЕЗИСОВ

BOOK OF ABSTRACTS

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USAGE OF RED FLUORESCENT PROTEINS FOR VISUALIZATION OF [*PSI*$^+$] AGGREGATES

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Prions are heritable self-assembled protein aggregates. [*PSI*$^+$] is one of the most studied prions described in *Saccharomyces cerevisiae*. [*PSI*$^+$] is the prion form of translational termination factor eRF3 (Sup35). Aggregated Sup35 causes defects in termination of translation, which results in nonsense suppression in strains, carrying premature stop-codons. N-terminal domain of Sup35 is necessary for maintaining [*PSI*$^+$] in cells, while middle (M) domain affects properties of the prion. Overproduction of N-domain or NM-domains (Sup35NM) leads to increase in frequency of prion formation. C-domain is responsible for translational release function, but doesn't affect the Sup35 prion properties.

Fluorescent proteins, fused with Sup35NM, are usually applied for visualizing [*PSI*$^+$] aggregates. Previously, the variants of Sup35NM, which carried substitutions in N-domain and fused to GFP, were obtained. To study colocalization of mutant and wild type Sup35NM, red fluorescent protein contrasting GFP is required. We constructed vector for *SUP35NM-mCherry* expression in yeast. However, instead of the expected aggregates we observed diffuse Sup35NM-mCherry fluorescence in [*PSI*$^+$] strains. Nevertheless, analysis of the phenotypes did not show any loss of the prion in these strains. We suggested that aggregates were not observable due to Sup35NM-mCherry proteolysis. We substituted mCherry for another red fluorescent protein yTagRFP-T, resulting in Sup35NM-yTagRFP-T construct. Production of this protein allowed detection of [*PSI*$^+$] aggregates in contrast to mCherry. On the other hand, Western-blot analysis of protein lysates showed that both Sup35NM-yTagRFP-T and Sup35NM-mCherry undergo more intensive degradation than Sup35NM-GFP. Thus, it is possible that degradation products of Sup35NM-mCherry may preserve their fluorescence properties, resulting in diffuse fluorescence despite the presence of the aggregates in the cells. Thus, using red fluorescent protein mCherry does not allow to visualize [*PSI*$^+$] aggregates in contrast to yTagRFP-T. However, both red fluorescent proteins cause increased Sup35NM-RFP degradation, so they are not optimal for visualization of [*PSI*$^+$] aggregates. Using Sup35NM-yTagRFP-T construct we showed colocalization of the wild-type protein with Sup35NM-M0 variant with two aminoacid substitutions (Q33K and A34K), which exhibits anti-prion properties.

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