The goal of this three-years project supported by OTKA-PD Grant is to accomplish a systems biological analysis to describe the decision making process of cells between autophagic dependent survival and apoptosis induced cell death with respect to endoplasmic reticulum (ER) stress generated by various harmful ER stressors. This response mechanism is controlled by a signal transduction pathway, called unfolded protein response (UPR). The primer role of UPR to induce autophagy-dependent survival by self-eating the damaged components, however sever ER stress results in apoptotic cell death.

To achieve a comprehensive study of ER stress response mechanism various human cell lines (HEK – human embryonic kidney, HepG2 – hepatocellular carcinoma) and various ER stressors (such as DTT, tunycamycin, thapsigargin) were used. First we identified a so called low and high level of ER stress. At low level only autophagy turns on, meanwhile high level of ER stress later results in apoptotic cell death. However we confirmed that autophagy-dependent survival always precedes apoptotic cell death even at excessive level of ER stress generating a "time window" for the control mechanism to try to survive the harmful effect. To understand the dynamical characteristic of the regulatory network a stochastic mathematical model was generated. The computational simulations suggest that apoptosis induction has an irreversible switch like characteristic when ER stress reaches a critical threshold. These results were confirmed by so called washout experiments, where the stressors were washed out after a certain time delay from the cells. Our novel results are published in *Biomed Res Int.* These data were also presented both on oral and poster presentations.

New scientific results have revealed that mTOR pathway has directly connected to ER stress response, although the proper mechanism is not known. To investigate the connection between the UPR and mTOR ER stress was induced in human cell line and the phosphorylation of the key targets of mTOR (p70s6, 4EBP1) was followed in time. Our results suggest that mTOR has a transient inactivation when autophagy is active during ER stress; however it comes back when apoptosis turns on. We also observed that the activity of UPR-induced Gadd34 seems to be connected to autophagy activation and mTOR inactivation during ER stress. This result was introduced on a poster presentation, and a manuscript is under preparation.

Meanwhile the role of both PERK-induced Gadd34 and CHOP in respond to ER stress controlled decision making between life and death is under investigation. The main proteins of both IRE-1- and PERK-dependent branches of UPR were followed by immunoblotting in both HEK and HepG2 cell lines with respect to ER stress. Our data suggest that both Xbp1 and Gadd34 get activated parallel to autophagy, while JNK-P and CHOP levels start to increase at fatal ER stress supposing that Gadd34 is connected to the survival mechanism. These effects were also followed both in PERK and Gadd34 silenced cell lines. Our experimental results were introduced on poster presentations, and a manuscript is also under preparation.

Our novel scientific results were presented on many conferences via both oral and poster presentations. The travel cost of OTKA-PD Grant was used to attend the following conferences:

- *Molekuláris Élettudományi Konferencia*, Eger, Hungary, March 27-29.,
- *45. Membrán-Transzport Konferencia,* Sümeg, Hungary, May 19-22.

Our novel scientific results were also presented in Norwich, UK, both at Institute of Food Research and The Genome Analysing Centre in October, 2015 (invited oral presentations).

Our publications and presentation in the 3rd year:

1. accepted manuscript
Holczer M, Márton M, Kurucz A, Bánhegyi G, Kapuy O (2015) Comprehensive Systems Biological Study of Autophagy-Apoptosis Crosstalk during Endoplasmic Reticulum Stress, *Biomed Research International*, 2015:319589.

2. manuscript under preparation
Holczer M, Bánhegyi G, Kapuy O (2015) The positive role of Gadd34-regulated crosstalk between UPR and mTOR pathways in autophagy induction with respect to endoplasmic reticulum stress

3. oral presentation (in English)
Kapuy O (2015) A comprehensive systems biological study of autophagy-apoptosis crosstalk during endoplasmic reticulum stress, *Molekuláris Élettudományi Konferencia*, Eger, Hungary, March 27-29.

4. poster presentation (in English)
Holczer M, Márton M, KuruczA, Bánhegyi G, Kapuy O (2015) A systemsbiological view of life and death decision after endoplasmic reticulum stress – the role of PERK pathway, *Molekuláris Élettudományi Konferencia*, Eger, Hungary, March 27-29.

5. poster presentation (in Hungarian)
Holczer M, Bánhegyi G, Kapuy O (2015) Az mTOR jelpálya és a „nem-feltekeredett fehérjeválasz" kapcsolata endoplazmás retikulum stressz esetén, *45. Membrán-Transzport Konferencia*, Sümeg, Hungary, May 19-22.

6. poster presentation (in Hungarian)
Márton M, Bánhegyi G, Kapuy O (2015) Az élet-halál közötti döntés UPR-szinten történő szabályozása endoplazmás retikulum stressz esetén, *45. Membrán-Transzport Konferencia,* Sümeg, Hungary, May 19-22.