The new PNM mutation that prevents [PSI+] prion propagation

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SUP35 gene encodes translation termination factor eRF3, and thus it is an essential gene of yeast Saccharomyces cerevisiae. During translation termination eRF3 interacts with a product of another essential gene, SUP45, which encodes another translation termination factor eRF1. Essential nature of both genes implies that nonsense mutations in each of them should be lethal. However, previously we isolated such mutations, designated them as sup35-n or sup45-n (n – from nonsense) and proposed that viability of such mutants can be explained by readthrough of preliminary stop codon (PTC) because of decreased amount of one of translation termination factor. In support of this assumption, we have shown that all sup35-n and sup45-n mutants contain decreased amount of full-length eRF3 or eRF1, respectively, together with short fragments synthesized in the case of translation termination on PTC. In yeast cells Sup35p is able to aggregate giving rise to [PSI+] prion. Aggregation of Sup35p in the [PSI+] cells results in defective translation termination that leads to omnipotent nonsense suppression. The N-terminal domain of Sup35p is responsible for [PSI+] induction, which is more efficient in the case of artificial introduction in yeast cell of short N-terminal fragments of Sup35p. However a generation of N-terminal fragments of Sup35p is possible in vivo in the case of sup35-n mutations.
Previously we have shown that combination of strong [PSI+] with sup45-n mutations leads to the synthetic lethality which was detected even in heterozygous state. This lethal effect was explained by too high readthrough level in the cells combining decreased amount of Sup45p with decreased amount of functional Sup35p.
Here, we demonstrate that, unlike sup45-n, sup35 nonsense mutations do not lead to synthetic lethality in heterozygous state. We have analyzed the effects of the combination of sup35-n mutations with [PSI+] prion in haploid and diploid cells. Among the mutations studied, we found one, sup35-240, that in all studied cases led to prion loss. This is the only mutation that could replace SUP35WT allele both in haploid and diploid [PSI+] strains, however only [psi-] cells emerged after such shuffle. We have demonstrated that short Sup35p1–56 protein producing from sup35-240 allele, is able to decorate and include in Sup35p aggregates. Thus we can propose that Sup35-240p included into prion aggregates is able to destabilize these aggregates. Alternatively, single molecules of Sup35p1–56 p can stick to aggregate ends, and thus interrupt the fibril growth. Thus we can conclude that sup35-240 mutation prevents [PSI+] propagation and can be considered as a new PNM (from

“[PSI+] No More”, Cox et al., 1988) mutation.
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