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CREATION OF MUTANT VARIANT K102R OF YEAST SACCHAROMYCES CEREVISIAE GENE SUP35

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Prions are altered, infectious forms of native proteins that can acquire new functions/lose old ones, aggregate, self-proliferate, spread and cause neurodegenerative diseases in humans and other mammals. In yeast prions are inherited through the cytoplasm, while in mammals — transmitted extracellularly. *Saccharomyces cerevisiae* is an excellent model organism because of its cellular machinery similarity to higher eukaryotes, universal DNA transformation system, and at least 10 different prion domains identified in them. Prion [*PSI*$^{+}$] of Sup35 protein is one of the best-studied yeast prions.

The fact that prions are resistant to various elimination factors encourages the search for reasons of this and possible solutions. It was found that *in vivo* Sup35 protein is accessible to proteases, but the resistance to degradation is determined by the arrangement of amino acids and their properties. Usually lysines in the N-domain of the protein must be ubiquitylated in order for a protein to be directed for proteosomal degradation. Therefore, this work attempts to change the only lysine in 102 position in the N-domain of Sup35 protein to arginine.

In order to create mutant variant K102R of yeast *SUP35* gene primers were created and site-directed mutagenesis was used to change one nucleotide in the sequence through three independent PCR reactions. Later — Sup35K102RGFP was inserted into pJET1.2/blunt cloning vector and successfully multiplied in *Escherichia coli* DH5α cells.

For the first time mutant variant of *SUP35* gene K102R was created. Sup35K102RGFP from pJET1.2/blunt vector can be used to ligate to yeast shuttle vector pRSCup. Then using fluorescent microscopy method native protein and mutated protein prionization can be compared. It is expected that mutant Sup35 protein variant K102R is more prone to prionization and therefore more resistant to degradation in proteasome but to affirm that more experiments has to be done.