FINAL PROGRESS REPORT (Project 3R01GM58763-10S1, period 12/17/2009-11/30/2010)

a. Specific Aims

Specific Aims of the parental grant remained as follows:

Aim 1. To study the role of chaperones in generation and clearance of the prion protein aggregates.
Aim 2. To study the role of the proteolytic pathways in generation and clearance of the prion protein aggregates.
Aim 3. To study the role of the cytoskeletal networks in generation and clearance of the prion protein aggregates.
Aim 4. To study the role of the Golgi-to-ER trafficking pathway in propagation and clearance of the prion protein aggregates.

Specific Research Objectives of the Administrative Supplement project were as follows:

Objective 1. *Analysis of the composition of cytosolic aggregates formed in the GET-deficient strains.*

Objective 2. *Role of Sgt2 in the TA/GET aggregate composition and modulation of the Hsp104 effect.*

b. Studies and Results

Objective 1.

Epitope-tagged Get proteins have been generated. Colocalization experiments show that in *get1Δ* or *get2Δ* cells, TA/Get aggregates (visualized by tagged Get3) and Sup35 prion aggregates co-localize only in about 10% of the cells that bear both type of aggregates, depending on the deletion strain, indicating that this is unlikely that Get/TA aggregates protect prion aggregates from curing by sequestering them. It is shown that Get3 aggregates formed in the *get*-defective cells do not colocalize with the autophagosomal marker Atg8, that distinguishes them from autophagosomes and autophagosome-associated quality control deposits (IPOD). Get3 aggregates also do not colocalize with with the stress granule marker Pub1 or P-bodies markers Edc3 and Dcp2. This proves that Get3 aggregates are not a result of a stress but caused specifically by a defect in the GET pathway. It is also shown that Get3 aggregates are benomyl-sensitive, pointing to the role of microtubular cytoskeleton in their formation. Induction of the Get3 aggregates by overproduction of the extended aggregating polyQ constructs in the wild-type prion-containing yeast strains is demonstrated, suggesting that formation of the aggresome-like deposits of the polyQ proteins interferes with the functioning of GET pathway. However, polyQ aggregation by itself does not protect [*PSI*$^{+}$] prion from curing by excess Hsp104.

It is shown that levels of Hsps (including Hsp70-Ssa) are increased in GET-deficient strains, indicative of stress response. However, overproduction of Hsp70-Ssa has no effect on prion curing in *get* deficient strains. It is shown that overproduction of the other Hsp70 chaperone, Ssb partly ameliorates effect of get deletions on Hsp104-mediated prion curing. Analysis of the triple deletion *ssb1/2Δ get2Δ* shows that effects of *ssb1/2Δ* and *get2Δ* on prion curing by excess Hsp104 are epistatic, indicating that Hsp70-Ssb and GET complex influence prion curing via the same pathway. It is also shown that *get* deficiencies antibiotic-sensitivity phenotypes that are similar to those detected in *ssb1/2Δ*, suggesting that deficiencies of the GET pathway may partly inactivate Hsp70-Ssb.

Objective 2.

It is shown that Sgt2 is induced in the strains with *get* deficiencies, further confirming the role of Sgt2 as mediator of the *get* effects on prions. However, Sgt2 overproduction failed to protect prion from curing by excess Hsp104 in a wild type strain, showing that decrease of prion curing in the get deficient strains is not caused directly by induction of Sgt2. The *sgt2Δ* deletion does not abolish stress response (including induction of Hsp70-Ssa) in the strains get deficient strains, indicating that restoration of prion curing by excess Hsp104 in the *sgt2Δ get2Δ* strain is not due to lack of induction of other Hsps. However, *sgt2Δ* deletion abolishes formation of Get3 foci in the *get2Δ* strain, indicating that Sgt2 is required either for the formation of TA/Get aggregates in the *get* deficient strains, or for incorporation of the Get3 protein into these aggregates. This

suggests that *sgt2Δ* might restore prion curing by excess Hsp104 via blocking TA/Get aggregate formation or altering TA/Get aggregate composition, and thus preventing sequestration of the Hsp104 cofactors by TA/Get aggregates. It is also shown that *sgt2Δ* increases rather than decreases the defect in prion curing by excess Hsp104 in the strains with defects of ubiquitin system. This shows that Sgt2 exhibits differential effects on different pathways participating in the clearance of prion aggregates.

Two-hybrid and tagged Sgt2 derivatives have been generated. Two-hybrid interactions between Sgt2 and Sup35 have been detected. N-terminal domain of Sup35, that is responsible for prion transformation, is also involved in the interaction with Sgt2. However, coimmunprecipitation failed to detect a complex of the Sgt2 with Sup35 in the yeast cell, indicating that if this interaction occurs, it is apparently of a transient nature.

c. Significance.

Overall, our data lead to the model stating that Sgt2 influences prion-curing by excess Hsp104 in *get* deficient strains via promotion of the formation of TA/Get aggregates, sequestering cofactors that participate in prion curing (possibly, chaperone Hsp70-Ssb and/or some co-chaperones). As most components of the *G*uided *E*ntry of the *T*ail-anchored (TA) proteins trafficking pathway (GET pathway), and specifically Sgt2/SGT protein are conserved between yeast and humans, and both Sgt2 induction and formation of the TA/Get aggregates are influenced by environmental stresses, our data uncover new important and potentially conserved link between intracellular trafficking and amyloid propagation.

d. Publications and meeting presentations

Research paper on the results of research supported by ARRA Supplement is currently being prepared for publication. Results of this work were also presented at the following scientific meetings:

1. D.A. Kiktev, Y. Nishida, S. Müller, J. Patterson, B. Bariar, H. Gong, T. Pan, A. V. Romanyuk & Y.O. Chernoff. Modulation of the effects of a chaperone protein on a yeast prion by the GET pathway and the ubiquitin system. Meeting on Molecular Chaperones and Stress Responses, Cold Spring Harbor Laboratory, 05/2010. (Abstracts of Papers Presented at 2010 Meeting on Molecular Chaperones and Stress Responses, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 82.)
2. Y.O. Chernoff. Prions and quality control in yeast. FASEB Summer Research Conference “Ubiquitin and Cellular Regulation”, Saxtons River, Vermont, 06/2010. (Invited lecture.)
3. D.A. Kiktev, Y. Nishida, S. Mueller, J. Patterson, B. Bariar, T. Pan, A.V. Romanyuk & Y.O. Chernoff. Modulation of the effects of a chaperone protein on a yeast prion by the GET pathway and the ubiquitin system. Yeast Genetics Society of America Meeting, 07/27-08/01/2010. University of British Columbia, Vanvouver, BC Canada. (Program and Abstracts, p. 98.)

e. Project-Generated Resources

Strains and plasmids generated in the course of this project were used to train students at Georgia Tech School of Biology, and also provided to a number of research organizations, including NIDDK, NIH (MD); Emory University (GA); University of Colorado; University of Tennessee at Knoxville; Universitat Autonoma de Barcelona, Spain; Max Delbrueck Center for Molecular Medicine, Berlin, Germany; and Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria.