

FINAL PROGRESS REPORT
R03TW006965-03 “Phenotypic detection of mouse PrP aggregation in yeast”

a. Specific Aims

Specific Aims of this proposal were as follows.

Aim 1: To check whether PrP-Sup35MC inactivation in yeast is due to prion formation.

Aim 2: To search for the agents that block [PrP^{S+}] formation and or propagation in yeast.

b. Studies and Results

Aim 1.

Our data have shown that transient overproduction of the PrP-Sup35MC protein in the yeast cells generates partially inactive state of this protein ($[PrP^{S+}]$) that is inherited in cell generations and may be detected in yeast phenotype. We hypothesized that this partial inactivation of PrP-Sup35MC is due to formation of the prion isoform of this protein. To check this hypothesis, the following experiments have been performed.

- It is shown that both $[PRP^{S+}]$ and $[prp^{S-}]$ strains contain insoluble (presumably aggregated) fraction of PrP-Sup35MC. The ratio of aggregated and soluble isoforms is not changed in $[PRP^{S+}]$ compared to $[prp^{S-}]$ (Figure 1A).
- It is shown that PrP-GFP fusion protein forms cytologically detected clumps in both $[PRP^{S+}]$ and $[prp^{S-}]$ strains (Figure 1B and 1C), but not in the strain lacking PrP-Sup35MC (Figure 1D).
- Semidenaturing detergent-agarose gel electrophoresis (SDD-AGE) demonstrates that PrP-Sup35MC polymers in $[prp^{S-}]$ strain are localized on the gel mainly in one zone whereas the localization of $[PRP^{S+}]$ polymers is more diffused and significant part of PrP-Sup35MC protein form the high molecular-weight aggregates (Figure 1E).
- It is shown that PrP-Sup35MC chimeric protein is more resistant to proteinase K in the $[PRP^{S+}]$ extracts, compared to the $[prp^{S-}]$ extracts (Figure 1F).
- It is shown that $[PrP^{S+}]$ and $[prp^{S-}]$ states exhibit unusual non-Mendelian segregation in meiosis. The $[PrP^{S+}]$ state is lost in the majority of spores obtained after the cross to isogenic $[prp^{S-}]$ strain followed by meiosis (see Table 1). Thus, $[prp^{S-}]$ isoform is preferentially inherited in meiosis. Diploids resulting from mating of the isogenic $[PrP^{S+}]$ ($MATa$) and $[prp^{S-}]$ ($MATa$) strains grew on $-Ade$ medium slower than $[PrP^{S+}]$ haploids., and after several generations, usually lost the nonsense-suppression phenotype., indicating the predominance of the $[prp^{S-}]$ state in mitosis as well.
- It is found that $[PrP^{S+}]$ factor is transmitted by cytoduction (cytoplasm transfer) albeit with low frequency (about 5-6%). Thus, $[PrP^{S+}]$ is infectious at cytoplasmic level. Relatively low efficiency of cytoduction agrees with the prevalence of $[prp^{S-}]$ over $[PRP^{S+}]$ in the mixed cytoplasm that has been mentioned above. No $[PRP^{S+}]$ colonies were recovered among 406 independent cytoductants analyzed in the control experiments where the $[prp^{S-}]$ donor was mated to the same $[prp^{S-}]$ recipient.

Taken together, these data confirm that PrP-Sup35 can be induced into a phenotypically detectable heritable proteinase-resistant state, possessing most of the major characteristics of a yeast prion such as reversible curability, non-Mendelian inheritance and cytoplasmic infectivity.

As a part of this Aim, analysis of PrP and PrP-GFP aggregation patterns in the yeast cells has also been performed. The following results were obtained.

- PrP and PrP-GFP proteins form the amyloid-like aggregates in the yeast cells, that are resistant to proteinase K, SDS and N-lauryl sarcosine. These aggregates are not toxic to the yeast cells.
- PrP-GFP fusion protein forms various types of visible aggregates in the yeast cytoplasm, that do not colocalize with nucleus, mitochondria, endosome or vacuole.

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Aim 2.

The following experiments were performed.

- Screening of mammalian cDNA library on the yeast expression vector has not produced any proteins influencing [*PrP^{S+}*] among 6,000 clones analyzed.
- It is shown that yeast chaperon Hsp104 that controls the propagation of endogenous yeast prion, is not required for propagation of [*PrP^{S+}*].
- Overproduction of the Hsp40 proteins Sis1 and Ydj1 (from yeast) or Hdj1 (from human) does not affect PrP-GFP aggregation in yeast.
- Overproduction of Hsp104 increases the amount of PrP-Sup35MC, PrP-GFP and GFP when the corresponding genes are under control of *CUP1* promoter (Rubel et al., Mol Biol. 2008, 42(1):123-30). In contrast, overproduction of Hsp104 decreases the amount of PrP-GFP and GFP is case of their expression under control of *GPD* promoter. The effects of Hsp104 are related to neither changes in mRNA content of the genes under investigation nor ability of the proteins to form aggregates. These data show that Hsp104 modulates expression of aggregated and non-aggregated proteins on the posttranscriptional level.

c. Significance

- 1) PrP-containing chimeric proteins are shown to form amyloid-like aggregates in the yeast cytoplasm.
- 2) Chimeric protein PrP-Sup35MC can be induced into a heritable proteinase-resistant state, possessing all the major characteristics of a yeast prion. Thus, at least some crucial components of the prion-propagating machinery are conserved between yeast and mammals.
- 3) PrP-Sup35MC conformational switches have detectable phenotypic effect in the yeast-based system. This provides a new experimental assay that could be applied to studying factors influencing prion properties of PrP.
- 4) Hsp104 regulates the expression of aggregated and non-aggregated proteins in the yeast cell.

d. PublicationsPapers.

Rubel A.A., Saifitdinova A.F., Lada A.G., Nizhnikov A.A., Inge-Vechtomov S.G., Galkin A.P. Yeast chaperone Hsp104 regulates gene expression on the posttranscriptional level. *Mol. Biol.* (Mosk). 2008, 42(1):123-130.

Meeting presentations.

Rubel A. A., Saifitdinova A. F., Lada A.G., Petrova I.T., Chernoff Y.O., Inge-Vechtomov S.G., Galkin A.P. Chaperone Hsp104 affects the mammalian amyloids aggregation in yeast. Prion Biology. Joint Cold Spring Harbor Laboratory/Wellcome Trust Conference. Hinxtion, UK, 2005, p. 71.

Galkin A.P., A.A. Rubel, A.F. Saifitdinova, A.G.Lada, I.N.Petrova, S.G. Inge-Vechtomov, Y.O.Chernoff. Phenotypic detection of the prion-like alterations of mammalian PrP protein in the yeast model. International conference "Prion 2006". Italy, Torino, 3-6 October. Book of the abstracts, p. 135.

Sayfitdinova A., Rubel A., Lada A., Inge-Vechtomov S., Galkin A. Evidence of amyloid nature of A β —GFP and PrP-GFP fusion proteins expressing in yeast. //Materials of XXIII international confrence on yeast genetics and molecular biology, Melbourne, Australia, 1-6 July 2007, Yeast; 24: SI-SI 75, p. S124.

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Saifitdinova A., Lada A., Rubel A., Urazalinov M., Inge-Vechtomov S., Galkin A.. Discovery of new prion-like factor in *Saccharomyces cerevisiae*. Materials of international conference Prion 2007. Book of the abstracts. NeuroPrion. P. 46.

e. Project-Generated Resources

Some plasmids generated within the framework of this project, are being used to teach the Methods of Gene Engineering course at the St.-Petersburg State University.

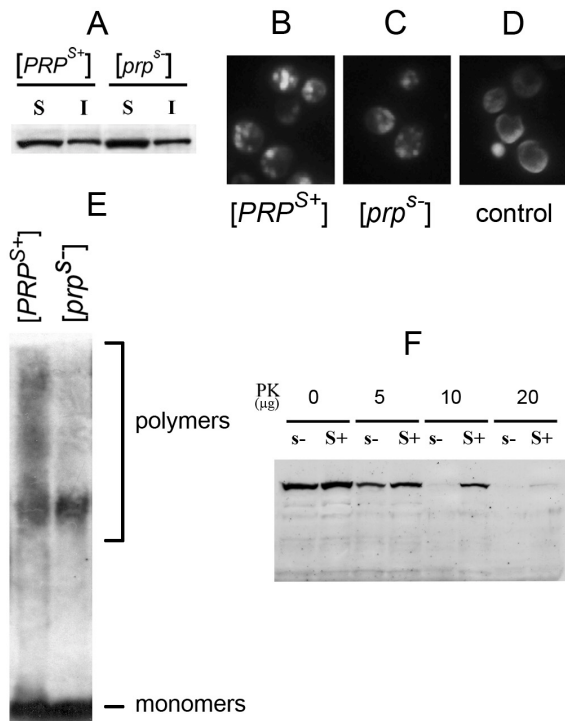


Fig. 1. Aggregation and proteinase resistance of PrP-Sup35MC protein. (A) Detection of PrP-Sup35MC aggregation in $[PRP^{S+}]$ and $[prp^{S-}]$ strains by centrifugation. Proteins were isolated, fractionated by centrifugation and analyzed. Both $[PRP^{S+}]$ and $[prp^{S-}]$ cultures contained both soluble and insoluble PrP-Sup35MC. **(B-D)** Fluorescence microscopy of the PrP-containing aggregates. $[PRP^{S+}]$ and $[prp^{S-}]$ strains were transformed with the plasmid bearing the *PrnP-GFP* gene under P_{SUP35} promoter. Isogenic strain expressing *PrnP-GFP* and bearing the *SUP35* gene instead of *PrnP-SUP35MC* was used as control. **(E)** The comparative analysis of PrP-Sup35MC polymers in $[PRP^{S+}]$ and $[prp^{S-}]$ strains was performed by using Semidenaturing detergent-agarose gel electrophoresis (SDD-AGE). Protein concentrations were determined by Bradford assay according to Bio-Rad (Richmond, CA) protocol. Equal amounts of total protein were loaded in each case. **(F)** Digestion of the PrP-Sup35MC protein by proteinase K. Proteins were isolated from $[PRP^{S+}]$ (S+) and $[prp^{S-}]$ (S-) cultures, digested with proteinase K, run in SDS-PAGE gel and reacted to mouse monoclonal PrP-specific antibodies 3F4.

Table 1.

Meiotic inheritance of the Ade^+_{Cu} phenotype

Viable spores per tetrad	Numbers of tetrads with the following number of Ade ⁺ _{Cu} spores					Total number of tetrads	Total number of viable spores	
	4	3	2	1	0		Ade ⁺ _{Cu}	Σ
4	-	-	-	4	4	8	4	32
3	-	-	1	9	5	15	11	45
2	-	-	-	3	12	15	3	30
1	-	-	-	1	5	6	1	6
Total	0	0	1	17	26	44	19	113