In the fourth year of the project we investigated the calcium sensitive regulation of ER redox homeostasis. The development of genetically encoded fluorescent redox sensors made it possible to examine the ER's redox state in living cells. Previous publications using the GSSG/GSH sensitive Grx1-roGFP1-iE$_{ER}$ and the general redox sensor HyPer$_{ER}$ probes revealed that depletion of luminal Ca^{2+} from the endoplasmic reticulum provokes a rapid and reversible shift towards a more reducing poise. We aimed to clarify the so far unknown underlying molecular mechanism of this phenomenon, and summarized our findings in an original research article.

We found that Ca^{2+} mobilization-dependent reduction was sensitive to inhibition of glutathione synthesis or dilution of cytosolic glutathione by selective permeabilization of the plasma membrane with digitonin. Increasing ER-luminal glutathione levels in response to Ca^{2+} efflux were further confirmed by an ER-targeted sCGrx1p, which specifically equilibrates with [GSH]:[GSSG] and Grx1-roGFP1-iE$_{ER}$ which indicates $[GSH]^2$:[GSSG].

The Ca^{2+}-binding chaperone calreticulin has been previously implicated in this process, however we observed that it was dispensable in inducible reduction of the ER lumen by glutathione flux in our experimental setup.

However, opening the translocon channel by puromycin or addition of cyclosporine A mimicked the glutathione-related effect of Ca^{2+} mobilization. The action of puromycin was ascribable to Ca^{2+} leakage from the ER, what was confirmed by silencing Sec61. Although the mechanism of cyclosporine A-induced glutathione flux was independent of calcineurin and cyclophilin A and B and remained unclear.

We concluded that influx of cytosolic glutathione rather than inhibition of local oxidoreductases is responsible for the reductive shift upon Ca^{2+} mobilization. We postulated the existence of a novel Ca^{2+}- and cyclosporine A-sensitive glutathione transporter in the ER membrane.

In the fourth year we also studied the dynamical characteristic of mTOR/AMPK balance regulated autophagy induction upon various stress events. It is well-known that autophagy-dependent self-eating is tightly regulated by mTOR and AMPK kinases. AMPK promotes autophagy by phosphorylating ULK1 kinase, the key inducer of autophagy activator complex, meanwhile mTOR down-regulates it under nutrient rich condition. However, the active ULK1 can inhibit both AMPK and mTOR. Interestingly, a periodic activation of ULK1 was also observed during prolonged stress.

By using systems biological methods, we analysed the essential feedback loops the AMPK-mTORC1-ULK1 regulatory triangle. We claim that these feedback loops guarantee the appropriate response mechanism when nutrient and/or energy supply changes in the cell. In our opinion, there is an essential double negative feedback loop between mTORC and AMPK. Namely, not only AMPK downregulates mTOR, but mTOR also inhibits AMPK and this inhibition is required to keep AMPK inactive at physiological conditions. Our study also suggests that a delayed negative feedback loop between active AMPK and ULK1 is essential to manage a proper cellular answer. AMPK kinase gets induced by generating prolonged starvation or rapamycin treatment followed by ULK1 activation, whereas active ULK1 kinase quickly down-regulates AMPK resulting in a delayed decrease in ULK1 activity. This periodic repeat of AMPK-ULK1 activation/inactivation due to the negative feedback between them generates an oscillatory activation of autophagy, as well. By computational simulations we also suggest various scenario to introduce "delay" on AMPK-P-dependent ULK1 activation (i.e. extra regulatory element in the wiring diagram or multi-phosphorylation).

According to our new scientific results a manuscript is under preparation. Hopefully this manuscript will be published in high impact factor international journal.

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

- *FEBS3+ From Molecules to living systesms*, Siófok, 2018. szeptember 2-5.,
- *49. Membrán-Transzport Konferencia,* Sümeg, 2019. Hungary, May 14-19.,
- *Molekuláris Élettudományi Konferencia*, Eger, 2019. március 29-31.,
- *Annual Meeting of COST Action - TRANSAUTOPHAGY,* Sofia, Bulgária, 2019. április 23-25.

Our publications and presentations in the 4th year:

1. accepted manuscripts

Beáta Lizák, Julia Birk, Melinda Zana, Gergely Kosztyi, Denise V. Kratschmar, Alex Odermatt, Richard Zimmermann, Miklós Geiszt, Christian Appenzeller-Herzog, Gábor Bánhegyi (2020) Ca2+ mobilization-dependent reduction of the endoplasmic reticulum lumen is due to influx of cytosolic glutathione, *BMC Biology* volume 18, Article number: 19

2. manuscript under preparation

Marianna Holczer, Bence Hajdú, Gábor Bánhegyi G, Orsolya Kapuy (2020) Fine-tuning of AMPK-ULK1-mTOR regulatory triangle is crucial for autophagy oscillation

3. oral presentation (in English)

O. Kapuy, M. Holczer, M. Márton, B. Boglárka, B. Hajdú, PK Vinod, G. Bánhegyi (2018), A Systems Biological Analysis of Cellular Life-and-death Decision in Neurodegenerative Diseases, *FEBS3+ From Molecules to living systesms*, Siófok, szeptember 2-5.

4. poster presentations (in English)

- M. Márton, G. Bánhegyi, O. Kapuy (2018) Studying the genes of PERK pathway during endoplasmic reticulum stress, *FEBS3+ From Molecules to living systesms*, Siófok, szeptember 2-5.

- M. Holczer, B. Hajdú, G. Bánhegyi, O. Kapuy (2018) A systems biological analysis of AMPK-mTOR–ULK1 module via controlling autophagy, *FEBS3+ From Molecules to living systesms*, Siófok, szeptember 2-5.

- M. Holczer, B. Hajdú, G. Bánhegyi, O. Kapuy (2019) A systems biological analysis of AMPK-mTOR–ULK1 module via controlling autophagy, *Molekuláris Élettudományi Konferencia*, Eger, március 29-31.

- M. Márton, G. Bánhegyi, O. Kapuy (2019) Connections between genes of PERK pathway upon endoplasmic reticulum stress, *Molekuláris Élettudományi Konferencia*, Eger, március 29-31.

- M. Holczer, B. Hajdú, G. Bánhegyi, O. Kapuy (2019) A systems biological analysis of AMPK-mTOR-ULK1 module via controlling autophagy, *Annual Meeting of COST Action - TRANSAUTOPHAGY,* Sofia, Bulgária, április 23-25.

- M. Márton, G. Bánhegyi, O. Kapuy (2019) The dynamical characteristic of PERK targets in the decision-making between life and death upon endoplasmic reticulum stress, *Annual Meeting of COST Action - TRANSAUTOPHAGY,* Sofia, Bulgária, április 23-25.

- M. Holczer, B. Hajdú, G. Bánhegyi, O. Kapuy (2019) Fine-tuning of AMPK-ULK1-mTOR regulatory triangle is crucial for periodic activation of autophagy, IX Conference of the Serbian Biochemical Society, Belgrád, Szerbia, november 14-16.

- M. Margita, G. Bánhegyi, O. Kapuy (2019) The dynamical characteristic of PERK targets in the decision-making between life and death upon endoplasmic reticulum stress, IX Conference of the Serbian Biochemical Society, Belgrád, Szerbia, november 14-16.